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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, processing or publication charges. Any processes and submissions about the journal can be made from the website: http://viralhepatitisjournal.org/. Archive of the journal is also available at this website. Manuscripts should be submitted online from https://mc04.manuscriptcentral.com/viralhepatj.

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

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 in 2013) (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The approval of the ethics committee and the fact that informed consent was given by the patients should be indicated in the Materials and Methods section (including approval number). All papers reporting experiments using animals must include a statement in the Material and Methods section giving assurance that all animals have received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" (www.nap.edu/catalog/5140.html) and indicating approval by the institutional ethical review board.

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

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.

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

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

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

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

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

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

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

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

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

• Author number for review articles should not exceed three.

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

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

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

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

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

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

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

ARTICLE SECTIONS

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

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

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

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

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

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

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

Keywords:

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

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

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

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

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 keywords. The main text should consist of introduction, case report, discussion, acknowledgment, conclusion and references. The entire text should not exceed 5 pages (A4, formatted as specified above).

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

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

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

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

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

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

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

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

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

• The manuscripts containing color figures/pictures/tables would be published, if accepted by the Journal. In case of publishing colorful artwork, the authors will be asked to pay extra printing costs.

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• Pictures/photographs must be in color, clear and with appropriate contrast to separate details.

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

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

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

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.

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CONTENTS

Research Article

DOI: 10.4274/vhd.galenos.2022.2022-6-2
Viral Hepatitis Journal 2022;28(3):94-99

Clinical and Laboratory Characteristics of Patients with Hepatitis B Patients with Atypical Serologic Profiles

Atipik Serolojik Profilli Hepatit B Hastalarının Klinik ve Laboratuvar Özellikleri

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ABSTRACT

Objectives: To investigate atypical serological profiles in patients diagnosed and followed up with hepatitis B virus (HBV) infection and to clinically evaluate patients with those have atypical profiles.

Materials and Methods: This study was a single-centre, retrospective cross-sectional cohort study. Six thousand five hundred and sixty-four hospital applications were evaluated. We reviewed 3,372 patient records, of which 145 met the inclusion criteria.

Results: Of the 3,372 patients followed up for HBV infection, 2,072 (61.4%) were male, and the mean age was 50.3±13.6 years. Data from 145 patients with atypical HBV serology were analyzed. Eighty-six (59.3%) of the patients were male. The mean age was 49.2±13.6 years. The prevalence of simultaneous positivity for hepatitis B surface antigen (HBsAg) and anti-HBs (group 1), dual positivity for hepatitis B e antigen (HBeAg) and anti-HBe (group 2), isolated positivity for anti-HBc-IgG (group 3) and isolated HBsAg positivity were found 2.13% (71/3,327), 1.47% (49/3,327), 0.75% (25/3,327) and 0.03% (1/3,327), respectively. Concomitant hypertension was more common in group 1; younger age, elevated alanine aminotransferase (ALT) and HBV-DNA levels and treatment with antiviral drugs were more common in group 2; comorbidities (p=0.07), and hematologic diseases were more common, HBV-DNA levels were negative and treatment with antiviral drugs were less in group 3.

Conclusion: Groups 1 and 2 cases had higher ALT and HBV-DNA levels and are at risk for developing cirrhosis, progressive liver disease and hepatocellular carcinoma. Patients with isolated anti-HBc-IgG should be treated when the risk of HBV reactivation exists.

Keywords: Hepatitis B virus, prevalence, serology, Turkey

ÖZ

Amaç: Hepatit B virüsü (HBV) enfeksiyonu tanısı konan ve takip edilen hastalarda atipik serolojik profilleri araştırmak ve bu hastaları klinik olarak değerlendirmektir.

Gereç ve Yöntemler: Bu çalışma, tek merkezli, retrospektif kesitsel bir kohort çalışmasıdır. Altı bin beş yüz altmış dört hastane başvurusu değerlendirildi. Yüz kırk beşi dahil edilme kriterlerini karşılayan 3.372 hastanın kayıtları incelendi.

Bulgular: HBV enfeksiyonu nedeniyle takip edilen 3.372 hastanın 2.072'si (%61,4) erkekti ve yaş ortalaması 50,3±13,6 yıldı. Atipik HBV serolojisi olan 145 hastanın verileri analiz edildi. Hastaların 86'sı (%59,3) erkekti. Ortalama yaş 49,2±13,6 yıldı. Hepatit B yüzey antijeni (HBsAg) ve anti-HBs için eş zamanlı pozitiflik (grup 1), hepatit B e antijeni (HBeAg) ve anti-HBe için ikili pozitiflik (grup 2), izole anti-HBc-IgG için pozitiflik (grup 3) ve izole HBsAg pozitiflik prevalansı hastalarda sırasıyla %2,13 (71/3.327), %1,47 (49/3.327), %0,75 (25/3.327) ve %0,03 (1/3.327) bulundu. Eşlik eden hipertansiyon grup 1'de daha sık görülürken; daha genç yaş, yüksek alanin aminotranferaz (ALT) ve HBV-DNA seviyeleri ve antiviral ilaçlarla tedavi grup 2'de daha yaygındı; grup 3'te komorbiditeler (p=0,07), hematolojik hastalıklar daha sık, HBV-DNA düzeyleri negatif ve antiviral ilaç tedavisi daha azdı.

Sonuç: Grup 1 ve 2 olguları daha yüksek ALT ve HBV-DNA seviyelerine sahiptir ve siroz, ilerleyici karaciğer hastalığı ve hepatoselüler karsinom geliştirme riski altındadır. İzole anti-HBc-IgG pozitif hastalarda HBV reaktivasyonu riski olduğunda tedavi edilmelidir.

Anahtar Kelimeler: Hepatit B virüs, prevalans, seroloji, Türkiye

Cite this article as: Ayaz ÇM, Başpınar B, Güner R. Clinical and Laboratory Characteristics of Patients with Hepatitis B Patients with Atypical Serologic Profiles. Viral Hepatitis Journal 2022;28(3):94-99

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Introduction

Infection with hepatitis B virus (HBV) attacks the liver and can cause both acute and chronic disease and it is a major global public health problem with significant morbidity and mortality (1). The World Health Organization estimates that 296 million people will live with chronic hepatitis B (CHB) infection in 2019, with 1.5 million new infections each year. In 2019, hepatitis B resulted in an estimated 820,000 deaths, mostly from cirrhosis and hepatocellular carcinoma (HCC) (2). Therefore, the diagnosis and treatment of patients is of great importance in the fight against HBV.

Serological tests are widely used for the diagnosing of HBV infection. During the natural course of HBV infection, four serological biological markers are observed: hepatitis B surface antigen (HBsAg) and its antibody (anti-HBs); surface antigens associated with HBsAg particles and their antibodies; HBV core antigen (HBc) and its antibody (anti-HBc); and an antigen structurally related to HBcAg, namely, hepatitis B e antigen (HBeAg) and its antibody (anti-HBe) (3).

The diversity of HBV antigens and of the antibodies production may vary during the infection natural course, impact of methodology, mutations of virus itself, the immune status and genetic factors of the hosts (3). This situation complicates the evaluation of serological results and affects the treatment orientation (4). Table 1 shows the antigen and counter-antibody serologic profiles and the interpretation of the profile, that can be encountered in the natural course of infection (3,4).

The purpose of this study was to investigate atypical serological profiles in patients diagnosed and followed up with HBV infection and to clinically evaluate patients with those have atypical profiles.

Materials and Methods

Study Design and Population

This study was a single-centre, retrospective cross-sectional cohort study. We included all aged 18 years and older patients who followed up in outpatient clinics of Ankara City Hospital with HBV infection between January 1, 2020 and December 31, 2020. The results of all included patients in all their applications until the end of December 31, 2021 were evaluated. Patients with atypical serological profile were selected for this study. We excluded patients with serologic profiles might have been in the natural course of infection. Patients who underwent plasmapheresis and received intense chemotherapy, had a history of liver and hematopoetic stem cell transplantation were also excluded due to higher rates of atypical serologic profiles. Six thousand five hundred and sixty-four hospital applications were evaluated. We reviewed 3,372 patient records, of which 145 met the inclusion criteria (Figure 1).

Study Variables

Serological markers of patients were analyzed and included according to HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc-immunoglobulin M (IgM), anti-HBc-IgG and HBV-DNA appearances from hospital automation systems. Patients with atypical serological profile were examined in terms of age, gender, underlying comorbidities (including diabetes mellitus, hypertension, malignancy, hematologic, rheumatic and renal diseases), treatment status of HBV infections, the presence of immunosuppression, cirrhosis, HCC and laboratory parameters.

Microbiological Evaluation

For this study, we obtained results of HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc IgM, anti-HBc-IgG, hepatitis delta antigen (HD-Ag) and antibodies against hepatitis delta virus (anti-HDV), human

Table 1. Serological profiles that can be encountered in the natural course of HBV infection and its interpretation

Profiles	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc IgM/IgG		HBV-DNA	Interpretation of infection
Profile 1	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Susceptible individual Never had contact with HBV
Profile 2	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Immune, vaccine responses
Profile 3	Negative	Positive	Negative	Negative	Negative	Positive	Negative	Immune, old infections
Profile 4	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Immune, recent infection
Profile 5	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Incubation period, early phase
Profile 6	Positive	Negative	Positive/ negative	Negative	Negative	Negative	Positive	Incubation period, late phase
Profile 7	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Acute infection
Profile 8	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Immunologic window period
Profile 9	Positive	Negative	Negative	Positive/ negative	Negative	Positive	Positive	Chronic HBV infection/end of recent infection
Profile 10	Positive	Negative	Positive	Negative	Negative	Positive	Positive	Chronic HBV infection
Profile 11	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Inactive HbsAg carrier
Profile 12	Negative	Positive/ negative	Negative	Negative	Positive/negative		Positive	Occult HBV infection

HBsAg: Hepatitis B surface antigen, Anti-HBs: Hepatitis B surface antibody, HBeAg: Hepatitis B e antigen, Anti-HBe: Hepatitis B e antibody, Anti-HBc IgM and IgG: Immunoglobulin M and G antibody to hepatitis B core antigen, HBV: Hepatitis B virus

immunodeficiency virus (anti-HIV) and hepatitis C virus (anti-HCV), HBV-DNA and HDV-DNA levels available in the hospital database. All the serum samples from patients were quantitatively tested for HBsAg and anti-HBs with the enzyme immunoassay method. HBeAg, anti-HBe, anti-HBc, anti-HIV and anti-HCV was detected by commercially available enzyme-linked immunoassay kits. The HBsAg ≥0.05 IU/mL, anti-HBs ≥10 mIU/mL, HBe antigen ≥10 IU/mL, anti-HBe ≥1.2 IU/mL, anti-HBc-IgM ≥1 s/c, anti-HBc-IgG ≥1 s/c, HD-Ag ≥1.1 s/c, anti-HDV ≥1.1 s/c, anti-HIV ≥1.0 s/c, and anti-HCV ≥1.1 s/c was defined to be positive, respectively. Alanine aminotransferase (ALT) was considered high when the lower level was above 50 U/L.

The concentrations of HBV-DNA and HDV-RNA levels were determined the Rotorgene® Q real-time polymerase chain reaction system (Qiagen, Germany) using Artus® HBV-Rotorgene Q kit (Qiagen, Germany, linear range: 31,6-2x10^7 IU/mL) and HDV Real-TM Quant® kit (Sacace, Italy, linear range: 30-10^8 copy/mL), respectively.

The methods and kits are routinely controlled according to the quality standards prepared by the Republic of Turkey Ministry of Health. This study was approved by the Medical and Health Research Ethics Committee of Ankara City Hospital (approval number: E-21-1893).

Statistical Analysis

Atypical serological profiles were presented in the groups. Nominal variables were given as numbers and percentage, whereas continuous variables were given as mean ± standard deviation or median and interquartile range. The distribution of the continuous variables was performed using the Kolmogorov-Smirnov test. Categorical variables were evaluated with Pearson's chi-square test or the Fisher's exact test; continuous variables were compared Student's t-test or Mann-Whitney U test, or Kruskal-Wallis test, where appropriate. The IBM SPSS version 24 (Chicago, USA) was used to perform all statistics.

Results

Of the 3,372 patients followed up for HBV infection, 2,072 (61.4%) were male, and the mean age was 50.3±13.6 years. Data from 145 patients with atypical HBV serology were analyzed. Eighty-six (59.3%) of the patients were male. The mean age was 49.2±13.6 years. Seventy (91.7%) patients presented with comorbidities. The demographics and clinical characteristics of the patients are shown in Table 2.

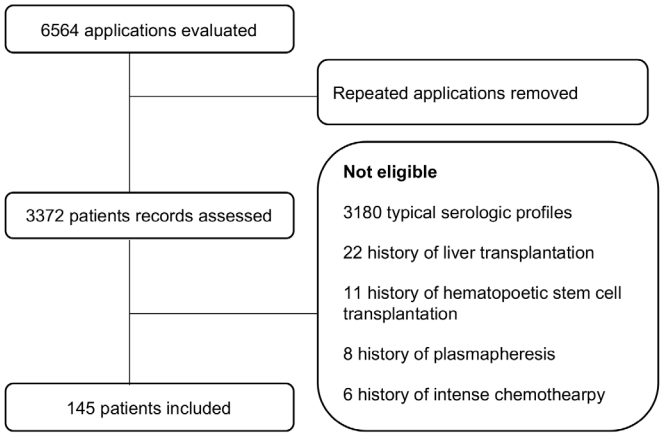

Figure 1. Patient flow chart of the study

The patients were stratified into three groups according to their serologic status as follows: group 1: simultaneous positivity for HBsAg and anti-HBs; group 2: simultaneous positivity for HBeAg and anti-HBe and group 3: positivity for anti-HBc-IgG without detection of HBV-DNA. Only one patient (0.7%) had isolated positivity for HBsAg with undetectable HBV-DNA. One patient was included in both group 1 and group 2 because of serological characteristics. The characteristics of patients with atypical profiles are shown in Table 2.

Patients in groups 1, 2, and 3 were compared with common hepatitis B patients in terms of age and gender. The patients in group 2 were younger than those with common HBV infection (p=0.001), and no difference was found in the groups.

The prevalence of simultaneous positivity for HBsAg and anti-HBs, simultaneous positivity for HBeAg and anti-HBe, positivity for anti-HBc-IgG with or without detection of anti-HBe and isolated HBsAg positivity was found 2.13% (71/3,327), 1.47% (49/3,327), 0.75% (25/3,327) and 0.03% (1/3,327) in all assessed patients, respectively (Table 3).

Concomitant hypertension was more common in group 1; elevated ALT and HBV-DNA levels and treatment with antiviral drugs were more common in group 2. In group 3, comorbidities (p=0.07), and hematologic diseases were more common, HBV-DNA levels were negative and treatment with antiviral drugs were found less (Table 2). Of the 15 (60.0%) patients in group 3, 7 (46.6%) had HBsAg positivity and 1 (6.6%) had anti-HBs positivity in the past years.

Anti-HDV positivity was found in 4 (2.7%) patients (in 2 patients from group 1 and 2) and HDV-RNA positivity was found in 3 (75.0%) out of 4 patients. Elevated ALT (in 2 patients) and positive HBV-DNA (in 1 patient) levels were found in patients with positive HDV-RNA. Anti-HIV and anti-HCV positivity was seen in groups 1 and 3, one patient (0.7%) each.

Discussion

HBsAg/anti-HBs coexistence is an unusual serologic profile seen in the course of HBV infection. Various antiviral treatments, mutations of the virus itself, vaccination and immune responses might be the reason for this coexistence (5). The prevalence of simultaneous anti-HBs and HBsAg positivity was 2.4-5.8% in China (6,7); 2.9-7.0% in South Korea (8,9); 5.0-8.9% in France (10,11) and 0.2-3.6% in Turkey (12,13). In the present study, we found that, of 3,371 HBV-infected patients, 2.13% had HBsAg/anti-HBs coexistence. We observed that, compared with other patients with atypical profiles, the patients with HBsAg/anti-HBs coexistence had higher hypertension rates.

The coexistence of HBsAg and anti-HBs might be associated with important clinical conditions and this profile could be linked to progressive liver disease, HCC, active replication, or reactivation of virus in previous reports (9,14). In our study, HBV-DNA and ALT

Table 2. The demographics and clinical characteristics of the patients

Characteristics	Group 1 71 (49.0)	Group 2 49 (33.8)	Group 3 25 (17.2)	Total, n (%) 145 (100)	p-value
Mean age (±SD), years Median age (IQR), years	- 55 (22)	44.3±11.8	54.1±12.2	49.2±13.6	**0.002**[a]
Male gender	38 (53.5)	32 (65.3)	15 (60.0)	86 (59.3)	0.50[b]
Comorbidities	36 (50.7)	18 (36.7)	16 (64.0)	70 (48.3)	0.07[b]
Hypertension	18 (25.4)	4 (8.2)	1 (4.0)	23 (15.9)	**0.008**[c]
Diabetes mellitus	11 (15.5)	2 (4.1)	2 (8.0)	15 (10.3)	0.12[c]
Malignancy	2 (2.8)	3 (6.1)	-	5 (3.4)	NA
Hematologic disease	6 (8.5)	1 (2.0)	5 (20.0)	12 (8.3)	**0.03**[c]
Rheumatic disease	1 (1.4)	1 (2.0)	2 (8.0)	4 (2.8)	NA
Renal disease	2 (2.8)	-	2 (8.0)	4 (2.8)	NA
Others[*]	4 (5.6)	3 (6.6)	-	7 (4.8)	NA
Immunosuppression	3 (4.2)	2 (4.1)	3 (12.0)	8 (5.5)	0.26[c]
Cirrhosis	6 (8.5)	5 (10.2)	-	11 (7.6)	0.31
Hepatocellular carcinoma	1 (1.4)	1 (2.0)	-	2 (1.4)	NA
Detectable HBV-DNA	32 (45.1)	24 (49.0)	-	56 (38.6)	**<0.001**[c]
Elevated ALT	26 (36.6)	24 (49.0)	3 (12.0)	52 (35.9)	**0.01**[c]
Treatment with antivirals	29 (40.8)	33 (67.3)	2 (8.0)	64 (44.1)	**<0.001**[c]
TDF	17 (58.6)	19 (57.6)	-	36 (56.3)	NA
TAF	2 (6.9)	2 (6.1)	-	4 (6.3)	NA
Entecavir	9 (31.0)	10 (30.3)	2 (100.0)	21 (32.8)	NA
Lamivudine	1 (3.4)	1 (3.0)	-	2 (3.1)	NA
Telbivudine	-	1 (3.0)	-	1 (1.6)	NA

Data were presented as mean ± standard deviation, median and interquartile range (IQR) or n (%). Percentages belong to columns. [*]Others: Thyroid, cardiovascular, cerebrovascular, chronic pulmonary diseases. NA: Not applicable, HBV: Hepatitis B virus, ALT: Alanine aminotransferase, TDF: Tenofovir disoproxil fumarate, TAF: Tenofovir alafenamide fumarate, [a]Kruskal-Wallis test, [b]Pearson's chi-square test, [c]Fisher's exact test

Table 3. The distribution of atypical serology in patients

Serology	Number of patients (n)	Percentages (%)
HBsAg (+) and anti-HBs (+)	71/3,372	2.10
HBeAg (+) and anti-HBe (+)	49/3,372	1.45
Isolated anti-HBc-IgG (+)	25/3,372	0.74
Isolated HBsAg (+)	1/3,372	0.03

Data are presented as n (%). HBsAg: Hepatitis B surface antigen, anti-HBs: Antibody against HBsAg, HBeAg: Hepatitis B e antigen, anti-HBe: Antibody against HBeAg, anti-HBc: Antibody against HBV core antigen immunoglobulin G

levels in group 1 were higher than that in group 3. Therefore, these patients should be closely followed up for the development of advanced liver disease in out-patient settings.

The detection of simultaneous HBeAg and anti-HBe positivity is a rare but well-established profile in HBV infected patients. Previous studies have shown that the prevalence of this profile is 0.2-5.9% in CHB patients and 10.4% in the immune-active phase of HBV infections (15-18). In our study, 1.47% of patients had dual positivity for HBeAg and anti-HBe, which was similar to the reported 1.5% in other study from Turkey (4).

The patients with simultaneous HBeAg and anti-HBe positivity were mostly male, slightly younger, had higher levels of ALT, HBV-DNA, a higher risk of developing liver failure and cirrhosis in the literature (15,18). In the present study, we also found that the mean age was 44.3 years and 65.3% of them were male, had less comorbidities (probably associated with young age), had higher biochemical indicators of liver and virus function and had a higher rate of treatment with antiviral drugs. Mutations, antigen-antibody complex and increased immunological response associated with these complexes may be the reasons for the high HBV-DNA and ALT levels and the increased risk of liver failure in patients with dual HBeAg and anti-HBe positivity (18). These patients are at higher risk of hepatic dysfunction, should be closely monitored.

Hepatitis B core antibody is a sensitive biomarker in identifying patients infected or exposed to HBV (19). Isolated anti-HBc-IgG can be seen in the natural course of HBV infection (Table 1) and this serology may also represent other clinical entities, including

the late stage of prior infections after HBsAg or anti-HBs has fallen down, cross-reactivity and false positivity (12,19). The importance of this serology arises in risk groups such as pregnant women, hemodialysis patients, co-infected patients with HCV and HIV, organ transplant recipients, intravenous drug users and immunosuppressive patients due to the possibility of HBV reactivation. Isolated anti-HBc-IgG positivity has been reported between 1.0%-32% in different populations (20). Studies from Turkey, this rate varies between 1.9% and 5.8% (12,21,22). In our study, the prevalence of isolated anti-HBc-IgG positivity was 0.75% and HBsAg or anti-HBs positivity was found in 8 (53.3%) out of 15 patients in previous years. This result was somewhat lower than that reported rates by prior studies.

Patients with isolated anti-HBc-IgG had more comorbidities, had higher rates of hematologic diseases and had lower ALT levels with undetectable HBV-DNA in our study. The above results were present, probably because anti-HBc positivity in these patients was found by chance because of research on underlying disease. Antiviral treatment was given to 2 (8.0%) patients to prevent reactivation due to the treatment of hematologic or rheumatic disease. It is strongly advised that hemato-oncological patients and candidates for transplantation and immunosuppressive treatment should be screened for anti-HBc-IgG markers with HBV-DNA.

Study Limitations

There are some limitations to our study. Firstly, it is single-center, retrospective study, and includes a specific follow-up period. Secondly, while patients with atypical profile are evaluated with clinical and laboratory results, these data are not available for patients with common HBV. Therefore, no larger scale comparison could be made.

Conclusion

Atypical serological profiles are not uncommon in patients with HBV infection. The interpretation of these results, patients follow-up and their treatment require care in clinical practice. Patients who are likely to develop liver failure, cirrhosis, HCC and HBV reactivation should be followed more closely and necessary active treatments are mandatory, where appropriate. In the presence of such atypical serologies, mutation analyzes should be requested if possible.

Ethics

Ethics Committee Approval: This study was approved by the Medical and Health Research Ethics Committee of Ankara City Hospital (approval number: E-21-1893).

Informed Consent: This was a retrospective study for which no formal consent was required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ç.M.A., B.B., R.G., Design: Ç.M.A., B.B., R.G., Data Collection and Processing: Ç.M.A., B.B., R.G., Analysis or Interpretation: Ç.M.A., B.B., R.G., Literature Search: Ç.M.A., Writing: Ç.M.A., B.B., R.G.

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

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Research Article

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Examination of COVID-19 and Vaccines in Patients with Chronic Hepatitis B

Kronik Hepatit B Hastalarında COVID-19 Hastalığının ve Aşılarının İncelenmesi

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ABSTRACT

Objectives: Coronavirus disease-2019 (COVID-19) has affected more than 16 million people around the worldwide so far. Simultaneously, it has made the follow-up of chronic diseases difficult. We examined the course of co-infection with COVID-19 and chronic hepatitis B in this article and to reveal the vaccination status of these patients.

Materials and Methods: Patients requiring oxygen therapy were classified as severe. Also, patients' demographic and vaccination information was scanned using the hospital data system.

Results: A total of 100 patients with chronic hepatitis B were included. There were 53 patients with polymerase chain reaction-confirmed COVID-19. Since these patients needed oxygen, 9 were admitted to clinics and 4 to the intensive care unit. Of 13 patients with severe disease, 5 had Sinovac, 2 had Pfizer-BioNTech, 1 had mixed vaccine, and 5 were unvaccinated. Severe disease was significantly lower in the Pfizer-BioNTech vaccinated group. Similarly, the longest interval between vaccine and COVID-19 disease was found in this group.

Conclusion: The effect of COVID-19 and hepatitis B co-infection on the severity of COVID-19 and the long-term effects of vaccine-induced immunity in these patients will be guided by epidemiological studies. According to our study, it can be said that the type of vaccine is one of the factors affecting the severity of the disease. Although the number of patients is small, severe acute respiratory syndrome-coronavirus-2 and hepatitis B co-infection do not affect the more severe outcomes.

Keywords: Chronic HBV infection, COVID-19, COVID-19 vaccines, HBV

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) şimdiye kadar dünya çapında 16 milyondan fazla insanı etkilemiştir. Aynı zamanda COVID-19 pandemisi sürecinde kronik hastalıkların takibi zorlaşmıştır. Bu yazımızda COVID-19 ve kronik hepatit B ko-enfeksiyonunun seyrini ve bu hastaların aşılanma durumlarını irdelemeyi amaçladık.

Gereç ve Yöntemler: Kronik hepatit B'si mevcut olup COVID-19 geçiren hastalardan oksijen tedavisi gerektiren hastalar şiddetli olarak sınıflandırıldı. Ayrıca hastane veri sistemi kullanılarak hastaların demografik ve aşı bilgileri tarandı.

Bulgular: Kronik hepatit B'li toplam 100 hasta dahil edildi. Polimeraz zincir reaksiyonu ile doğrulanmış COVID-19 olan 53 hasta vardı. Bu hastaların oksijene ihtiyacı olduğu için 9'u kliniklere, 4'ü de yoğun bakıma yatırıldı. Şiddetli hastalığı olan 13 hastanın 5'inde Sinovac, 2'sinde Pfizer-BioNTech, 1'inde karma aşı vardı ve 5'inde aşı yoktu. Şiddetli hastalık, Pfizer-BioNTech ile aşılanmış grupta önemli ölçüde daha düşüktü. Benzer şekilde aşı ile aşı sonrası COVID-19 geçirme arasındaki en uzun aralık da bu grupta bulundu.

Sonuç: COVID-19 ve hepatit B ko-enfeksiyonunun COVID-19'un ciddiyeti üzerindeki etkisi ve bu hastalarda aşı kaynaklı bağışıklığın uzun vadeli etkileri epidemiyolojik çalışmalara ışık tutacaktır. Çalışmamıza göre hastalığın şiddetini etkileyen faktörlerin birinin aşı türü olduğu söylenebilir. Hasta sayısı az olsa da COVID-19 ve hepatit B ko-enfeksiyonu kliniğin daha ciddi olmasına yol açmamıştır.

Anahtar Kelimeler: Kronik HBV enfeksiyonu, COVID-19, COVID-19 aşıları, HBV

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Introduction

A coronavirus strain known as severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has spread throughout the worldwide and emerged as a significant cause of morbidity and mortality. The outbreak of coronavirus disease-2019 (COVID-19) has been declared a pandemic on March 11, 2020 (1). On the other hand, with approximately 300 million people chronically infected worldwide, the hepatitis B virus (HBV) is a serious threat to public health (2). There have been problems in the follow-up of chronic diseases due to the quarantine of countries, the overcapacity of hospitals with COVID-19. Regarding chronic hepatitis B, Turkey is a middle -endemic region, because of that co-infection with COVID-19 and chronic hepatitis B is also common (3).

COVID-19 does not only affect the respiratory system. The cause of hepatic manifestations is unclear at this stage. Cholangiocytes and hepatocytes have entry cell receptors for SARS-CoV-2 known as angiotensin-converting enzyme 2 receptors (4). According to a recent study, asymptomatic liver function tests are elevated in 50% of patients hospitalized for COVID-19 (5). Possible causes of liver impairment are the direct cytopathic effect of the virus, ischemic liver injury due to hypoxia developing during the disease, and immune-mediated liver injury (6). Additionally, hepatitis B reactivation and drug hepatotoxicity may develop because of the drugs used for treating COVID-19. The management of SARS-CoV-2 and hepatitis B co-infection is challenging because of them (7).

There are many studies have evaluating the severity of COVID-19 in patients with chronic hepatitis B patients. Several studies have shown that COVID-19 is not severe in patients with chronic hepatitis B (7). However, the mortality of COVID-19 is higher in patients with advanced liver disease. Also, patients with advanced liver disease have a poor immune system, and as a result, they are a vulnerable population that should prioritize COVID-19 vaccines. There are inactivated coronavirus vaccine (Sinovac) and mRNA vaccine (Phizer-BionTech) in our country. In this study, we determined the vaccination preference and vaccination rates of chronic hepatitis B patients, as well as to examine the effect of co-infection on the course of the COVID-19.

Materials and Methods

Subjects

One hundred people were included in the study who had chronic hepatitis B. The diagnosis of chronic hepatitis B was made according to the European Association for the Study of the Liver 2020 hepatitis B guideline. Patients' demographics and vaccination information were manually collected from electronic health records.

Ethical Approval

The study protocol was approved by the Ethics Committee of Kocaeli University (approval number GOKAEK-2022/10.10). Since the study was retrospective, informed consent was not obtained from the patients.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 20.0. The values $p<0.05$ were considered statistically significant. Data are presented as mean and percentages. The Kolmogorov-Smirnov test was used to assess the normality of the distribution of the investigated parameters. All parameters in our study were not distributed normally. Differences were tested by Mann-Whitney U test and Wilcoxon test.

Results

One-hundred patients with chronic hepatitis B were included in our study. Among these patients, males (n=56, 56%) predominated over females (n=44, 44%). The mean age of the individuals was calculated as 44 years (range: 12-78). All patients were taken antiviral treatment. Elevated aspartate aminotransferase and alanine aminotransferase were recorded in patients on 7/18 and 2/18, respectively, during COVID-19. The demographics and medical findings of the individuals are shown in Table 1.

Of all chronic hepatitis B patients, 50 were vaccinated with mRNA vaccine (Pfizer-BioNTech, 50%), and 22 with inactivated coronavirus vaccine (Sinovac, 22%). While 11 people (11%) had mixed immunotherapy, 17 (17%) were unvaccinated. In the Pfizer-BioNTech vaccinated group, 15 individuals had COVID-19 after vaccination. In the Sinovac vaccinated group, there were 7 patients after vaccination. Four people who vaccinated with mixed immunotherapy had also COVID-19 disease after vaccines. The time between the vaccination and polymerase chain reaction (PCR) positivity is shown in Table 2. The mean day count between the last vaccine date and SARS-CoV-2 PCR positivity date 127 (Pfizer-BioNTech group), 64 (Sinovac group), and 91 (mixed vaccine group) days, respectively. Those vaccinated with Pfizer-BioNTech were statistically significantly less infected with COVID-19 after vaccination ($p<0.05$).

There were 53 patients with PCR-confirmed COVID-19. Among them, 9 patients were admitted to clinics and 4 to the intensive care unit due to hypoxia. Of 13 patients with severe disease, 5 had Sinovac, 2 had Pfizer-BioNTech, one had mixed, and 5 were unvaccinated. When the 2 patients who died were examined, it was found that 1 of them was unvaccinated and the other was inactivated coronavirus vaccine. Forty patients (74%) had mild disease. On the other hand, among patients with severe diseases, 10 of them had negative current HBV-DNA values.

Table 1. Demographic and medical findings of the patients

Characteristic	Patient
Age, mean	44
Gender, M/F, n	56/44
SARS-CoV-2 PCR positivity, n	53
Mild disease, n (%)	40 (75)
Severe disease, n (%)	13 (25)
Pfizer-BioNTech vaccinated, n (%)	43 (54)
Sinovac vaccinated, n (%)	17 (21)
No vaccinated, n (%)	13 (16)
Mixed vaccinated, n (%)	7 (9)

M: Male F: Female, SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2, PCR: Polymerase chain reaction

Table 2. The time between the vaccination and PCR positivity

	SARS-CoV-2 PCR positivity
Pfizer-BioNTech, day (range)	127 (29-333)
Sinovac, day (range)	64 (7-149)
Mixed, day (range)	91 (51-129)

SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2, PCR: Polymerase chain reaction

Discussion

Data on the prevalence of COVID-19 and chronic hepatitis B confection are limited. In a meta-analysis conducted in China where chronic hepatitis B is hyperendemic, approximately 25,000 COVID-19 patients were examined, and the prevalence of co-infection was found to be 3% (8). Recent reports showed that about 2-11% of patients with COVID-19 had underlying chronic liver disease (9). Hepatic involvement in COVID-19 is multi-factorial. Many reasons such as the direct cytopathic effect of the virus, uncontrolled immune reaction, drug-related injury, sepsis, and hypoxia can cause damage in the spectrum from asymptomatic liver enzyme abnormalities to fatal acute liver injury. During this pandemic, hepatic dysfunction has been seen in 14-53% of patients (10).

In our study, although the number of patients is small, SARS-CoV-2 and hepatitis B co-infection do not affect the severe outcomes. Similar findings were found by Lv et al. (11), who discovered that COVID-19 patients with HBV infection had a lower risk of serious events such as intensive care unit admission or death. However, Jothimani et al. (10) said that COVID-19 may cause worsening of underlying chronic liver disease, leading to hepatic decompensation and acute-on-chronic liver failure, with higher mortality. In a study from China, where hepatitis B is highly endemic, COVID-19 is related to more severe outcomes in patients with chronic liver disease (8).

The administration of vaccines against SARS-CoV-2 will help control the pandemic, especially to populations at high risk of developing severe COVID-19. Although it varied according to the variants, the efficacy of CoronaVac and Pfizer-BioNTech vaccines was found to be 60% and 90%, respectively (12).

Study Limitations

Main limitations are the small number of our patients and the inability to do subgroup analyses.

Conclusion

The effect of COVID-19 and hepatitis B co-infection on the severity of COVID-19 and the long-term effects of vaccine-induced immunity in these patients will be guided by epidemiological studies. In our study post-vaccine COVID-19 disease and severity of disease are less in Pfizer-BioNTech vaccinated group. Additionally, co-infection with SARS-CoV-2 and hepatitis B has no impact on the more serious outcomes.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Kocaeli University (approval number GOKAEK-2022/10.10).

Informed Consent: Since the study was retrospective, informed consent was not obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Research Article

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Hepatitis A Seroprevalence and Factors Affecting Hepatitis A Vaccination Among Healthcare Workers in a University Hospital

Bir Üniversite Hastanesinde Sağlık Çalışanlarında Hepatit A Seroprevalansı ve Hepatit A Aşılamasını Etkileyen Faktörler

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ABSTRACT

Objectives: Although there is an effective and valid vaccine, hepatitis A is an important public health problem, especially in underdeveloped countries. Ensuring high vaccination rates can help reduce the burden of hepatitis A. The aim of our study was to investigate hepatitis A seroprevalence, vaccination status, and barriers to vaccination among healthcare professionals.

Materials and Methods: This is a cross-sectional descriptive study. The study was carried out in the Staff Health Screening Outpatient Clinic of Mengücek Gazi Training and Research Hospital, and the hepatitis A immunoglobulin G (IgG) results of 226 people included in the study were evaluated. A 20-question questionnaire prepared by the researchers, which scanned the participants' occupations, hepatitis infection status, coronavirus and hepatitis A vaccination status, barriers to vaccination, and their relationship with primary care physicians, was filled in by face- to-face interview method.

Results: The mean age was 30.02. Anti-hepatitis A virus IgG value was positive in 65.5% (n=148) of the participants. Only 36.7% (n=83) of the participants had previously been vaccinated against hepatitis A. The biggest obstacle to vaccination was the lack of time with 32.1% (n=46). Hepatitis A vaccination rate of physicians were statistically significantly higher than the others (p=0.018). The communication of the participants with their family physicians positively affected the vaccination rates positively (p=0.001).

Conclusion: The vaccination rate among healthcare workers was relatively low, indicating the need for increased efforts to improve

ÖZ

Amaç: Etkili ve geçerli bir aşı olmasına rağmen hepatit A özellikle az gelişmiş ülkelerde önemli bir halk sağlığı sorunudur. Yüksek aşılama oranlarının sağlanması, hepatit A yükünün azaltılmasına yardımcı olabilir. Çalışmamızın amacı, sağlık çalışanları arasında hepatit A seroprevalansını, aşılama durumunu ve aşılama engellerini araştırmaktır.

Gereç ve Yöntemler: Bu, kesitsel tanımlayıcı bir çalışmadır. Çalışma Mengücek Gazi Eğitim ve Araştırma Hastanesi Personel Sağlık Tarama Polikliniği'nde gerçekleştirildi ve 226 kişi çalışmaya dahil edildi. Katılımcıların hepatit A immünoglobulin G (IgG) sonuçları hastane sisteminden değerlendirildi. Katılımcıların mesleklerini, hepatit enfeksiyon durumlarını, koronavirüs ve hepatit A aşılanma durumlarını, aşılamadaki engelleri ve birinci basamak hekimleri ile ilişkilerini tarayan, araştırmacılar tarafından hazırlanan 20 soruluk anket yüz yüze görüşülerek doldurduldu.

Bulgular: Yaş ortalaması 30,02 idi. Anti-hepatitis A virüs IgG değeri katılımcıların %65,5'inde (n=148) pozitifti. Katılımcıların sadece %36,7'si (n=83) daha önce hepatit A aşısı olmuştu. %32,1 (n=46) ile aşılamanın önündeki en büyük engel zaman yetersizliğiydi. Hekimlerin hepatit A aşılama oranları diğerlerine göre istatistiksel olarak anlamlı derecede yüksekti (p=0,018). Katılımcıların aile hekimleri ile iletişimi aşılanma oranlarını olumlu yönde etkiledi (p=0,001).

Sonuç: Sağlık çalışanları arasında aşılama oranının nispeten düşük olması, aşılama oranlarını iyileştirmek için daha fazla çaba

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vaccination rates. At this stage, family physicians should take a more active role in public health.

Keywords: Hepatitis A, hepatitis A vaccines, seroprevalence, vaccination

gösterilmesi gerektiğini göstermektedir. Bu aşamada aile hekimleri toplum sağlığı açısından daha aktif rol almalıdır.

Anahtar Kelimeler: Hepatit A, hepatit A aşıları, seroprevalans, aşılama

Introduction

Hepatitis A virus (HAV) is an RNA virus belonging to the picornavirus family and the hepatovirus genus (1). It is a significant public health problem in developing countries and countries with low socio-economic status and is directly linked to poor sanitation and socio-economic conditions (2). HAV is transmitted through contaminated food, water or close contact with an infected person (3). The clinical course of HAV infection tends to be milder in children, but it can become more severe with age (4,5). Due to properties such as being disinfectants-resistant and heat, HAV is highly contagious and can survive for long periods outside the body (3). Symptoms may persist for months after the resolution of the infection because HAV can be transmitted through the fecal-oral route for a long time (6). Despite the availability of safe and effective vaccine, HAV continues to play a significant role in the etiology of acute viral hepatitis (3).

According to the World Health Organization (WHO), although the global incidence of hepatitis A has decreased significantly over the past two decades, with an estimated 1.4 million cases reported in 2018, HAV remains the most common form of acute hepatitis worldwide (7). It is believed that the actual incidence of HAV infection is much higher than reported. HAV infection, usually subclinical, anicteric, or icteric, can cause significant morbidity, even if it does not become chronic. In rare cases, fulminant hepatitis can occur, which can lead to high mortality (8). Complications are more common when infection occurs at an older age (4). In terms of seroprevalence, the rates of hepatitis A vary widely depending on the population being studied. In general, the prevalence of hepatitis A antibodies (indicating past infection or vaccination) tends to be higher in countries with lower socio-economic status and poorer sanitation. Poor hygiene practices and crowded living conditions increase the likelihood of transmission (1).

The importance of hepatitis A vaccination cannot be overemphasized. Hepatitis A can cause serious illness, including liver failure and death, and most cases occur in unvaccinated individuals. In addition, outbreaks of hepatitis A can have a significant impact on public health because it can quickly spread in close-knit communities or settings with inadequate hygiene practices. The WHO recommends that all children receive the hepatitis A vaccine as part of their routine childhood immunization schedule (9). However, vaccination rates vary widely worldwide, with coverage range from less than 10% in some countries to above 90% in others (10).

The hepatitis A vaccine is safe, effective and provides long-lasting immunity, and it is recommended for individuals at increased risk of contracting the virus. Ensuring high vaccination rates can help reduce the burden of hepatitis A and protect both individual and public health. WHO reduces the incidence of hepatitis A by 90% by 2030 and increasing vaccination rates is vital to this effort (10).

Our study investigated the seroprevalence of hepatitis A and vaccination status among healthcare workers, who are role models for the community in the health field.

Materials and Methods

This is a retrospective cross-sectional descriptive study. The study was conducted between 01.01.2022 and 01.11.2022 in Erzincan Mengücek Gazi Training and Research Hospital Personnel Health Screening Polyclinic. The research population consists of allied health personnel and physicians working in Erzincan Mengücek Gazi Training and Research Hospital whose hepatitis markers have been checked in the last 6 months.

Although 890 people applied to the polyclinic, hepatitis serology was not requested from health workers who were not in risky groups due to the procedure (medical secretaries, hospital security, patient transport personnel etc.). Hepatitis serology was requested from approximately 450 people. Those who had deficiencies in their analyses and those who did not accept participating in the study were excluded. Without performing a sample calculation, we tried to reach all the patients and reached 226 patients.

The results of hepatitis A immunoglobulin G (IgG) checked in the past six months for the participants were evaluated from the hospitals information management system. The presence of anti-HAV antibodies in serum samples collected from patients has been investigated using a chemiluminescent microparticle immunoassay method. When interpreting the results, samples with values below 1 S/CO were considered negative and samples with ≥1 S/CO were considered positive.

Written informed consent forms were obtained from healthcare personnel who met the inclusion criteria for the study and agreed to participate. The researchers prepared a questionnaire consisting of 20 questions that screened for the participants' occupations, hepatitis infection status, coronavirus (COVID) and hepatitis A vaccination status, barriers to vaccination, and relationships with primary care physicians. The questionnaire was filled out using face-to-face interviews.

Approval for the study was obtained from the Erzincan Binali Yıldırım University, Clinical Research Ethics Committee (approval number: 2022/07-86, date: 06.06.2022). The procedures were followed to comply with the ethical standards of this committee responsible for human experimentation and the principles of the Declaration of Helsinki as revised.

Statistical Analysis

The data were entered into the IBM SPSS Statistics 23 (SPSS, Chicago, IL) package program, and descriptive statistics, chi-square test, Mann-Whitney U test, and Student's t-tests were performed. The statistical significance level was taken as $p<0.05$.

Results

The average age of the 226 people included in the study was 30.02 (minimum: 19, maximum: 55), the average number of siblings was 2.97 (minimum: 0, maximum: 13) and the average number of children was 0.62. (minimum: 0, maximum: 4). Other demographic data of the participants are given in Table 1.

96.4% (n=218) of the participants had at least one dose of the coronavirus disease-2019 (COVID-19) vaccine (mean: 2.75±1.05). The most preferred combination was the 2 Sinovac 1 Biotech combination with 27% (n=61). 25.2% (n=57) of the participants preferred only Sinovac, 22.6% (n=51) only Biotech, and 48.7% (n=110) both vaccines.

The hepatitis A and hepatitis A vaccination status of the participants are given in Table 2.

The relationship between being vaccinated against hepatitis A and gender, occupation, educational status, presence of hepatitis patients in the same household, presence of chronic diseases, and being vaccinated against COVID-19 are given in the Table 3.

The relationship between the anti-HAV IgG values of the participants and age groups, gender, education level, presence of hepatitis patients at home, COVID vaccination status, and previous hepatitis A is given in Table 4.

The relationship between the communication levels of the participants with their family physicians and the vaccination variables is given in the Table 5.

Discussion

HAV is an RNA virus that belongs to the picarnovirus family and is a significant public health problem, particularly in developing countries, due to poor sanitation and socio-economic conditions. The hepatitis A vaccine is safe, effective and provides long-lasting immunity, and it is recommended for individuals at increased risk of contracting the virus. This study investigated the seroprevalence of hepatitis A and vaccination status among healthcare workers at the Mengücek Gazi Training and Research Hospital in Turkey.

The hepatitis A vaccine on the World Health Organization List of Essential Medicines was first approved in Europe in 1991 and in the United States in 1995 (11). In Turkey, the hepatitis A vaccine was introduced as part of the national immunization program in 1998. Since then, it has been consistently administered to children and adults at high risk of infection, including travelers to countries with high rates of hepatitis A, healthcare workers, and individuals with liver disease. It was included in the expanded immunization program in November 2012 (12).

HAV seroprevalence is decreasing in Turkey because of the inclusion of the vaccine in the national immunization program and improved socio-economic conditions (13,14). Although there are positive decreases, Turkey is still in the middle endemic region regarding HAV infection. In middle endemic areas, HAV infection usually coincides with adolescence and early adulthood, and its prevalence increases with age, as expected. In our study, it was observed that anti-HAV IgG positivity increased significantly with increasing age.

Although Turkey is located in the middle endemic region, there are also regional differences due to its wide geographical structure. For example, while anti HAV IgG positivity was found to be 10,18%

Table 1. Demographic characteristics of the participants

	n	%
Gender		
Man	91	40.3
Woman	135	59.7
Duty		
Nurse	111	49.1
Doctor	58	25.7
Other allied health personnel	57	25.2
Education		
Primary-secondary school	9	4.0
High school	22	9.7
University and above	195	86.3
The presence of chronic disease		
Yes	28	12.4
No	198	87.6
Presence of patients with hepatitis in the same household		
Yes	18	8
No	208	92

Table 2. Previous hepatitis A and vaccination status of the participants

	n	%
Anti-HAV IgG positivity		
Positive	148	65.5
Negative	78	34.5
Have you ever had hepatitis A?		
Yes	40	17.7
No	186	82.3
Have you had the hepatitis A vaccine?		
Yes	83	36.7
No	143	63.3
If it was vaccinated, where was it?		
Family health center	29	34.9
Hospital	47	56.6
Other	7	8.4
Would you consider getting the hepatitis A vaccine if you do not?		
Yes	47	32.8
No	54	37.3
I'm undecided	42	29.3
The biggest obstacle to vaccination		
The lack of time	46	32.1
The lack of information	27	18.8
The fear of side effects	31	21.6
Other	39	27.2

HAV: Hepatitis A virus, IgG: Immunoglobulin G

in a study conducted in İzmir, this rate was found to be 90,3% in another study conducted in Şırnak (15,16). In a study by Kutlu et al. (17) on dentistry students in the Central Anatolian region, this rate was found to be 24.9%. In our study, this rate was determined to be 65.5%, and it can be said that there is an average positivity.

Considering the hepatitis A vaccination status although the population of our study consisted of health professionals, vaccination rates were quite low (63.3%). In a study conducted by Bolatkale et al. (18) with 402 people in our country, it was seen that 86.6% of the participants had not had the hepatitis A vaccine before. Although the rates are better, it is expected that the vaccination rates of health professionals who should be role models to society will be much better.

The study also identified several barriers to hepatitis A vaccination among healthcare workers. When questioned about the barriers to getting the vaccine, they included lack of time, lack of awareness about the importance of vaccination, and concerns about the safety and efficacy of the vaccine. These barriers highlight the need for improved education and vaccine access for healthcare workers.

On the other hand, there was no significant relationship between hepatitis A vaccination status and gender, education, presence of hepatitis at home, presence of chronic disease, and COVID-19 vaccination status. There was only a significant relationship between occupation and hepatitis A vaccination status. The percentage of vaccination was higher in physicians in other healthcare professionals, and this level was statistically significant. This could be due to various factors, including differences in access to vaccination, knowledge about the importance of vaccination, or personal beliefs about vaccination. It is also possible that physicians may be more likely to encounter hepatitis A in their work and may therefore have a greater incentive to be vaccinated.

Our study found a significant relationship between age groups and anti-HAV IgG in accordance with the literature (6,8,13,19). Since the probability of encountering hepatitis A infection increases with age, positivity was higher at later ages. However, no significant relationship was found between gender, occupation, education, having hepatitis at home, and preferences for the COVID vaccine. Although the vaccination rates of allied health personnel are quite low, relatively high antibody positivity is an exciting finding. This may be related to hepatitis A infection in childhood to to low socioeconomic conditions.

It is likely that the relationship between other healthcare workers and family physicians could play a role in determining hepatitis A vaccination rate. Family physicians are often the primary point of contact for individuals seeking medical care, and they can play a crucial role in educating patients about the importance of

Table 3. Relationship between being vaccinated against hepatitis A and gender, occupation, educational status, presence of hepatitis patients in the same household, presence of chronic diseases, and being vaccinated against COVID-19

	The status of hepatitis A vaccination					**p**
	Yes		**No**		**Total**	
	n	**%**	**n**	**%**		
Gender						
Man	38	41.8	53	58.2	91	0.198
Woman	45	33.3	90	66.7	135	
Duty						
Nurse	37	33.3	74	66.6	111	**0.018**
Doctor	30	51.7	28	48.3	58	
Other allied health personnel	16	28.0	41	72.0	57	
Education						
Primary-secondary school	2	22.2	7	77.8	9	0.281
High school	11	50	11	50	22	
University and above	70	35.9	125	64.1	195	
Presence of patients with hepatitis in the same household						
Yes	3	16.7	15	83.3	18	0.066
No	80	38.5	128	61.5	208	
The presence of chronic disease						
Yes	9	32.1	19	67.9	28	0.591
No	74	37.3	124	62.7	198	
COVID vaccination status						
None	1	12.5	7	87.5	8	0.143
Only Sinovac	25	43.9	32	56.1	57	
Only Biotech	14	27.5	37	72.5	51	
Both of them	43	39.0	67	61.0	110	

COVID-19: Coronavirus disease-2019

vaccination and helping them access vaccines. Therefore, a positive relationship between healthcare workers and family physicians may be beneficial in promoting vaccination among healthcare workers. This could include collaborating on education and outreach efforts and coordinating vaccine delivery and administration.

Study Limitations

Our study has some strengths and weaknesses. Although participants' vaccination information and medical records have been reviewed, there may still be incomplete records (especially if it has been ten years or more since vaccination). In these cases, the answers given by the participants were accepted as correct. Although the participants are generally a group with high health literacy, some deficiencies may exist. In this respect, it may be considered in the future to conduct a study only with participants whose medical records are up-to-date.

On the other hand, there are many studies on hepatitis seroprevalence in the literature but few on vaccination rates, especially in the hepatitis A vaccine. Although there are many studies on hepatitis B vaccination in the literature, less attention may be given to hepatitis A vaccinations, probably because they are seen as more harmless and included in vaccination programs later. In this respect, our study is a study that can contribute to the literature. However, the fact that the study was conducted only on health workers can be considered a shortcoming. There is a need for advanced community-based studies that include primary care.

Conclusion

Overall, the study found that the seroprevalence of hepatitis A and the vaccination rate among healthcare workers in Mengücek Gazi Training and Research Hospital in Turkey were relatively low,

Table 4. Relationship between the anti-HAV IgG values of the participants and age groups, gender, education level, presence of hepatitis patients at home, COVID vaccination status, and previous hepatitis A

	Hepatitis A IgG positivity					p
	Positive		Negative		Total	
	n	%	n	%	n	
Age groups						
19-24	28	52.8	25	47.2	53	**0.022**
25-27	34	60.7	22	39.3	56	
28-32	39	67.2	19	32.8	58	
33-55	47	79.6	12	20.4	59	
Gender						
Woman	86	63.7	49	36.3	135	0.492
Man	62	68.1	29	31.9	91	
Duty						
Nurse	75	67.5	36	32.5	111	0.771
Doctor	36	62.0	22	38.0	58	
Other allied health personnel	37	64.9	20	35.1	57	
Education						
Primary-secondary school	9	100	0	0	9	0.085
High school	14	63.6	8	36.4	22	
University	125	64.1	70	35.9	195	
Do you have patients with hepatitis in your home?						
Yes	12	66.6	6	33.3	18	0.913
No	136	65.3	72	34.7	208	
Have you had COVID vaccine?						
Yes	142	65.1	76	34.9	218	0.564
No	6	75	2	25	8	
Which COVID vaccine						
Only Sinovac	37	65	20	35	57	0.640
Only Biontech	30	58.8	21	41.2	51	
Both of them	75	68.1	35	31.9	110	
Passing hepatitis A						
Yes	38	95	2	5	40	**0.001**
No	110	59.1	76	40.9	186	

HAV: Hepatitis A virus, IgG: Immunoglobulin G, COVID: Coronovirus

Table 5. Relationship between the participants' communication levels with their family physicians and vaccination variables

	Communication level with family physicians									
	Very bad-bad		Intermediate		Good		Excellent		Total	p
	n	%	n	%	n	%	n	%	n	
Have you been informed about vaccinations by your family physician?										
Yes	0	0	12	14.4	35	42.1	36	43.3	83	0.001
No	13	12.8	47	46.5	41	40.5	13	12.8	101	
Partly	1	0.4	9	42.8	11	52.3	8	38	21	
Have you been vaccinated against hepatitis A?										
Yes	2	2.4	21	25.3	27	32.5	33	39.8	83	0.001
No	12	8.4	47	32.9	60	42	24	16.7	143	
If there were vaccine, where did it happens?										
Family Health Center	0	0	8	27.6	7	24.1	14	48.2	29	0.022
Hospital	2	4.2	11	23.4	18	38.2	16	34.0	47	
Other	0	0	2	28.5	2	28.5	3	42.9	7	
The biggest obstacle to vaccination										
The lack of time	3	10.7	15	53.6	5	17.9	5	17.9	28	0.009
The lack of information	4	14.8	9	33.3	7	25.9	7	25.9	27	
The fear of side effects	1	5.5	11	61.0	3	16.6	3	16.6	18	
Other	4	11.7	12	5.9	9	26.4	9	26.4	34	

indicating a need for increased efforts to improve vaccination rates and protect healthcare workers from hepatitis A infection. This could include improved education about the importance of vaccination, increased access to the vaccine, and efforts to address concerns about the safety and efficacy of the vaccine.

Ethics

Ethics Committee Approval: Approval for the study was obtained from the Erzincan Binali Yıldırım University, Clinical Research Ethics Committee (approval number: 2022/07-86, date: 06.06.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Research Article

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The Effect of the Administration of Interferon and Steroids on Regulatory T-cells in the Liver, Spleen, and Bone Marrow of Mice

Farelerde İnterferon ve Steroid Uygulamasının Karaciğer, Dalak ve Kemik İliğindeki Regülatuvar T-hücrelerine Etkisi

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ABSTRACT

Objectives: Regulatory T-cells (T-regs) maintain immune tolerance by affecting other cells of the immune system. They play an important role in autoimmune diseases and the prevention of graft rejection. Steroids suppress the immune system, especially inhibiting cytokine secretion of T-lymphocytes, initiation of the cell-mediated immune response, and stimulation of T-regs. Interferons (IFN) also have immunomodulatory, antiviral, and anti-proliferative effects. They activate macrophages and cytotoxic T-cells and stimulate the differentiation of T-regs. The aim of this study was to evaluate the effects of IFN and steroids on T-regs in the liver, spleen, and bone marrow in a mouse model, and to determine if they exert their immunosuppresive/immunomodulatory effects through T-regs.

Materials and Methods: A total of 24 mice were randomly separated into 3 groups and administered an intraperitoneal injection for five days. The control group received 0.1 mL saline every day, the IFN group received IFN-alpha-2b 20,000 IU on the first, third, and fifth days, and only 0.1 mL saline on the other days, and the steroids group received 5 mg/kg dexamethasone in 0.1 mL

ÖZ

Amaç: Regülatuvar T-hücreleri (T-reg) immün sistemde görevli birçok hücre çeşidine etki ederek immünolojik toleransı sağlayan hücrelerdir. Otoimmün hastalıklar, greft rejeksiyonunun önlenmesi ve enfeksiyon hastalıklarında önemli role sahiptirler. Steroidler, immün sistemi baskılarlar; özellikle T-lenfositlerin sitokin salgılamasını ve hücresel immünolojik yanıtın başlamasını önlerler ve T-reg'leri de stimüle ederler. Diğer yandan interferonlar (İFN) immünomodülatör, antiviral ve anti-proliferatif etkiye sahiptirler. Makrofajları ve sitotoksik T-hücrelerini aktive ederler ve T-reg'lerin diferansiyasyonunu uyarırlar. Biz bu çalışmamızda İFN ve steroidin karaciğer, dalak ve kemik iliğindeki T-reg'lere etkisini, bilinen immünosüpresif/immün düzenleyici etkilerini T-reg'ler üzerinden yapıp yapmadıklarını değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Bunun için 24 fareye 5 gün boyunca intraperitoneal enjeksiyon yapıldı. Kontrol grubuna 0,1 cc serum fizyolojik her gün uygulandı. İFN grubuna İFN-alfa-2b 20.000 IU 0,1 cc olacak şekilde serum fizyolojik ile sulandırılarak gün aşırı 3 kez diğer günler 0,1 mL serum fizyolojik uygulandı. Steroid grubuna deksametazon 5 mg/kg 0,1 mL olacak şekilde serum fizyolojik

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saline every day. Two days after the end of therapy, each mouse was anesthetized, the portal vein was explored via laparotomy, and 5 mL bovine serum albumin (BSA) was administered through the portal vein. The inferior vena cava was cut to allow BSA perfusion of the liver, and then the mice were sacrificed. The liver, spleen, and bone marrow were removed for analysis. T-regs were identified and counted using flow cytometry.

Results: The flow cytometry count results showed no significant difference between the IFN, steroid, and control groups.

Conclusion: IFN and steroid use do not seem to affect the quantity of T-regs.

Keywords: Regulatory T-cells, interferon, steroid, immunology

ile sulandırılarak her gün uygulandı. Enjeksiyonlar bittikten sonra 2 gün beklendi. Farelere genel anestezi uygulandı, laparotomi yapılıp portal ven açığa çıkarıldı, portal venden 5 mL bovine serum albümin (BSA) verildi, inferior vena kava kesilerek karaciğerin BSA ile perfüzyonu sağlandı, fareler feda edilmiş oldu. Karaciğer, dalak ve kemik ilikleri elde edildikten sonra T-reg'lerin ayrımı yapıldı ve akım sitometrisi ile sayıldı.

Bulgular: Akım sitometrisi ile sayımda İFN, steroid ve kontrol grubunda T-reg sayılarında istatiksel olarak anlamlı bir farlılık bulunamadı.

Sonuç: Sağlıklı farelerde İFN ve steroid kullanımının karaciğer, dalak ve kemik iliğindeki T-reg'lerin miktarına etki etmediği düşünüldü.

Anahtar Kelimeler: Regülatuvar T-hücresi, interferon, steroid, immünoloji

Introduction

Lymphocytes are a single cell group in the immune system that carry receptors specific to antigens. Non-response of lymphocytes to self-antigens is known as immune tolerance. The absence of immunological tolerance results in autoimmune diseases. In 1969, Nizhizuka and Sakakura (1) demonstrated that one cell type was especially affected in autoimmune diseases. In 1995, Sakaguchi et al. (2) demonstrated the role of CD4(+) T-cells that show CD25 positivity in immunological tolerance. Regulatory T-cells (T-regs) are a heterogeneous cell group which carry CD4(+) CD25(+) surface molecules expressing forkhead box P3 (FOXP3) transcription factor (3,4). This is indispensable for the reliable functioning of the immune system. T-regs are located among CD4(+) and CD8(+) T-cells, B-cells, natural killer cells, natural killer T-cells, and dendritic cells, and suppress the activation, proliferation, differentiation, and effect functions of many cell types. In this way, they control the immune responses that develop against pathogens, alloantigens, and tumors, with self-antigens (5).

Interferons (IFN) have antiviral, immunomodulatory, and antiproliferative effects and stimulate the differentiation of T-regs (6).

Corticosteroids are the most frequently used drugs because of their anti-inflammatory, antiallergic, and immunosuppressive effects. Glucocorticoids have been shown to upregulate FOXP3 expression and regulatory T-cells (7,8,9).

The aim of this study was to evaluate the effect of IFN and steroids on T-regs in the liver, spleen, and bone marrow and whether they produce their immunosuppressor/immunomodulatory effects through T-regs.

Materials and Methods

Mouse Model

Approval for this study was granted by the Animal Experiments Local Ethics Committee of İstanbul University. Adult male Balb-C mice at least 8 weeks old and each weighing 25-35 gr were obtained from İstanbul University Experimental Medical Research Institute. Throughout the experiment, the mice were kept in a cage at standard room temperature and humidity with a daily provision of food and drinking water.

The protocol was approved by the Committee on the Ethics of Animal Experiments of İstanbul University (approval number: 119).

Applications

The animals were separated into 3 groups and intraperitoneal (ip) injections were performed daily for 5 days. The control group (n=6) received 0.1 cc isotonic saline (0.9% NaCl) (IS) each day. The IFN group (n=9) received IFN-alpha (α) 2b 20,000 IU (Intron-A flacon, 10 M IU/1 mL) diluted in saline to a dose of 0.1 cc on days 1, 3, 5 and 0.1 cc IS only on days 2 and 4 (10,11,12). The steroid group (n=9) received 5 mg/kg dexamethasone (Dekort-im/iv ampoule 8 mg/mL) diluted in IS to a dose of 0.1 cc, every day for 5 days (1). At 2 days after completion of all the injections, general anesthesia was administered ip to all the mice with atropine sulfate 0.3 mg/kg (atropine ampoule), xylazine 10 mg/kg (rompun flacon), and ketamine 200 mg/kg (ketalar flacon). Laparotomy was performed, and the portal vein was exposed. Bovine serum albumin (BSA) 5 mL was administered, and by cutting the inferior vena cava, perfusion of BSA to the liver was achieved. All mice were then euthanized.

Obtaining the T-regs

After euthanasia of the mice, the liver, spleen, and bone marrow were removed from each animal for evaluation of T-regs. The liver was removed and weighed, then placed in Roswell Park Memorial Institute (RPMI) 1640 medium solution and cut into small pieces with surgical scissors. The pieces were made homogenous by crushing with a syringe piston and then passed through a tea strainer. After leaving for 1 min in a flacon, the supernatant was withdrawn. This was made up to 40 cc with RPMI, then centrifuged at 500 rpm for 3 mins and the supernatant was withdrawn. Thus, leukocytes within the liver were obtained. The spleen was removed and placed in RPMI solution. RPMI solution was administered between the capsule and the spleen, and when the capsule was swollen, the spleen was separated from the capsule using the tip of an insulin syringe with gentle movements. This was made homogenous by crushing with a syringe piston and

after leaving for 1 min in a flacon, the supernatant was withdrawn. This was made up to 40 cc with RPMI, then centrifuged at 500 rpm for 3 mins and the supernatant was withdrawn and passed through a tea strainer. Thus, leukocytes in the spleen were obtained.

To obtain bone marrow, the tibia and femur bones were removed, the surrounding muscle tissues were cleaned, and the bones were placed in an RPMI solution. The RPMI solution was administered with a syringe from one end of the bone. The bone marrow was separated from the bone with the pressure of injection. This was made homogenous by crushing with a syringe piston, and after leaving for 1 min in a flacon, the supernatant was withdrawn and passed through a tea strainer. Thus, leukocytes in the bone marrow were obtained.

All procedures were performed over a dish of ice. For separation of the T-regs from the lymphocytes obtained, the CD4(+) CD25(+) Regulatory T-cell Isolation Kit (Miltenyi Biotec), MS column (Miltenyi Biotec) and MiniMACS separator (Miltenyi Biotec) were used.

The leukocytes obtained were counted on a Thoma slide separately for each organ, and the cell count was determined as millimeters cubed, the ratio of cells in the suspension was calculated, and the total leukocyte count was determined. First, the CD4(+) lymphocytes was separated. For this, the cell suspension was centrifuged at 1393 rpm for 10 min. The supernatant was completely aspirated, and the precipitate remaining at the bottom was made a suspension again with 90 µL buffer for every 10^7 cells, then 10 µL CD4 microbead for every 10^7 cells was added, mixed well, and then cooled at 4-8 °C for 15 mins. The cells were washed with 1-2 mL buffer for every 10^7 cells, then centrifuged at 1393 rpm for 10 mins, the supernatant was completely aspirated and the precipitate remaining at the bottom was made a suspension again with 500 µL buffer for every 10^8 cells. The MS column was washed with 500 µL buffer and the cell suspension was applied to the column, then the column was washed 3 times with 500 µL buffer, the column was removed from the separator and placed in an appropriate collection tube, then 1 mL buffer was applied to the column. The cells adhering to the column were shed and thus the CD4(+) cells were obtained, and the CD25(+) cells within these cells were marked to be counted in flow cytometry. For this, cells shed from the column were counted on a Thoma slide. The cell suspension was centrifuged at 1393 rpm for 10 min. The supernatant was completely aspirated. The precipitate remaining at the bottom was made a suspension again with 100 µL buffer for every 10^7 cells, then 10 µL CD25 antibody for every 10^7 cells was added, mixed well, and then left in the dark for 10 min at 2-8 °C. The cells were then washed with 1-2 mL buffer for every 10^7 cells, then centrifuged at 1393 rpm for 10 mins, the supernatant was completely aspirated and then made a suspension again with 1 mL buffer for flow cytometry counting. Thus the CD4(+) CD25(+) cells (T-regs) were prepared for counting with flow cytometry. After counting a total of 10,000 cells on flow cytometry, the lymphocytes were gated and the CD4(+) CD25(+) cells within the gated lymphocytes were counted (Figure 1).

Statistical Analysis

Data obtained in the study were analyzed using SPSS 16.0 software. For the comparison of categorical variables, the One-Way ANOVA (post-hoc Scheffe) test was used.

Results

All parameters examined in the IFN, steroids, and control groups were calculated as mean ± standard deviations values. The results are shown in Table 1.

No statistically significant difference was determined between the IFN, steroid, and control groups regarding the leukocyte count in the liver, spleen, and bone marrow, the CD4(+) cell count, the CD4(+) CD25(+) cell count, lymphocyte count gated on flow cytometry, ratio of CD4(+) CD25(+) cells to lymphocytes gated on flow cytometry, and ratio of CD4(+) CD25(+) cells to cells counted on flow cytometry.

Discussion

T-regs were first identified in 1969, and especially after their role in immunological tolerance was shown in 1995, questions were asked about T-regs and an increase was seen in research (1,2,3). It is understood that T-regs have been studied in healthy individuals and in peripheral blood samples of patients reflecting a specific disease model (autoimmune disease, after transplant, etc). There are few studies in the literature that have evaluated T-regs within tissue. A previous study that investigated T-regs in tissue compared the intrahepatic T-reg distribution in 43 patients with chronic hepatitis C, a control group of 31 healthy adults, and 8 organ donors. Extensive leukocyte infiltration containing CD4(+) FOXP3 (+) T-regs was determined at a high level in livers infected with hepatitis C virus (HCV), and almost no T-regs were determined in healthy livers (13). In another study, a high level of CD4(+) FOXP3 (+) T-regs was determined in livers infected with hepatitis B virus (HBV) (14).

Claassen et al. (15) evaluated the T-reg count in the liver during and after IFN-α and ribavirin treatment in 22 naive chronic hepatitis C patients and showed that the intrahepatic T-reg count was inversely proportional to the level of fibrosis. It was determined that the intrahepatic T-regs examined during antiviral treatment were relatively increased compared to the CD4(+) T-cell count in 10 patients and to the CD8(+) T-cell count in 12 patients. It was reported that this increase in the T-reg ratio could be due to an increase in the T-reg count or a decrease in the effector cells. Of these patients, 20 completed the treatment, and the treatment resulted in success in 13 patients. Of the 13 successfully treated patients, the intrahepatic T-reg count examined 4 weeks after the end of treatment was seen to be increased compared with the total liver lymphocyte, CD4(+), and CD8(+) T-cell counts. In 3 successfully treated patients, no change was determined in the intrahepatic CD4(+) CD25(+) FOXP3 (+) T-regs examined 24 weeks after the end of treatment, and although there was a decrease in the T-reg count in other patients, it was seen to be higher than that of healthy livers (15).

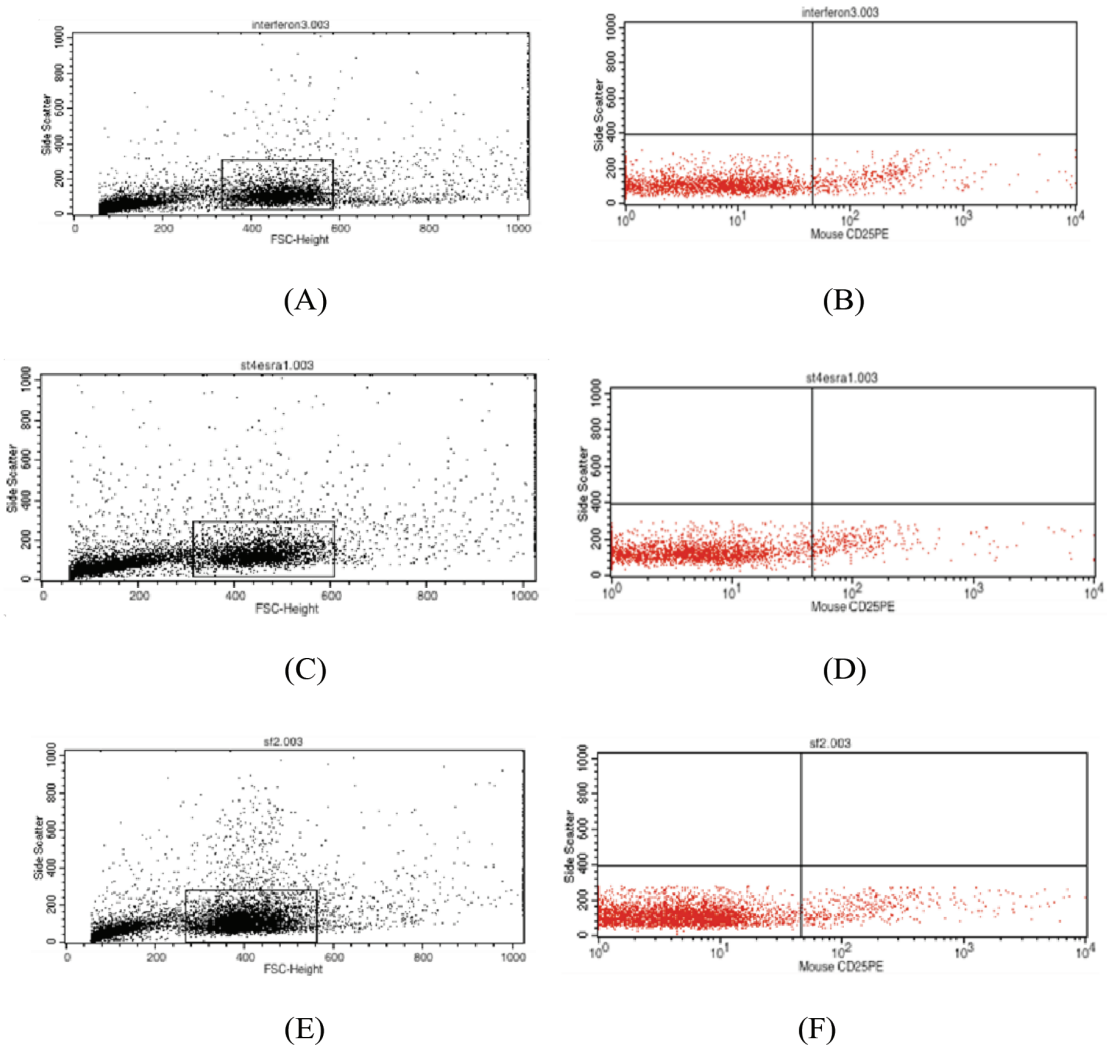

Figure 1. Counts and gating of lymphocytes on flow cytometry. Gating of lymphocytes in the liver on flow cytometry of the interferon, steroid, and control groups (A, C, E, respectively) and the CD4(+) CD25 (+) cell count on flow cytometry (B, D, F, respectively)

In a study by Demirkiran et al. (16), the intrahepatic T-reg level was examined in liver transplantation patients, and immunosuppressive treatment of anti-CD25 monoclonal antibody (basiliximab), calcineurin inhibitor (cyclosporin A or tacrolimus) and prednisone was applied to the patients. FOXP3 was examined with polymerase chain reaction in liver tissue obtained with fine-needle aspiration biopsy at 6 or 12 months after transplantation and an increase in FOXP3 expression was detected. Recurrence occurred in 5 HCV (+) recipients within the first 3 months, and in these patients, FOXP3 expression was found to be higher compared to HCV (-) patients. Of the 15 HCV (-) recipients, acute rejection developed in 3 within the first 3 months, and FOXP3 expression was determined to be higher in these patients than in those without rejection (16).

In another study that evaluated intrahepatic T-regs in 24 liver transplantation patients who developed cirrhosis associated with chronic HCV or HBV infection, immunosuppressive treatment of steroids and tacrolimus was administered, and early acute rejection developed in 10 patients. The T-reg levels of patients who developed acute rejection were found to be lower than those of patients who did not. Although the reason for the decrease in T-reg level could not be explained, it was thought that it could have been related to the immunosuppressive treatment or genetic factors of the recipient (17).

In an experimental mouse model study that examined the T-reg level in the spleen tissue, adult male mice were treated with dexamethasone and/or interleukin-2 (IL-2). After the mice were euthanized, the spleens were removed and spleen cell culture was performed, and then ip was administered to adult female mice that had undergone total-body irradiation. The development of graft-versus-host disease was followed up. The results determined an increase in CD4(+) CD25(+) spleen T-cells and in the ratio of CD4(+) cells to CD25(+) FOXP3 (+) T-cells gated on flow cytometry in the group given dexamethasone and IL-2. When dexamethasone or

IL-2 were administered separately, it was reported that although an increase was observed compared to the control group, it did not result in an increase as great as in the group where they were administered together (18).

In another experimental mouse model study that evaluated the effect of immunosuppressive drugs on T-regs in the spleen and lymph nodes, the spleen and lymph nodes were removed from adult male mice, CD+(+) CD25(+) T-cells were obtained, and cell culture was performed. Cell cultures of T-cells obtained from another lineage of mice were treated with suppressed splenocytes. When there was no immunosuppressive drug in the medium and methylprednisolone was given, an increase was determined in the T-reg level. The rate of increase in the methylprednisolone group was determined to be less compared to the group not given any immunosuppressive drug (19).

Starting from the hypothesis that glucocorticoids act as a co-stimulant in increasing IL-2-mediated selective T-reg expansion, a previous study used healthy mice and an experimental autoimmune encephalomyelitis (EAE) model formed of mice. These patients were given dexamethasone and/or IL-2, and then the rate of T-regs in the spleen and inguinal and mesenteric lymph nodes were compared. An increase was observed in the T-reg percentage in the spleen and in the inguinal and mesenteric lymph nodes both daily and at the end of 5 days of treatment in healthy mice given dexamethasone and IL-2 together. When the effects of the administration of dexamethasone alone or IL-2 alone or the two together were evaluated after 3 days, there was seen to be an increase in the ratio of CD25(+) T-cells in the CD4(+) cell population and an increase in the ratio of CD4(+) CD25(+) T-cells to CD4(+) CD25(-) T-cells, and despite the increase in this ratio, there was observed to be a decrease in splenic CD4(+) CD25(+) T-cell count in the group given dexamethasone alone. Unexpectedly, a decrease was determined in the CD4(+) FOXP3 (+) cell percentage in the lymph nodes, spleen, and peripheral blood in the group applied with dexamethasone alone. Similar results were obtained in mice forming the EAE model. It was demonstrated that dexamethasone significantly strengthened IL-2-mediated FOXP3 (4) T-reg cell growth in both normal healthy mice and the mice forming the EAE model (20).

Another study also showed that dexamethasone treatment in normal naive mice increased the T-reg ratio in lymphoid tissues (21).

In an experimental animal study using New Zealand Black mice, which evaluated the effect of IFN-on the T-reg level in splenic tissue, spleen cell cultures were performed with 48-h 1000 U/mL IFN-α, and IFN-gamma (γ), and phosphate buffer solution was applied to the control group. In the flow cytometry analysis, it was seen that IFN-γ led to a lesser change in the mean T-reg ratio, while IFN-α resulted in a 35% decrease. In addition, of the major cytokines produced by T-regs, the IL-10 level was examined, while there was no change in the IL-10 mRNA level in the group given IFN-γ, there was an 18% decrease in the IFN-α group (22).

Table 1. The leukocytes, CD4(+) cells, CD4(4) CD25 (+) cells, and lymphocyte counts gated on flow cytometry, and the ratios to each other in the liver, spleen, and bone marrow, in the interferon, steroid, and control groups

	Steroid group, (n=9)	Interferon group, (n=9)	Control group (n=6)
Liver: leukocyte count (x10^6):	8.83±3.94	12.1±6.92	9.3±2.4
Liver: CD4+ cell count	250,000±62,449.98	560,000±364,965.75	70,000±28,284.27
Liver: lymphocyte count gated on flow cytometry	3783.33±1912.76	4628.67±1846.57	3676±776.4
Liver: CD4+ CD25+ cell count	343.67±199.2	131.67±219.4	405.5±125.16
Liver: ratio of CD4+ CD25+ cells to lymphocytes gated on flow cytometry	10.97±9	4.18±7.06	11.65±5.87
Liver: ratio of CD4+ CD25+ cells to cells counted on flow cytometry	3.44±1.99	1.32±2.19	4.04±1.22
Spleen: leukocyte count (x10^6)	15.07±4.37	47.87±18.08	46.3±30.69
Spleen: CD4+ cell count	166,666.67±109,696.55	696,666.67±667,108.19	400,000±14,1421.36
Spleen: lymphocyte count gated on flow cytometry	3730.67±2291.36	5022.67±914.12	3966.5±649.83
Spleen: CD4+ CD25+ cell count	222.33±88.58	87.67±135.4	144±38.18
Spleen: ratio of CD4+ CD25+ cells to lymphocytes gated on flow cytometry	6.45±1.45	1.8±2.78	3.76±1.58
Spleen: ratio of CD4+ CD25+ cells to cells counted on flow cytometry	2.22±0.89	0.88±1.35	1.44±0.38
Bone marrow: leukocyte count (x10^6):	5.43±1.1	13.03±1.89	8.4±3.68
Bone marrow: CD4+ cell count	116,666.67±66,583.28	83,333.33±66,583.28	160,000±113,137.08
Bone marrow: lymphocyte count gated on flow cytometry	2384±1545.88	1539.67±610.68	2327±671.75
Bone marrow: CD4+ CD25+ cell count	132±156.12	82±63.55	89±60.81
Bone marrow: ratio of CD4+ CD25+ cells to lymphocytes gated on flow cytometry	4.65±3.2	6.9±6.11	4.39±3.88
Bone marrow: ratio of CD4+ CD25+ cells to cells counted on flow cytometry	1.45±1.44	0.82±0.64	0.89±0.61

In multiple sclerosis patients treated with IFN-beta (β), an increase in T-reg levels was determined in the 3rd-6th months of treatment, but this increase could not be fully explained (23,24). An experimental animal study was conducted with mouse EAE model to explain this increase. One group was administered ip IFN-β treatment every other day for 2 months, and no treatment was given to the control group. At the end of the treatment, the T-regs in the spleen were examined and an increase was determined in the CD4(+) CD25(+) FOXP3 (+) T-regs in the treated group compared to the control group (25).

Prasad et al. (26) examined patients aged 2-16 years with a diagnosis of childhood idiopathic nephrotic syndrome. The frequency of CD4(+) CD25(+) FOXP3 (+) T-regs was examined in peripheral blood before treatment in patients given prednisolone treatment, at 4 weeks after the treatment was stopped as remission had been entered, and before re-starting immunosuppressive treatment because of relapse. Compared with pre-treatment, it was seen that T-regs increased in remission and decreased during relapse (26).

The increase in T-reg activity and function in autoimmune diseases is discussed as one of the potential treatment options. Although IFN-β treatment did not increase the T-reg frequency in the peripheral blood of multiple sclerosis patients, it increases the suppressing function of T-regs. Therefore, further research is needed to explore the mechanism of this effect (27).

In an experimental animal study (Balb-C mice) by Prenek et al. (28), the response of T-regs to apoptosis caused by glucocorticoid hormone was evaluated. After 4 weeks of dexamethasone treatment, the mice were euthanized and thymic and splenic T-regs were obtained. The rate of thymic T-regs was seen to increase but this was a relative increase (as thymocytes are sensitive to glucocorticoid) and there was no change in the absolute T-reg count (28).

Study Limitations

The limitation of the study is that the disease model was not used in the study.

Conclusion

In the current study, IFN-α and dexamethasone did not change the T-reg count in the liver, spleen, and bone marrow of mice. No disease model was used in the study, and the effect was investigated in healthy mice. Repeating this study with a disease model (especially autoimmune disease) would be able to more clearly reveal the relationship between the immunosuppression and immunomodulatory effects of IFN and dexamethasone and T-regs.

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Ethics

Ethics Committee Approval: The protocol was approved by the Committee on the Ethics of Animal Experiments of İstanbul University (approval number: 119).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Z., E.Ö., E.A., R.Ö., Concept: E.Z., E.Ö., E.A., B.M., N.S., F.T., R.Ö., Design: E.Z., E.Ö., E.A., B.M., N.S., F.T., R.Ö., Data Collection or Processing: E.Z., E.Ö., E.A., B.M., N.S., F.T., R.Ö., Analysis or Interpretation: E.Z., E.Ö., E.A., B.M., N.S., F.T., R.Ö., Literature Search: E.Z., E.Ö., E.A., B.M., N.S., F.T., R.Ö., Writing: E.Z., E.Ö., E.A., B.M., N.S., F.T., R.Ö.

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Insidious Hepatitis B Virus and Risk of Community Transmission: Case Report

Sinsi Hepatit B Virüsü ve Toplumda Bulaşma Riski: Olgu Sunumu

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ABSTRACT

Hepatitis B virus (HBV), which causes serious health problems worldwide, causes millions of people to get sick and hundreds of thousands to die every year, despite the precautions and vaccination campaigns. Health professionals providing services are at serious risk, particularly in terms of undiagnosed HBV carriers. The hepatitis B surface antigen value of five children who were born at home in a family living in rural areas, whose mother's were hepatitis B patient's, and who were not vaccinated against hepatitis B, were found to be positive. These individuals, who are excluded in any treatment and education program are an important risk factors for both health workers and society.

Keywords: Hepatitis B virus, risk factor, health professionals

ÖZ

Dünya genelinde ciddi sağlık sorunlarına yol açan hepatit B virüsü (HBV), alınan önlemlere ve aşılama kampanyalarına rağmen her yıl milyonlarca insanın hastalanmasına ve yüz binlercesinin hayatını kaybetmesine neden oluyor. Hizmet sunan sağlık çalışanları, özellikle tanı konulmamış HBV taşıyıcıları açısından ciddi risk altındadır. Kırsal kesimde yaşayan bir ailede evde doğan, anneleri hepatit B hastası olan ve hepatit B aşısı yapılmamış beş çocuğun hepatit B yüzey antijeni değeri pozitif bulunmuştur. Herhangi bir tedavi ve eğitim programında alınmayan bu bireyler hem sağlık çalışanları hem de toplum için önemli bir risk faktörüdür.

Anahtar Kelimeler: Hepatit B virüsü, risk faktörü, sağlık çalışanları

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Introduction

Hepatitis B virus (HBV) is a small, double-stranded, and deadly DNA virus from the hepadnaviridae family that can cause liver disease in both the acute and chronic stages. It can usually be transmitted from mother to baby during childbirth. It can also be transmitted through blood and sexual intercourse. The use of uncontrolled blood or blood products, medical interventions with non- sterilized tools, injections, use of sharp/piercing materials, sharing of items such as razors, toothbrushes, tattooing, and applying body jewelry with non-sterilized tools are also the most common ways of transmission. Hepatitis B can remain in the body for many years without any symptoms after infection (1,2).

According to the World Health Organization data, in 2015, HBV its prevalence was found to be 3.5%. In other words, an average of 257 million people live with HBV infection, and an average of 1-2 million new patients are added to this number annually (1,3). As of 2015, only 8% (1.7 million) of the diagnosed patients could be included in the treatment. Additionally, considering that 25.3% of these are women of childbearing age, 65 million women of childbearing age have the risk of transmitting the disease to their babies (4,5). In 2015 alone, 885,000 people died from HBV-related liver disease (3).

In a study conducted in Turkey in 2009 on individuals over the age of 18, hepatitis B surface antigen (HBsAg) positivity was 4% and anti-hepatitis B core antigen (anti-HBc) positivity was 30.6%.

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It has been reported that one out of every three people over the age of 18 in Turkey has encountered HBV. It is estimated that there are more than 2 million HBsAg positivity in adult individuals. It was found that only 12% of these people were aware of the situation (2). This is important in terms of demonstrating the extremely low level of awareness in our country (6). According to the data of the Ministry of Health of the Republic of Turkey, approximately half (40-50%) of liver transplantations performed between 2012 and 2016 constitute acute-chronic liver failure and liver cancer due to HBV infection (7).

Those working in risky occupational groups, especially health professionals, should be more careful and have a high level of awareness about HBV. Studies have reported that the prevalence of hepatitis B among healthcare professionals in Turkey is between 0.5 % and 1%. In the world, studies have stated that this rate rises up to 10% (8,9).

Many hepatitis B carriers lead their lives uncontrolled, and there may be periods when they may pose a risk both for themselves and for their environment. When these patients are identified, they should be promptly directed to the relevant departments and followed up (10).

Case Report

Mother who is 62 years old, has a positive HBsAg value, has not received any treatment, is illiterate and gave birth to all her children in a home environment with normal birth, has chronic hepatitis B disease and strengthens the assumption that the mother transmitted the HBV to the children at birth. Additionally, because the family lives in a rural part of a province in the Southeastern Anatolia Region of Turkey, the fact that four children aged 44, 41, 37, and 34, respectively, were not vaccinated at all or were not directed in any way, caused these five children to have chronic hepatitis B. Therefore, these people, who are not followed -up and treated, constitute a serious risk factor for health service providers and other individuals in the society. In the serological tests of the mother and her five children, the HBsAg value was positive (+) and the anti-HBs value was (-). All five children have 6, 4, 2, 5 children, respectively; However, since all children are born in a hospital environment, the HBsAg value is not positive in any of them, as they are vaccinated. These patients, who are still living their daily lives, were not involved in any control and treatment process. The risk that these individuals may pose, who do not even have the slightest knowledge about the transmission and spread of the HBV, poses a serious problem for public health. Informed consent was obtained.

Discussion

In the literature review, public awareness of the HBV in Turkey is at a very low level (1,2,4). Saatçi et al. (11) in their study with high school students in Turkey, it is emphasized that awareness of hepatitis B is not at the desired level, and therefore, it is necessary to implement education programs on this subject urgently. In our case, the mother and her five children learned by chance that they had hepatitis B by the results of the general examination. According to Tozun et al. (2) In their population-based viral hepatitis prevalence study conducted in Turkey in 5,471 people over the age of 18, HBsAg positivity was 4%, anti-HBc total positivity was 30.6%, and anti-HB positivity was 31.9%. Additionally, it was determined that HBsAg positivity was significantly higher in Southeastern Anatolia, Eastern Anatolia, and Central Anatolia regions and lower in western regions (2). As a matter of fact, the fact that our case lived in the rural part of the Southeastern Anatolia Region of Turkey increased the risk of HBsAg positivity in line with the literature studies. Mahamat et al. (12), in which a meta-analysis of 227 studies (224,936 health professionals in 71 countries) published between 1970 and 2019 were examined; found HBsAg positivity as 2.3% and acute HBV infection as 5.3% in healthcare professionals. This situation shows us that health professionals are at a serious risk.

Conclusion

Undiagnosed patients who receive services in the public sphere are mixed into society and benefit from all common areas, including healthcare services. Considering the ways of transmission of the HBV, they can increase the rate of spread of the virus in all public spaces, especially healthcare providers. The presence of these undiagnosed patients causes the virus to be transmitted, but the fact that their exact numbers cannot be determined is a worrying situation. To raise awareness about the HBV in Turkey and to be more vigilant against it, it is necessary to conduct screenings throughout the country, to identify and follow-up infected people, to conduct regular vaccination campaigns, especially in rural areas, and to organize national training. Health professionals should also take the necessary precautions in service delivery and ensure that infected patients are guided quickly and effectively.

Ethics

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: O.P., A.Y., A.K., Concept: O.P., A.Y., A.K., Design: O.P., A.Y., Data Collection, or Processing: O.P., A.Y., Analysis, or Interpretation: O.P., A.Y., A.K., Literature Search: O.P., A.K., Writing: O.P., A.Y., A.K.

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