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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.

Efforts are being made to be recognized of Viral Hepatitis Journal by indexes. Online article acceptance through website of the journal and all published volumes can be reached as full text without fee through the web site http://viralhepatitisjournal.org/.

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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.

The ORCID (Open Researcher and Contributor ID) number of the correspondence author should be provided while sending the manuscript. A free registration can be done at http://orcid.org.

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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.

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

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

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

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

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

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

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

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

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

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.

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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.

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

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

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

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

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

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

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

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

Figures, Pictures, Table 's and Graphics:

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

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

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

• 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.

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

• For figures, pictures, tables and graphics to be reproduced relevant permissions need to be provided. This permission must be mentioned in the explanation.

• 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.

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RESEARCH ARTICLES

Research Article

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Viral Hepatitis Journal 2019;25(2):35-39

Is There an Association Between Hepatitis B and Atherosclerosis?

Kronik Hepatit B Enfeksiyonu Ateroskleroza Yol Açar Mı?

Zuhal Çalışkan, Özge Telci Caklılı

Kocaeli State Hospital, Clinic of Internal Medicine, Kocaeli, Turkey

ABSTRACT

Objectives: Although hepatitis B infection can cause chronic disease, its association with atherosclerosis is a matter of debate. Retinopathy is an early marker for microvascular abnormalities of retinal circulation and some reports state that it can predict cardiovascular events. In this study, we aimed to evaluate early atherosclerosis in patients with chronic hepatitis B (CHB) using retrobulbar flow velocities.

Materials and Methods: The study included 56 patients with CHB and 45 controls. Patients with hepatitis B who attended Ümraniye Training and Research Hospital Gastroenterology outpatient clinics were screened. Pulsed Doppler documentation of flow velocity was obtained from ophthalmic artery, posterior ciliary artery and central retinal artery (CRA). The resistive index for each artery was calculated using the following formula: peak systolic velocity (PSV)-end-diastolic velocity (EDV)/PSV.

Results: The mean age of the patients and controls was 46.18±13.7 and 45.89±8.6 years, respectively. There were 29 males and 27 females in CHB group and 16 males and 29 females in control group. The patients with CHB had statistically lower PS flow and ED flow of CRA compared to controls [(10.9±3.1 vs 12.1±3.09, p=0.047) and (2,8±1,1 vs 3,2±1,1, p=0.027), respectively].

Conclusion: Our findings show no increase in retrobulbar flow velocities of patients with CHB compared to controls.

Keywords: Hepatitis B, atherosclerosis, retrobulbar blood flow velocity

ÖZ

Amaç: Hepatit B enfeksiyonu kronik karaciğer hastalığına neden olmaktadır. Buna rağmen kronik hepatit B'ye (KHB) bağlı enflamasyona ikincil ateroskleroz ile ilişkisi kesin değildir. Retinal arterlerin dolaşımının erken aterosklerozu öngörebilme yeteneği olduğuna dair literatürde yayınlar mevcuttur.

Gereç ve Yöntemler: Bu çalışmamızda KHB hastalarında retrobulbar akım hızları ile erken aterosklerozu değerlendirmeyi amaçladık. Çalışmaya 56 KHB hastası ve 45 sağlıklı kontrol katıldı. 2019 yılı içerisinde (kör değerlendirme için belirtilmedi) Ümraniye Eğitim ve Araştırma Hastanesi, Gastroenteroloji Kliniği'nde takip edilen Hepatit B tanısı olan karaciğer biyopsisi pozitif hastalar tarandı. Pulsed Doppler yardımı ile oftalmik arter, posterior silier arter ve santral retinal arter (SRA)gözlendi. Her arter için direnç indeksi formüle göre hesaplandı: Tepe sistolik akım (TSA)-diyastol sonu akım (DSA)/TSA.

Bulgular: Hastaların ortalama yaşı 46.18±13.7 iken kontrol katılımcıların 45.89±8.6 idi. Yirmi dokuz erkek ve 27 kadın hasta KHB grubunda iken, bu oran kontrol grubunda sırasıyla 16'ya 29 idi. KHB hastalarının kontrol grubuna göre daha düşük TSA ve DSA akımlarının olduğu gözlendi [(10,9±3,1 vs 12,1±3,09, p=0,047) ve (2,8±1,1 vs 3,2±1,1, p=0,02), sırasıyla].

Sonuç: Çalışmamızda KHB hastalarının retrobulbar akım hızlarında kontrol grubuyla karşılaştırıldığında artış gözlenmemiştir.

Anahtar Kelimeler: Hepatit B, ateroskleroz, retrobulbar kan akımı

Çalışkan Z, Telci Caklılı Ö. Is There an Association Between Hepatitis B and Atherosclerosis? Viral Hepat J. 2019;25:35-39.

Introduction

Hepatitis B virus infection is a common disease in the world. The World Health Organization estimated that 257 million people were living with hepatitis B in 2017 (1). Chronic hepatitis B (CHB) infection can cause extrahepatic manifestations such as polyarthritis nodosa, glomerulonephritis and skin disorders (2).

Atherosclerosis is now considered a systemic disease related to chronic inflammation (3). In patients with chronic inflammatory diseases, such as rheumatoid arthritis, seronegative polyarthritis

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or Behçet's disease, atherosclerosis is more common than in the general population (4,5,6). Although CHB infection can cause chronic disease, its association with atherosclerosis is controversial. In their study, Völzke et al. (7) reported that there was no association between hepatitis B and C virus infection and atherosclerosis risk, however, Targher et al. (8) have reported higher carotid intima media thickness (cIMT) values in patients with CHB compared to controls.

Most of the studies used cIMT to evaluate atherosclerosis. Retrobulbar blood flow velocity measurement is a relatively new method to assess early atherosclerosis (9). Retinopathy is an early marker for microvascular abnormalities of retinal circulation and some reports stated that it could predict cardiovascular events (10). Current literature shows increased resistance index in patients with atherosclerosis compared to controls (11). To assess retinal circulation, retrobulbar velocities of central retinal artery (CRA), posterior ciliary artery (PCA) and ophthalmic artery are measured and their resistance index is calculated. In this study, we aimed to evaluate early atherosclerosis in patients with CHB using both retrobulbar flow velocities and cIMT.

Materials and Methods

Study Population

The study included 56 patients with CHB and 45 controls. Patients with hepatitis B who were followed at Ümraniye Training and Research Hospital Gastroenterology outpatient clinics between on 2019 were screened. Ethics Committee approval was obtained from İstanbul Medeniyet University Ethics Committee (approval number: 2019/0239). All the patients were hepatitis B surface antigen (HbsAg)-positive, none of the patients were positive for anti-HBs, and all patients had liver biopsy-proven CHB. Both patients and controls were inquired regarding their coronary artery disease history and those with a history of coronary artery disease were excluded. Patients with diabetes mellitus, chronic kidney disease and any other systemic disease and pregnant patients were also excluded. Informed consent was obtained from all the patients at every step of the study. The study was conducted in accordance the principles of the Helsinki Declaration.

Biochemical Assessment

Venous blood samples were obtained from each study participant after an overnight fast for biochemical analysis. The spectrophotometric method (Aeroset Automated Analyzer, Abbott Laboratories, Abbott, IL) was used for blood glucose measurement. Albumin, urea, and creatinine levels were determined. Enzymatic methods were used to measure triglyceride levels and total cholesterol as well as high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

Echocardiographic Examination

A GE Vivid 7 (Horten, Norway) echocardiography unit was used for echocardiographic examination. Echocardiographic evaluations were performed in parasternal long-axis view, using two-dimensional, M-mode, standard- and pulsed-tissue Doppler. Measurements were performed with M-mode images. One blinded investigator completed the echocardiography, while a pair of blinded cardiologists performed the analysis of the echocardiographic recordings.

Carotid Intima Media Thickness Measurement

A GE Logic 5 ultrasound scanner (General Electric Medical Systems, Wallingford, CT) was employed in measuring cIMT. A trained radiologist, who was blinded to patient data, performed the sonic evaluations. The head was in midline position and tilted a little bit upward during the left common carotid artery evaluations. A 7.5 MHz linear probe was positioned parallel to both the near and the far wall of the carotid artery and lumen diameter was amplified as much as possible along the longitudinal plane (12). cIMT measurements were taken at approximately one centimeter proximal to the common carotid artery bifurcation. The distance between the media-adventitia interface and the lumen-intima interface served as the definition of cIMT (12).

Retrobulbar Blood Flow Velocities and Resistivity Index Measurement

Examinations were performed on the right eye. Color Doppler ultrasonography examinations were performed by an operator who received training with Toshiba Aplio XU Ultrasound device (Toshiba America Medical Systems, Inc., Tustin, CA) using a 12.5 MHz linear array probe. A uniform protocol governed ultrasound technique and subject positioning as well as arterial vessel location (13). The subjects were positioned in the supine position with their left, unexamined eye trained on a point directly above their head. Examinations were performed with the operator behind the subject and the probe and gel lightly applied to the eyelids. Care was taken to optimize the quality of the ultrasound imaging while the settings of the ultrasound device was kept constant throughout the operation. The ophthalmic artery was located where it ran on the optic nerves' medial side using the mode for color imaging, which was similarly employed concerning the PCA and CRA. Pulsed Doppler documentation of flow velocity was obtained with a 1.5 mm gate size and the angle of the Doppler under 60, as previously identified (13). The resistive index for each artery was calculated using the following formula: peak systolic velocity (PSV)-end-diastolic velocity (EDV)/PSV. The same operator performed the measurements again to calculate intraobserver of coefficient of variation (CV). CV was subsequently calculated using the results of all 10 measurements with the following formula: 100 mean ± standard deviation. CVs were computed for PSV, EDV, as well as resistivity index (RI).

Statistical Analysis

All analysis was performed with SPSS 9.0 (SPSS for Windows 9.0, Chicago, IL). Variables were expressed as mean ± standard deviation. To test normality of variables, Shapiro-Wilk and Kolmogorov-Smirnov tests were used. The Student's t-test or Mann-Whitney U test was used for comparison of two groups. A p value below 0.05 was considered statistically significant.

Results

Study Population

The mean age of the patients and controls was 46.18±13.7 and 45.89±8.6 years, respectively. There were 29 males and 27

females in CHB group and 16 males and 29 females in control group. Table 1 shows the demographic characteristics of both groups. There was a statistically significant difference in bilirubin, C-reactive protein, HDL cholesterol, LDL cholesterol, aspartate aminotransferase (AST) and albumin levels (Table 1).

Echocardiographic Examination

Interventricular septum, posterior wall thickness, left ventricle-ED diameter, left ventricle-end-systolic diameter and left ventricular ejection fraction were similar in two subgroups (Table 2).

CIMT Measurement

There was no difference in cIMT values between the patients and controls (0.53±0.17 vs 0.51±0.14; p=0.808).

Retrobulbar Blood Flow Velocities and Resistivity Index Measurement

There was a significant difference in PS flow and ED flow of CRA between the groups. Patients with CHB had lower PS flow and ED flow compared to controls [(10.9±3.1 vs 12.1±3.09, (p=0.047) and (2.8±1.1 vs 3.2±1.1, (p=0.027), respectively]. There was no difference in resistivity index of CRA. The PSV, EDV, RI of PCA and ophthalmic artery did not differ between the groups (Table 3).

Discussion

This study aimed to investigate the difference in retrobulbar blood flow velocity between patients with CHB and healthy controls. To our knowledge, this is the first study to test this hypothesis. Our findings show no increase in blood flow velocities and in resistance indices.

Although CHB can become a chronic disease, association between CHB and atherosclerosis is not clear in the literature. Observational studies revealed conflicting results. Völzke et al. (7) investigated the association of CHB with myocardial infarction, cIMT and stroke. They reported no independent association between hepatitis B infection and atherosclerotic endpoints. Complicating the subject more, a study has proposed a decrease in ischemic stroke

Table 1. Demographic characterizations of the groups

Variables	HBV	Controls	p
	Mean ± Standard deviation	Mean ± Standard deviation	
Age, (years)	46.18±13.7	45.89±8.6	0.8
Height, (cm)	163.8±10.9	166.1±6.9	0.39
Weight, (kilograms)	70.2±10.7	73.5±15.3	0.51
Waist circumference, (cm)	92.0±11.9	94.3±13.8	0.6
Systolic blood pressure, (mmHg)	129.0±21.1	131.5±13.3	0.26
Diastolic blood pressure, (mmHg)	76.0±11.3	76.0±4.5	0.49
C-reactive protein, (mg/dL)	0.3±0.3	0.2±0.18	0.003*
Total cholesterol, (mg/dL)	179.4±33.9	166.0±79.4	0.3
High density cholesterol, (mg/dL)	46.8±14.08	71.4±36.2	<0.001*
Low density cholesterol, (mg/dL)	111.1±30.8	132.1±44.0	0.021*
Triglyceride, (mg/dL)	104.5±59.5	102.8±112.9	0.26
Alanine aminotransferase, (U/L)	47.2±81.7	25.8±17.3	0.059
Aspartate aminotransferase, (U/L)	36.5±37.3	23.2±10	0.001*
Bilirubin, (mg/dL)	0.7±0.21	0.54±0.32	0.03*
Albumin, (g/dL)	4.2±0.19	4.49±0.25	<0.001*
Platelets, (K/uL)	209.604±58.093	225.729±44.730	0.152
Creatinine, mg/dL	0.88±0.6	0.96±0.9	<0.001*

HBV: Hepatitis B virus

Table 2. Echocardiographic measurements

Variables	HBV	Controls	p
	Mean ± Standard deviation	Mean ± Standard deviation	
LA, (mm)	3.05±0.44	3.5±0.4	0.006*
LV systolic diameter, (mm)	4.6±0.46	4.7±0.42	0.6
LV diastolic diameter, (mm)	2.8±0.32	3.02±0.39	0.24
Septum, (mm)	0.9±0.14	0.87±0.13	0.5
Posterior Wall, (mm)	0.84±0.14	0.78±0.09	0.053
EF, (%)	66±4.35	65.2±4.3	0.8

HBV: Hepatitis B virus, LA: Left atrial diameter, LV: Left ventricle, EF: Ejection fraction

Table 3. Retrobulbar blood flow velocities

Variables	HBV	Controls	p
	Mean ± Standard deviation	Mean ± Standard deviation	
CRA PSV (cm/s)	10.9±3.1	12.1±3.09	0.047*
CRA EDV (cm/s)	2.8±1.1	3.2±1.1	0.027*
CRA RI	0.7±0.007	0.7±0.06	0.7
PCA PSV (cm/s)	11.7±3.2	11.7±3.4	0.9
PCA EDV (cm/s)	3.7±1.2	3.9±1.6	0.6
PCA RI	0.6±0.06	0.66±0.07	0.3
OA PSV (cm/s)	37.2±9.1	38.5±9.7	0.4
OA EDV (cm/s)	10.1±3.6	10.9±4.05	0.28
OA RI	0.7±0.07	0.69±0.11	0.18
cIMT (cm)	0.53±0.17	0.51±0.14	0.808

HBV: Hepatitis B virus, CRA: Central retinal artery, PSV: Peak systolic velocity, EDV: -end diastolic velocity, RI: Resistivity index, PCA: Posterior ciliary artery, OA: Ophthalmic artery, cIMT: Carotid intermedial thickness test

rates in patients with CHB (14). The authors have associated this decrease with lower age of CHB patients compared to controls. Another study has suggested a protective effect of CHB against atherosclerosis (15). In our study, age- and sex-matched controls were included, therefore, no age-related bias was present.

A meta-analysis has reported an increase in atherosclerosis-associated disease morbidity in patients exposed to HBV but this increase was not statistically significant (16). Targher et al. (8) showed increased cIMT in patients with CHB compared to controls (0.9±0.1 vs 0.8±0.1) and the difference was statistically significant. In our study, cIMT in both patients and controls did not exceed 0.6 mm. The mean age of the patients was almost the same in both studies, therefore, this difference in cIMT may be attributed to ethnic differences.

Our study is the first in the literature to investigate retrobulbar blood flow velocities in patients with CHB and lack of a difference between patients and controls is consistent with some of the literature reporting no relationship between CHB and atherosclerosis. Although not definitive, surrogate markers of atherosclerosis are convenient tools to identify patients at risk. In this study, we used two surrogate markers to investigate the relationship and neither of them showed a difference. However, Ishizaka et al. (17) reported increased plaque formation in HBsA carriers. They have also used a surrogate marker-carotid ultrasound and they found HBsAg positivity to be a risk factor for carotid atherosclerosis that was independent from other confounding risk factors.

There were significant differences in biochemical markers showing liver capacity such as bilirubin and albumin, therefore, we can say that our study cohort had sufficient number of patients with liver associated-disease to test the hypothesis. Also, no difference was observed between patients and controls with regard to height, weight and waist circumference.

Study Limitations

This study has some limitations that should be noted. Our study cohort was relatively small. With a larger sample size, findings may differ. Also, this is a cross-sectional case-control study. A study with follow-up of these patients may obtain more information regarding their metabolic status and atherosclerotic events.

Conclusion

In this first study to test retrobulbar blood flow velocity in patients with CHB, we found no increase in patients compared to controls. Our findings are consistent with some of the literature reporting no association between CHB and atherosclerosis, however, further studies are needed to reach a clear conclusion.

Ethics

Ethics Committee Approval: Ethics Committee approval was obtained from İstanbul Medeniyet University Ethics Committee (approval number: 2019/0239).

Informed Consent: Informed consent was obtained from each patient.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.Ç., Concept: Z.Ç., Design: Z.Ç., Data Collection or Processing: Z.Ç., Ö.T.C., Analysis or Interpretation: Ö.T.C., Literature Search: Z.Ç., Ö.T.C., Writing: Ö.T.C.

Conflict of Interest: Authors declare no conflict of interest.

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Research Article

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Molecular Characterization of Hepatitis B Virus Strains Isolated from Chronic Hepatitis B Patients in Southeastern Region of Turkey

Türkiye'nin Güneydoğu Bölgesi'ndeki Kronik Hepatit B Hastalarından İzole Edilen Hepatit B Virüsü Suşlarının Moleküler Karakterizasyonu

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ABSTRACT

Objectives: The aim of the present study was to assess the molecular aspects of HBV strains isolated from chronic hepatitis B (CHB) patients, in the Southeastern region of Turkey.

Materials and Methods: The study involved a total of 110 patients, 57 of them were treatment naive. 53 were undergoing nucleos(t)ide analogue (NUC) therapy, whom were diagnosed with CHB between July 2010 and April 2011 in the Southeastern region of Turkey. We analysed the HBV pol gene by amplification and direct sequencing with using polymerase chain reaction.

Results: The phylogenetic and genotype analysis showed that all (100%) of the patients were infected with HBV genotype D. The prevalence of antiviral drug-associated potential vaccine-escape mutant was 10.5% among treatment naive and 15% NUC therapy group. S gene mutation among treatment naive group and NUC therapy group were 19% and 26.4%.

Conclusion: Determination of genotypes/subgenotypes of HBV may provide robust epidemiological data related to their circulation as well as their transmissibility. However, the findings of HBV pol gene mutations may be helpful in the management of rescue strategies in NUCs resistant patients in Southeastern region of Turkey.

Keywords: Hepatitis B virus, HBV polymerase gene mutation, Nucleos(t)ide analogue

ÖZ

Amaç: Türkiye'deki hepatit B enfeksiyonunun prevalansı Güneydoğu Bölgesi'nde diğer bölgelere göre daha yüksektir. Hepatit B genomundaki mutasyonlar kronik hepatit B hastalarının tanı ve tedavisinde güçlüklere sebep olmaktadır. Bu çalışmanın amacı, yüksek endemisiteye neden olan sebeplerinin ve belirli bir suşun bölgede yaygın olup olmadığının değerlendirilmesidir.

Gereç ve Yöntemler: Haziran 2010 ve Nisan 2011 tarihleri arasında Türkiye'nin Güneydoğu Bölgesi'nde kronik hepatit B tanısı alan toplam 110 hasta çalışmaya katıldı. Hastaların 57'si tedavi deneyimsiz iken 53'ü nükleosid analog (NUC) tedavisi almaktaydı.

HBV pol geni amplifikasyon ve direct sekanslama polimeraz zincir reaksiyonu ile analiz edildi.

Bulgular: HBV virusunun filogenetik ve genotip analizi sonucunda hastaların hepsinin (%100) HBV genotip D ile enfekte olduğu görüldü. Antiviral ilaç ilişkili potansiyel aşı kaçak mutantlarının prevalansının tedavi deneyimsiz grupta %10,5 NUC tedavisi alan grupta %15 olduğu bulundu. S gen mutasyonu tedavi deneyimsiz grupta ve NUC tedavisi alan grupta sırasıyla %19 ve %26,4 olarak saptandı.

Sonuç: HBV'nin genotip/subgenotip tayini, virusun yaygınlığı ve bulaşıcılığı hakkında güçlü epidemiyolojik veriler sağlayabilir. Bununla birlikte HBV pol gen mutasyonları ile ilgili bu bulgular, Türkiye'nin Güneydoğu bölgesindeki NUC dirençli hepatit B hastalarında tedavi stratejilerinin yönetiminde yararlı olabilir.

Anahtar Kelimeler: Hepatit B virüsü, HBV polimeraz gen mutasyonu, Nucleos(t)ide analoğu

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Introduction

Hepatitis B virus (HBV) infection is endemic at an intermediate to high range within Turkey. The regional hepatitis B surface antigen (HBsAg) prevalence is ranging from 2.5% to 9.1% with the higher values concentrated in the Southeastern part of Turkey (1).

The HBV polymerase *(pol)* gene completely overlaps the envelope gene (2). Mutations in and around the major neutralization domain of HBV known as the "a" determinant, may cause HBV reactivation, diagnostic problems, failure in prophylaxis through vaccination or failure in prophylaxis by administering hepatitis B immunoglobulin (3).

The usage of low genetic barrier/potency drugs such as lamivudine (LAM) in medium-to-high HBV prevalence countries, antiviral drug-associated potential vaccine-escape mutant (ADAPVEM), is becoming a growing health concern (4). The public health significance of such mutant pol-envelop overlaps, were recently highlighted when the up to then, theoretical concerns about NUC-resistant HBV, potentiating behaviour as a vaccine escape virus was actually observed in chimpanzees. This drug resistant virus strain was genetically fit and stable, however its' altered envelope escapes the anti-HBs neutralization (5). Transmissions of ADAPVEMs are of critical concern in the control of HBV infections. The generally accepted method for the control of the latter is prevention through vaccination. A secondary approach is the prevention of clinical complications of chronic HBV infections through specific and effective oral antiviral treatments. These being significantly more potent, with higher genetic barriers to resistance, compared to LAM, telbivudine or adefovir (ADV) (5,6).

In this study our aim was to identify the molecular aspects of HBV strains isolated from CHB patients in the Southeastern Region of Turkey where the endemicity is particularly high. We have focused our attention to identify some of the reasons for this high endemicity and whether it is due to a particular strain being prevalent in the region.

Materials and Methods

The informed consents (in Turkish) were obtained from all participants before blood sampling. This study was approved by the Ethical Committee Harran University Faculty of Medicine (approval number: 06/10, date: 02.12.2010).

Patients

Between the dates of July 2010 and April 2011, a total of 110 CHB patients, ages between from 5 to 70 years, with a mean age of 32 years and having a 26% male and 74% female ratio, were enrolled to this retrospective study. CHB infection is defined as the persistence of HBsAg ongoing for 6 months from the date of its first detection. In the beginning of the study 53 patients were already undergoing nucleos(t)ide analogue (NUC) therapy and 57 of the patients were treatment naive.

The patient group undergoing NUC-therapy were receiving LAM (18/53), ADV (1/53), entecavir (ETV) (18/53) and tenofovir (TDV) respectively (16/53).

Inclusion criteria:

- CHB infection with hepatitis B e antigen (HBeAg)-positive or negative
- Treatment naive CHB patients: No previous treatment with Interferon-alpha or NUC,
- CHB Patients already on NUC treatment,
- CHB Patients with compensated liver functions.

Exclusion criteria:

- Co-infection with hepatitis C, hepatitis D, or the human immunodeficiency virus;
- The presence of other forms of liver disease.

Procedures;

The liver damage was classified with Knodell et al. (7) and scaled from 0 to 18 by the histology activity index. Blood samples were separated by centrifugation and the serum was stored at -20 ^{0}C until testing. The respective aspartate aminotransferase and alanine aminotransferase levels were measured in the serum by spectrophotometric analysis using standard diagnostic kits (Roche Diagnostics, Mannheim, Germany).

Serological markers of HBV (HBsAg), HBeAg, and antibodies to HBeAg were tested using commercially available micro particle enzyme immunoassay kits, (Axsym, Abbott Laboratories, IL, USA and Elecsys, Roche Diagnostics, Mannheim, Germany).

HBV-DNA Detection

The HBV-DNA was isolated from the serum sample using the bio-robot workstation, with magnetic-particle technology (QIA symphony SP, Qiagen GmbH, Hilden, Germany). HBV-DNA was detected and quantified by polymerase chain reaction (PCR) assay (artus HBV QS-RGQ test, Qiagen GmbH, Hilden, Germany) on the real-time platform (Rotor-gene Q, Qiagen GmbH, Hilden, Germany).

HBV Sequencing

A pair of primers (forward: 5'-TCGTG GTGGACTTCTCTCAATT-3' and reverse: 5'-CGTTGACAGACTTTCCAATCAAT-3') were used for the amplification of the HBV *pol* gene region. The up mentioned *pol* gene sequence was already being utilized in our laboratory, for routine HBV genotyping and genotypic resistance analysis). The PCR conditions were determined as in preceeding study (8).

The Determination of HBV Genotypes and Pol/Surface Gene Mutations

We used a phylogenetic analysis and genotyping tool (Gheno2pheno) which accepts nucleic acid sequences for the determination of the HBV genotypes as input. The Geno2pheno has a database that is specifically designed for rapid computer-assisted virtual phenotyping of HBV, (Centre of Advanced European Studies and Research, Bonn, Germany, http://coreceptor.bioinf.mpi-inf.mpg.de/).

The Geno2pheno searches for homology between the input sequences and others already stored in its database. Additionally stores relevant clinical data for HBV genotypes, drug resistance and S-gene mutations. The tool also searches for HBV drug resistance mutations in the rt domain of the *pol* gene (9).

The genotypic resistance mutations to the NUCs have been categorized as primary or compensatory (3). In our study the overlapping S-gene segment of HBV strain was searched by Geno2pheno and in parallel was checked against previously recorded ADAPVEM HBsAg amino acid substitutions

within its database (10). Some mutations especially ADAPVEMs which were not located in the "a" determinant of the HBsAg protein were observed. The other major neutralising domains of the HBsAg proteins were analysed.

Statistical Analysis

Data entries, determining the mean and the median of different parameters and other preliminary calculations were done on Microsoft Excel. There is no comparison since the prevalence and frequency of the data were given in our study. Genomic values based on bioinformatics were evaluated using bioinformatics-based genotypic rules Geno2Pheno (SVMs) (Centre of Advanced European Studies and Research, Bonn, Germany). Our study does not include statistical significance.

Results

The Gheno2pheno identified that all (100%) of the patients were infected with the HBV genotype D. Among these, the 96.4% of the patients were infected with the sub genotype D1 in the phylogenetic tree. The remaining, 2.7% and 0.9% of the strains were identified as the sub genotypes D2 and D3 respectively (Table 1).

Compensatory (23%) and primary drug-resistance mutations (7%), ADAPVEM (10.5%) and S-gene mutations (11%) were detected in 34/57 (60%) among treatment naive group (Table 2). The ratio of these mutations among patients with viral breakthrough under NUC therapy were 28%, 16.6%, 16.6% and 0% in 18 LAM group, 5.5%, 5.5%, 11%, 22% in 18 ETV group, 0%, 0%, 0% and 100% in ADV group and 0%, 12.5%, 18.7%, 43.7% in TDV group, respectively (Table 3). Six different motifs of ADAPVEM were detected among the CHB patients: rtM250R/sW172L, rtT184G/sL176V, rtM204I/sW196L, rtM204I/sW196S, rtM204V/sI195M, and rtA181V/sL173F. The frequency of ADAPVEM was 12.7% (14/110) in the total CHB patients. The prevalence of S-gene mutation among treatment naive group and NUC therapy group were 19% (11/57) and 26.4% (14/53) respectively (Table 2,3).

Discussion

Eight different genotypes (A-H) of the HBV genome are endemic in different regions of the world (11). The genotype D is prevalent around the Mediterranean Region, the Middle East, and India (12). Sheldon and Co showed that, the mutations in the HBsAg among LAM treated patients, were higher in the HBV genotype A compared to the HBV genotype D (3). Previous studies have identified the genotype D as being the dominant genotype among CHB patients in Turkey (13). We have similarly observed that all (100%) of our patients were infected with the HBV genotype D.

Replication defects in HBV caused by mutations under the NUC therapy, can be partially repaired by compensatory mutations (3). In our study the prevalence of compensatory mutations among untreated patients were 23%. The mutation patterns are listed as L91I, Q149K, I169V/X, V191F, and Q215H/P/S. The prevalence of compensatory mutations among NUC treated patients were 28% in the LAM treated group. The mutation patterns were L91I or N139K or Q215P or N238T. A sole mutation pattern (A194X) was observed in ETV treated patients and the prevalence was measured as 5.5%. The rtQ215H/Q/P/S compensatory mutations are frequently detected both in treatment naive and NUC treated patients (8,13). We, as well, have detected the rtQ215H/Q/P/S mutations both in treatment naive and LAM treated groups. In a previous paper, the rtL180M mutation was found to be the most common compensatory mutation in a general study among Turkish patients (14). Interestingly, in our study we weren't able to detect any rtL180M mutation.

In our study the prevalence of rt gene mutations among untreated patients was observed as 7%. The mutation patterns were V173M or A181P or I233V or M250R. The same mutation patterns were observed both in the LAM (L80I + rtL180M + rtM204I, T184E/G, M204I) and the ETV (L180M + T184V + M204V) treated patients. However the frequency was higher in the LAM (16.6%) treated patients compared to ETV (5.5%) treated patients. RtL180M + rtM204I, mutations were found among LAM treated patients in a previous study as well. Differing from our results, in this same previous study rt A181V and rt Q215S had also been observed (15).

Some of the mutations in the polymerase gene of HBV are associated with alterations in the "a" determinant of the HBsAg protein. These mutations change the antigenicity of the HBsAg. Such changes may reduce the efficiency of the antibodies induced by the recombinant vaccine (16). In our study we have identified that the vaccine escape HBsAg mutations consist of F161L/H, S193L, M250R/W172, T184G/L176V, M204I/W196L, M204I/W196S, M204V/I195M, M204V/I195M, A181V/L173F or W172L. The prevalence of ADAPVEM was observed to be 10.5% among untreated patients while it was 16.6% with LAM, 11% with ETV, and 18.7% with TDF treatment. Compared to those found in another study held in the northwest region of Turkey, the frequencies of ADAPVEM among untreated and treated patients were found to be higher in our study (15). Additionally, the mutation patterns of the ETV treated patients in our study were different from another previous study. The latter study identified rt I169T, rtT184C, rtT184L/S, rtT184G/M, rtS202C/G and rt S202I mutations. Interestingly, our study identified completely different M204V/I195M, S193L mutation patterns (17). The TDF treated ADAPVEMs were M204V/I195M, A181V/L173F, W172L in our patients. Both studies had only one matching mutation, which was identified as rtA181V (17).

Table 1. Clinical and laboratory characteristics of the study population

Male %, Female %	26%, 74%
Mean age, (range)	32 (5-70)
ALT, median IU/L (range)	81 (23-367)
AST, median IU/L (range)	55 (16-298)
HBV-DNA, median copies/mL (range)	1.2+E9 (2+E4 - 3.2+E10)
HBV genotype (%)	D (100)
- Sub genotype (%)	- D1 (96.4)
	- D2 (2.7)
	- D3 (0.9)

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HBV: Hepatitis B virus

Table 2. Hepatitis B virus pol gene mutation patterns and frequencies among treatment naïve chronic hepatitis B patients

Treatment naive group (n=57)	Compensatory mutation	Primary drug resistance mutation	ADAPVEM	S-gene mutation
	L91I Q149K I169V/X V191F Q215H/P/S 13 (23%)	V173M A181P I233V M250R 4 (7%)	F161L/H* S193L* M250R/W172L 6 (10.5%)	Q101R I110L T118A G119I P120S P127T G130R S132S/Y T140I S143L D144E 11 (19%)

ADAPVEM: Antiviral drug-associated potential vaccine-escape mutant

Table 3. Hepatitis B virus pol gene mutation patterns and frequencies among nucleos(t)ide analogue treated chronic hepatitis B patients

NUC treated (n=53)	Compensatory mutation	Primary drug resistance mutation	ADAPVEM	S-gene mutation
Lamivudine (n=18)	L91I N139K Q215P N238T 5 (28%)	L80I + L180M + M204I T184E/G M204I 3 (16.6%)	T184G/L176V M204I/W196L M204I/W196S 3 (16.6%)	Q101R Y134F 2 (11%)
Entecavir (n=18)	A194X 1 (5.5%)	L180M + T184V + M204V 1 (5.5%)	M204V/I195M S193L* 2 (11%)	T118A P127T M133I Y134F S143L 4 (22%)
Adefovir (n=1)	-	-	-	S132F 1 (100%)
Tenofovir (n=16)	-	L180M + S202G + M204V A181V + N236T 2 (12.5%)	M204V/I195M A181V/L173F W172L* 3 (18.7%)	P120S P127T T131I Y134*/H 7 (43.7%)

*Naturally present ADAPVEMs; F161L/H, W172L and S193L,
NUC: Nucleos(t)ide analogue, ADAPVEM: Antiviral drug-associated potential vaccine-escape mutant

Study Limitations

The number of the subjects could have been increased.

Conclusion

In this study we evaluated the mutations involving the polymerase/surface gene sequence changes in HBV patients with pre-existing, naturally occurring or with undergoing NUC treatments.

These findings are important to determine the prevalence and type of developing variants to NUCs. Further studies are needed to understand the clinical significance of these polymerase/surface gene sequence changes. We strongly suggest that, every patient who has been diagnosed with CHB, should be checked for the baseline polymerase/surface gene sequence changes, before initiating treatment. This report on the molecular characterization of HBV is the first of its kind within the Southeastern Region of Turkey. We wish that the results of our study will contribute to the decision-making processes and the choise of the treatment in the future.

Ethics

Ethics Committee Approval: This study was approved by the Ethical Committee Harran University Faculty of Medicine (approval number: 06/10, date: 02.12.2010).

Informed Consent: The informed consents (in Turkish) were obtained from all participants before blood sampling.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Research Article

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Relationship Between Basic Laboratory Results and Fibrosis in Chronic Hepatitis B Patients

Kronik Hepatit B Hastalarında Temel Laboratuvar Sonuçları ile Fibrozis Arasındaki İlişki

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ABSTRACT

Objectives: Liver biopsy (LB) is an important cornerstone in decision to start treatment for chronic hepatitis B (HB). Viral load and liver function tests are performed to determine the most appropriate time. In this study, factors affecting liver fibrosis in hepatitis B e antigen (HBeAg) negative chronic hepatitis B patients were investigated.

Materials and Methods: LB and the other laboratory results such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and hepatitis B virus (HBV)-DNA were collected from patient files, retrospectively. According to study protocol, necroinflammatory scores ≤4 and fibrosis scores ≤1 were accepted as normal liver (NL), and the high value in one of these scores was accepted as chronic hepatitis (CH).

Results: A total of 234 patient's LBs included in the study. A total of 74 (31.6%) patients' LB was evaluated as NL. In univariate analysis, age, gender, ALT, AST, GGT, AFP and HBV-DNA >100.000 IU/mL, and in multivariate analysis, age, AST level >29 U/L, and AFP level >2.5 ng/mL were the independent risk factors for CH.

Conclusion: According to our results, age, AST and AFP predict CH. Doctors who follow up chronic hepatitis B patients should be carefully evaluate these parameters when giving LB decision.

Keywords: Chronic hepatitis B, Liver biopsy, Fibrosis

ÖZ

Amaç: Kronik hepatit B tedavisine başlama kararını vermede karaciğer biyopsisi önemli bir mihenk taşıdır. Uygun zamanı belirlemek için viral yük ve karaciğer fonksiyon testleri ile takip yapılmaktadır. Bu çalışmada hepatit B e antigen (HBeAg) negatif kronik hepatit B hastalarında karaciğer fibrozisini etkileyen faktörler araştırıldı.

Gereç ve Yöntemler: Karaciğer biyopsisi ve alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), gama-glutamil transpeptidaz (GGT) ve hepatit B virüs (HBV)-DNA gibi laboratuvar testleri geriye dönük olarak hasta dosyalarından toplandı. Çalışma protokolüne göre nekroenflamatuvar skor ≤4 ve fibrozis skoru ≤1 tespit edilenler normal karaciğer (NK), bu skorlardan birindeki yüksek değer kronik hepatit (KH) olarak kabul edildi.

Bulgular: Çalışmaya toplam 234 hastanın karaciğer biyopsisi dahil edildi. Toplam 74 (%31,6) hastanın biyopsi sonucu NK olarak kabul edildi. Tek değişkenli analizlerde yaş, cinsiyet, ALT, AST, GGT, AFP ve HBV-DNA >100,000 IU/mL düzeyi NK ve KH arasında istatistiksel olarak farklıydı. Çok değişkenli analizlerde ise yaş, AST >29 U/L ve AFP >2,5 ng/mL düzeyi KH için bağımsız risk faktörüydü.

Sonuç: Sonuçlarımıza göre, yaş, AST ve AFP, KH'yi öngörmektedir. Kronik hepatit B hastalarını izleyen doktorlar, karaciğer biyopsisi kararı alırken bu parametreleri dikkatli bir şekilde değerlendirmelidir.

Anahtar Kelimeler: Kronik hepatit B, karaciğer Biyopsisi, Fibrozis

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Introduction

Hepatitis B virus (HBV) infect the liver and can lead to a broad spectrum of disease, ranging from an inactive carriage to cirrhosis and hepatocellular carcinoma (HCC) (1). The overall hepatitis B surface antigen (hBsAg) seropositivity in Turkey was 4% (2.3% in the western and 7.3% in the eastern regains (2).

Chronic hepatitis B (CHB) may present either as hepatitis B e antigen (HBeAg)-positive or HBeAg-negative. HBeAg-negative form of the disease has been increasing. In Turkey, HBeAg-negative form is the most prevalant form (90%) (3). European Association for the Study of the Liver guidelines recommended antiviral therapy for HBeAg-negative CHB patients if their liver biopsy showing at least moderate necroinflammation and/or at least moderate fibrosis when their alanine aminotransferase (ALT) level higher than the upper limit of normal (ULN) and HBV-DNA levels above 2.000 IU/mL. Patients with HBV-DNA >20.000 IU/mL and ALT >2 x ULN can start treatment even without a liver biopsy (1). However, approximately one third of patients who have persistently normal ALT levels have moderate inflammation and/or advanced fibrosis, particularly patients older than 35 years old (4,5). So, the sufficiency of monitoring HBeAg-negative patients with ALT is controversial. Liver biopsy is often recommended for determining the degree of necroinflammation and fibrosis since hepatic histology can asist the decision to start treatment (6). In this study, we aimed to investigate factors that affect the degree of necroinflammation and fibrosis in liver biopsiy, in HBeAg-negative CHB patients.

Matherials and Methods

This study was conducted in a tertiary care hospital. Liver biopsy results between 2009 and 2013 were collected from patient files retrospectively. Demographic characteristics and laboratory parameters which were made with in one year before liver biopsy were obtained from the patients' files, too.

Inclusion criteria for patients;

- Being HBsAg-positive for at least six months,
- Being HBeAg-negative and anti-HBe positive,
- Being older than 18 years,
- Antiviral and interferon therapy naive patients.

Exclusion Criteria for Patients

- Coinfection with hepatitis C virus, hepatitis D virus or human immunodeficiency virus,
- A co-existing disease (Wilson disease, hemochromatosis, autoimmune disease, HCC or other malignant diseases
- A history of using systemic corticosteroid, antineoplastic or immunomodulator drugs.

The Biopsy Decision

The indications for liver biopsy are based mainly on the combination of three criteria; serum HBV-DNA levels, serum ALT levels and age. ULN of ALT was accepted as 35 U/L.

- Liver biopsy was performed in all patients with ALT above 2 times ULN and serum HBV-DNA above 20.000 IU/mL.
- In patients who have serum HBV-DNA above 2.000IU/mL but normal ALT levels, liver biopsy was performed when the second HBV-DNA determination was found above 2.000IU/mL again during 3-6 months period and the patients' age was above 35 years.

Biopsy procedure: Percutaneous liver biopsy was performed with tru-cut biopsy method. Disposable 16-18 G semi-automatic tru-cut biopsy needles (Geotek Healthcare products, Turkey; Matek Medical Inc, Turkey) was used in the biopsies. Obtained specimens were sent to pathology laboratory in formalin. Grading and staging of histological activity index was scored with the modified Ishac score system (7).

Necroinflammatory scores ≤4 and fibrosis scores ≤1 were accepted as normal or minimally affected liver (NL) (8). The high value in one of these scores was accepted as CH.

The study protocol was approved by the Ethics Committee of Sakarya University Faculty of Medicine (approval number: 2013/71522473.050.01.04/37). This study was carried out in accordance with the principles of the Helsinki Declaration).

Statistical Analysis

Kolmogorov-Smirnov test was used to evaluate whether the distribution of variables were normal. Therefore, two independent Sample t-test was used to compare the normal distributed continuous variables between groups. The normal distributed continuous variables were presented as the mean ± standard deviation. Mann-Whitney U test was used to compare the non-normal distributed continuous variables between groups. The non-normal distributed continuous variables were presented as the median and interquartile range (quartile 1 to 3). Categorical variables were compared by Pearson's or Yates corrected chi-square tests. Categorical variables were presented as a count and percentage.

Receiver operating characteristic (ROC) curve analysis was performed to establish the most accurate diagnostic method (biomarker) to discriminate between CH and normal patients. ROC curves were constructed for ALT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and alpha-fetoprotein (AFP) to test the various biomarkers in predicting CH (Figure 1). The areas under the ROC curves (AUC) were calculated and the specificity, sensitivity, positive-predictive value, negative-predictive value, accuracy, for the ALT, AST, GGT and AFP of the most appropriate cut-off point were calculated for predicting CH. A multivariate logistic regression model was implemented to determine ALT, AST, GGT and AFP and other covariates associated with CH. A p-values <0.05 were considered significant. Analyses were performed using commercial software (IBM SPSS Statistics 20, SPSS inc., an IBM Co., Somers, NY; MedCalc 12.7, MedCalc Software bvba, Ostend, Belgium).

Results

In total, 268 liver biopsies were performed during the study period. Among these patients, 234 (87.3%) of them were HBeAg negative (140 males, 94 females). So, further evaluation was done with these patients according to our inclusion criteria. Mean age of HBeAg negative patients was 41.5±11.3 years. Mean ALT value was 56.9 U/L and 116 (49.6%) of them have ALT in the normal range. Of the patients, 142 (60.7%) had HBV-DNA >20.000IU/mL. Baseline demographic and other characteristics of patients are shown in Table 1.

Table 1. Baseline characteristics of hepatitis B e antigen-negative patients

n	234
Age	41.50±11.3
HBV-DNA IU/mL	33682 (7756.5-939050.5)
Log (HBV-DNA) IU/mL	4.53 (3.89-5.97)
Alanine aminotransferase U/L	56.9±69
Aspartate aminotransferase U/L	39.8±34
Gamma-glutamyltransferase U/L	25.5±19.6
Alpha-fetoprotein ng/mL	4.5±7.5
Median of histology activity index (range)	5 (1-16)
Median of fibrosis (range)	2 (0-5)

Data were shown as mean±standard deviation and median (Interquartile range)
HBV: Hepatitis B virus

Patients' total necroinflammatory score median was 5 (range: 1-16) and the fibrosis component was 2 (range: 0-5). Of the total, 31 (13.2%) patients had no fibrosis, 162 (69.2%) had portal fibrotic expansion (stages 1 and 2), 38 (16.2%) had bridging fibrosis (stages 3 and 4), and 3 (1.3%) had cirrhosis (stage 5). Among 31 patients who had no fibrosis, 28 patients had also ≤4 necroinflammatory scores, 12 patients had <20.000 IU/mL HBV-DNA, and 20 patients had normal ALT levels.

A total of 74 (31.6 %) patients' liver biopsy was evaluated as in normal or minimally affected liver. In univariate analysis, all baseline characteristics except mean HBV-DNA value were statistically different between NL and CH (Table 2). When patients analyzed by grouping with HBV-DNA level, meaningful statistical difference was observed between NL and CH if the cut off value was accepted as 100.000 IU/mL (p=0.04) or 1.000.000 IU/mL (p=0.005). Statistical difference was not found when the cut off value of HBV-DNA was accepted as <20.000 IU/mL (p=0.65) (Table 2).

Table 2. Demographic characteristics and laboratory findings of normal or minimally affected liver and chronic hepatitis groups

Characteristics		Normal or minimally affected Liver group (n=74)	Chronic hepatitis group (n=160)	p
Gender (male)		37 (50.0)	103 (64.4)	0.037
Age (years)		37±11.4	43.5±10.8	<0.001
HBV-DNA IU/mL		$25.9x10^3$ ($7.7x10^3$-$134.6x10^3$)	$45.8x10^3$ ($7,7x10^3$-$1696,2x10^3$)	0.161
ALT U/L		27.5 (20-39.5)	45 (23-83)	<0.001
AST U/L		24 (22-33)	34 (24-49)	<0.001
GGT U/L		17 (12-23)	23 (15-32)	<0.001
AFP ng/mL		2.5 (1.7-3.4)	3.2 (2.35-4,8)	0.002
HAI		3 (2-4)	6 (5-8)	<0.001
HAI (>4)		0	122 (76.3)	<0.001
Fibrozis		1 (0-1)	2 (2-3)	<0.001
Fibrozis (>1)		0	123 (76.9)	<0.001
HBV-DNA Level	**>20.000**	44 (59.4)	100 (42.7)	0.65
	>100.000 IU/mL	20 (27)	65 (40.6)	0.04
	>1.000.000 IU/mL	9 (12.2)	46 (28.8)	0.005

Data were shown as mean±standard deviation, median (Interquartile range) and n (%)
HBV: Hepatitis B virus, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transpeptidase, AFP: Alpha fetoprotein, HAİ: Histological activity index

Table 3. Results of receiver operating characteristic analysis for various biomarkers in predicting chronic hepatitis compared to normal or minimally affected liver

	HBV-DNA	ALT	AST	GGT	AFP
AUC	0.557	0.658	0.694	0.649	0.646
CI 95% of AUC	0.491-0.622	0.594-0.719	0.631-0.753	0.582-0.712	0.570-0.717
p	0.141	**<0.001**	**<0.001**	**<0.001**	**0.002**
Cut-off point	$>789.3x10^3$	>45	>29	>25	>2.5
Sensitivity	32.5	49.38	63.52	42.38	71.90
Specificity	87.84	83.78	67.57	82.86	50.98
PPV	85.2	86.8	80.8	84.2	77.7
NPV	37.6	43.4	46.3	40.0	43.3

HBV: Hepatitis B virus, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transpeptidase, AFP: Alpha-fetoprotein, AUC: Area Under the ROC Curve, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value

Table 4. A multivariate logistic regression model of biomarkers and other covariates associated with chronic hepatitis

Independent variables	β	SE of β	p	OR	95% CI for OR
ALT U/L	0.001	0.015	0.971	1.001	0.971-1.031
AST U/L	0.072	0.034	**0.034**	1.074	1.005-1.148
GGT U/L	0.004	0.016	0.785	1.004	0.974-1.036
AFP ng/mL	0.167	0.083	**0.045**	1.182	1.004-1.392
Age (years)	0.056	0.020	**0.005**	1.058	1.017-1.099
Gender (male)	0.453	0.434	0.296	1.574	0.673-3.681

β: Regression coefficient, SE: Standard error, OR: Odds Ratio, CI: Confidence interval, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transpeptidase, AFP: Alpha-fetoprotein

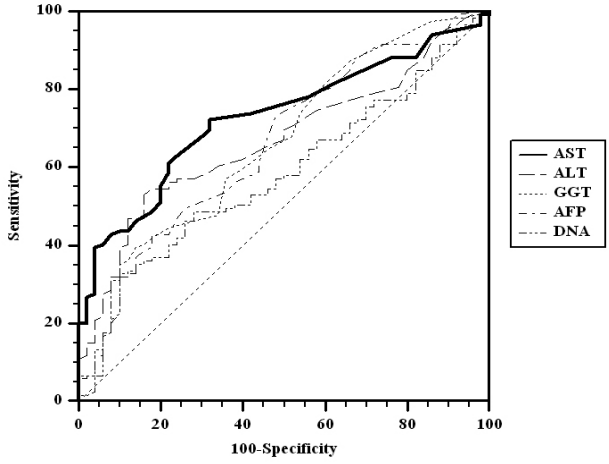

Figure 1. ROC analysis of laboratory results

ROC: Receiver operating characteristic, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transpeptidase, AFP: Alpha-fetoprotein

ROC curve analysis was performed and the optimal cut-off ALT, AST, GGT, and AFP values were determined for identifying CH. The AUC values were derived as 0.658 (95% confidence interval (CI)=0.594 to 0.719) for ALT, 0.694 (95% CI=0.631 to 0.753) for AST, and 0.649 (95% CI=0.582 to 0.712) for GGT. AST levels higher than 29 U/L have 63.52% sensitivity and 67.57% specifity for CH. Similarly AFP levels higher than 2.5 U/L have 71.9% sensitivity and 50.98% specifity for CH. The results of ROC curve analysis are shown in Table 3.

Multivariate logistic regression analysis was performed in order to identify factors associated with CH. Age, AST, and AFP were the significant independent risk factors for CH. The results of multivariate logistic regression analysis are shown in Table 4.

Discussion

In this study, we investigated associations between CH and some demographic and laboratory parameters such as age, gender, ALT, AST, GGT, AFP, and HBV-DNA in HBeAg-negative CHB patients. According to our results, age, gender, ALT, AST GGT, AFP, and HBV-DNA levels >100.000 IU/mL were associated with CH in univariate analysis. However in multivariate analysis only age, AST and AFP levels were associated with CH.

AST and ALT are normally contained in liver cells. In liver diseases such as in viral hepatitis, the liver cells spill the enzymes into the blood, raising the enzyme levels in the blood and signaling that the liver was damaged (8). In a study which was investigated relationship between histopathological features of liver and serum transaminase levels, AST was found a better laboratory screening test for finding the severity of liver injury than ALT in HBeAg-negative CHB patients (9). Similarly, our results showed that AST levels are more useful in showing liver damage than ALT. The normal limits of AST have been investigated in very few studies. It is recommended to adopt 40 U/L as the upper limit of AST (10). This value may be different in cases who have stage 3 or higher fibrosis, which was very low in our study. Inthis study the cut off level of AST was found to be 29 U/L. This suggests that normal AST levels in our country should be determined by large epidemiological studies.

Our results indicate that high levels of AFP was the second independent laboratory parameter related with CH. AFP is a glycoprotein that normally produced in early pregnancy by the fetal yolk sac, liver and gastrointestinal tract (11). In adults, AFP levels are elevated in acute or chronic viral hepatitis, chronic liver disease, non-alcoholic fatty liver disease, and especially in gonadal tumors and hepatocellular carcinoma (12). Also, it was shown that elevated serum AFP levels are associated with hepatic steatosis and ≥stage 2 fibrosis (13). Elevated serum AFP levels in these hepatic diseases are depend on the ongoing inflammation, altered hepatocyte-hepatocyte interaction or the loss of normal achitectural arrangements (14). According to our results, the cut off point of AFP is 2.5 ng/mL. However, this cut-off value is under the ULN value for AFP. This situtation might be related with observer difference in our pathologists.

Age was another parameter found as a prognostic factor for CH. The relationship between age and fibrosis was found in multiple studies. the average age was found lower in patients with mild fibrosis than in those with severe fibrosis (15). In another study, positive correlation was found between age and fibrosis, too (16). It should be noted that the duration of the disease may also be related to the fibrosis score. Our results revealed that the average age was higher in HBeAg-negative CHB patients with CH than in those with NL (43.5 years vs 37 years). Interestingly, according to our results, HBV-DNA level does not predict the necroinflammation and fibrosis. This finding has also been mentioned in other studies. Lu et al. (17) and Aktug Demir et al. (16) reported no correlation between the viral load, inflammatory activity, and the fibrosis score. As the risk of CH increases with age, patients should be followed up very closely after the age of 40 years and liver biopsy should be considered even if HBV-DNA levels and other liver tests such as

ALT are not too high. This is also important in determining the age at which treatment begins. Patients more than 40 years old should be evaluate for treatment be carefully because this age is critical for fibrozis.

Study Limitations

Before any conclusion we should declare our limitations. One of our limitation is that, we could not evaluate the effect of platelet results for fibrosis. Our other limitation is that, we did not investigate the pathologist observation difference. İf we did it, we could been say more accurate results.

Conclusion

As a result, age, high AST anf AFP levels are associated with hepatic necroinflammation in HBeAg-negative CHB. Specialist doctors who follow up these patients should evaluate these parameters more carefully.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Sakarya University Faculty of Medicine (approval number: 2013/71522473.050.01.04/37).

Informed consent: Retrospective study.

Peer-review: External and internal peer-reviewed.

Author Contributions

Concept: E.G., Design: E.G., A.Ö., O.K., Data Collection or Processing: E.G., A.Ö., M.K., O.K., Analysis or Interpretation: E.G., Ü.E., O.K., Literature Search: E.G., A.Ö., Writing: E.G., A.Ö., O.K.

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Research Article

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The Importance of Antiviral Prophylaxis against Hepatitis B Virus in Patients under Immunosuppressive Therapy

İmmünosupresif Tedavi Alan Hastalarda Hepatit B Virus Profilaksisinin Önemi

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ABSTRACT

Objectives: Immunosuppressive (IS) therapies present a risk of reactivation in patients with previous or known hepatitis B virus (HBV) infection and may cause mortality and morbidity. Before starting these therapies, patients should be tested for HBV serology and evaluated for antiviral therapy.

Materials and Methods: hepatitis B surface antigen (HBsAg)-positive or HBsAg-negative and Anti-HBs and/or anti-HBc immunoglobulin-positive patients aged over 18 years old who were scheduled to undergo or who were already on IS therapy due to underlying diseases were evaluated retrospectively. The study included patients who had monthly transaminase levels during the first six months of antiviral prophylaxis, and then who had transaminase and HBV-DNA levels every three months during subsequent follow-ups.

Results: Sixty-three patients were included in the study. Forty-eight patients (76%) received prophylaxis with IS therapy and 15 patients (24%) did not receive prophylaxis at the appropriate time. HBV reactivation (HBVr) was observed in three patients who did not receive prophylaxis at the appropriate time. The incidence of HBVr in all our patients was 4.8%, but was 20% in patients with delayed prophylaxis.

Conclusion: IS therapies represent a major risk in terms of HBVr. Before starting these therapies, patients should be evaluated for antiviral prophylaxis by testing their HBV serology.

Keywords: Hepatitis B virus, prophylaxis, immunosuppressive therapy

ÖZ

Amaç: İmmünosupresif tedaviler, önceki veya bilinen hepatit B virüsü (HBV) enfeksiyonu olan hastalarda reaktivasyon açısından bir risk oluşturur ve mortalite ve morbiditeye neden olabilir. Bu tedavilere başlamadan önce, hastalar HBV serolojileri test edilerek antiviral tedavi açısından değerlendirilmelidir.

Gereç ve Yöntemler: Altta yatan hastalıklar nedeniyle immünosupresif tedavi planlanan veya daha önce başlanan 18 yaş üstü hepatit B yüzey antijeni (HBsAg)-pozitif veya HBsAg-negatif ve anti-HBs ve/veya anti-HBc immünoglobulin-pozitif hastalar retrospektif olarak değerlendirildi. Çalışmaya antiviral proflaksi başlanan hastalardan ilk 6 ay boyunca aylık transaminaz, sonraki takiplerinde her üç ayda bir transaminaz ve HBV-DNA seviyeleri bakılan hastalar dahil edildi.

Bulgular: Altmış üç hasta çalışmaya alındı. Kırk sekiz (%76) hastaya immünosupresif tedavi ile birlikte profilaksi başlandı, 15 (%24) hastada profilaksi uygun zamanda başlanmadı. Uygun zamanda profilaksi alamayan hastaların üçünde HBV reaktivasyonu (HBVr) görüldü. Tüm hastalarımızda HBVr insidansı %4,8 idi, ancak gecikmiş profilaksi olan hastalarda %20 idi.

Sonuç: İmmünsupresif tedaviler HBV reaktivasyonu açısından önemli bir risk oluşturmaktadır. Bu tedavilere başlamadan önce, hastalar HBV serolojilerini test ederek antiviral profilaksi açısından değerlendirilmelidir.

Anahtar Kelimeler: Hepatit B virüs, profilaksi, immünosupresif tedavi

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Introduction

Hepatitis B virus (HBV) infection is one of the world's most important health problems. Immunosuppressive (IS) therapies constitute a risk in terms of HBV reactivation (HBVr) and can cause mortality and morbidity in patients with previous or known HBV infection (1,2). Patients receiving these must therefore first be tested in terms of HBV serology (1,2). Cancer chemotherapy, autoimmune diseases, IS therapies in patients receiving solid organ and stem cell transplantation, glucocorticoids, and biological agents frequently used in recent years are all risk factors for reactivation (1,2,3,4,5,6). HBVr is characterized by a symptomatic or asymptomatic increase in serum aminotransferase (alanine aminotransferase or aspartate aminotransferase) levels. An increase in HBV-DNA frequently accompanies that manifestation (1,2,3,7). HBVr is defined by the European Association for the Study of the Liver and the Asian Pacific Association for the Study of the Liver as hepatitis B surface antigen (HBsAg) seroreversion and an increase in HBV-DNA levels (2,8). According to the American Association for the Study of Liver Diseases, active necroinflammatory disease of the liver in inactive HBsAg carriers or subjects with histories of HBV infection is defined as reactivation (4). HBVr can be prevented in subjects receiving IS therapy with antiviral prophylaxis. HBV prophylaxis should be initiated 1-3 weeks before the IS therapy, if possible, or at least concomitantly with the IS therapy (1,2,4,7,8,9,10,11). However, this is known to be less effective on liver damage when given after IS therapies have already been started (1,2,4,7,8,9,10,11). According to the American Gastroenterological Association (AGA) guideline, the risk of reactivation in continuing or previous HBV infection varies depending on serology and/or immunosuppression (1). Subjects such as the prevention of reactivation, the most appropriate population for screening, who should use prophylaxis, the best specific agent, duration of prophylaxis, and monitoring when prophylaxis is not employed are still unclear (1,2). However, the consensus in all guidelines is that it is essential for patients to be evaluated in terms of antiviral therapy before IS therapy begins in order to prevent progression of HBVr and underlying disease (since IS therapy may be discontinued when HBVr develops) (1,2,7,8,9,10,11).

The purpose of this study was to assess the effect on HBVr development of prophylactic antiviral therapy in patients receiving IS therapy. While there have been previous case reports from Turkey, we encountered no studies concerning HBVr, and our study is thus the first of its kind from Turkey.

Materials and Methods

This study was conducted at our clinic between 01.01.2010 and 30.10.2016. The data were analyzed retrospectively. We evaluated patients diagnosed with chronic hepatitis B that received IS therapy or planned. HBsAg-positive or HBsAg-negative and anti-HBs and/or anti-HBc Immunoglobulin G (IgG)-positive patients aged over 18 age scheduled to be or already started on IS therapy due to underlying diseases (patients with solid or hematological malignity receiving chemotherapy, with autoimmune and/or rheumatological diseases, patients undergoing solid organ or stem cell transplantation, or patients using IS therapy, glucocorticoids, or biological agents for any reason) were enrolled in the study. Patients with known transaminase and HBV-DNA levels were included. Patients with human immunodeficiency virus (HIV), HCV, Delta co-infection were not included. Antiviral prophylaxis has started according to the guidelines of the period (1,4,7). Risk assessment performed according to AGA guidelines (1). No additional examination was requested except for the recommendations of the guidelines in the follow-up of the patients. Data of patients were obtained from electronic records. The study included patients who had received antiviral prophylaxis for the first 6 months of transaminase monthly, followed by transaminase and HBV-DNA levels every three months. One of lamivudine, tenofovir and entecavir was used as antiviral. Patients were divided into two groups. Group 1: Patients had been started on prophylaxis together with IS therapy (appropriate time), Group 2: Patients had not been started on prophylaxis timely (patients who did not receive prophylaxis at the appropriate time.). Data analysis was performed by using frequencies for the descriptive statistics.

Statistical Analysis

Data analysis was performed by using frequencies for the descriptive statistics.

Results

Sixty-three patients were included in the study, 33 men (52.3%) and 30 women (47.6%). Patients' mean age was 52.2±14.2 years (24-86). HBsAg, anti-HBc IgG was positive and anti-HBs-negative in 54 patients (85.7%). Forty-eight patients (76%) had been started on prophylaxis together with IS therapy (group 1), while 15 (24%) had not been started on prophylaxis timely (group 2). Patients' characteristics are shown in Table 1. In terms of IS drugs, 29 (46.1%) patients received anti-TNF, 24 (38.1%) chemotherapy, 5 (%7.9) took steroids, and 5 (7.9%) received chemotherapy combined with steroids. Based on the AGA guideline (1). In all groups, prophylaxis was evaluated by considering IS risk group, HBV serology and underlying diseases (1).

27 (42.9%) of the patients were in the high risk group, 31 (49.2%) were in the moderate risk group and 5 (7.9%) were in the low risk group. Of the high-risk patients, 18 (66.7%) received chemotherapy, 3 (11.1%) received anti-TNF, 3 (11.1%) received steroid and 3 (11.1%) received steroid and chemotherapy. In twenty-seven (100%) patients were HBsAg positive/anti-HBc IgG positive of these 26 (96.3%) were HBeAg negative and 1 (3.7%) was HBeAg positive. Eighteen (66.7%) lamivudine, 7 (25.9%) tenofovir and 2 (7.4%) entecavir were used as antiviral prophylaxis in high risk patients. HBV-DNA levels were <2000 IU/mL in 15 (55.6%) patients and HBV-DNA >2000 IU/mL in 12 (44.45) patients. 86.2% of the patients receiving anti- tumor necrosis factor (TNF) were moderate risk, 10.3% were high risk, and 3.5% were low risk.

Lamivudine, tenofovir and entecavir were used as prophylactic therapy by 46 (73%), 11 (17.4%) and 6 (9.5%) patients, respectively. Eight patients (72.7%) receiving tenofovir had experience of lamivudine.

The underlying diseases of HBeAg negative patients were rheumatological disease (n=38), hematological malignity (n=14), solid tumor (n=2), renal transplantation (n=1) and bone marrow transplantation (n=1). The prophylactic therapies of HBeAg

Table 1. The characteristics of the patients who take prophylaxis timely or not		
	Group 1, n=48	**Group 2, n=15**
Gender (Male/Female)	25/23	8/7
Underlying diseases		
- Rheumatological disease	31	7
- Hematological malignity	14	4
- Solid tumor	2	1
- Bone marrow transplantation	-	3
- Renal transplantation	1	-
HBV infection history, (HBsAg positivity)		
<1 year	6	3
1-5 year	13	4
>5 year	20	8
HBV serology		
- HBsAg positivity/anti-HBc IgG positivity	n=39	n=15
- HBsAg negativity/anti-HBs positivity/anti-HBc IgG positivity	3	-
- Isolated anti-HBc IgG positivity	6	-
HBV infection definitions		
HBeAg positivity	4	3
- HBV-DNA >20000 IU/mL, ALT: normal or elevated	3	3
- HBV-DNA < 20000 IU/mL, ALT: normal or elevated	1	-
HBeAg negativity	35	12
- HBV-DNA <2000 IU/mL, ALT: normal or elevated	18	3
- HBV-DNA >2000 IU/ml, ALT: normal or elevated	17	9
Risk group		
- High	20	7
- Moderate	25	6
- Low	3	2
Immunosuppressive drugs		
- Anti-TNF	24	5
- Steroid	4	1
- Chemotherapy	17	7
- Steroid and Chemotherapy	3	2
Antiviral prophylaxis		
- Lamivudine	40	6
- Tenofovir	5	6
- Entecavir	3	3
Reactivation	-	3
HBV: Hepatitis B virus, HBsAg: Hepatitis B surface antigen, Ig: Immunoglobulin, ALT: Alanine aminotransferase, TNF: Tumor necrosis factor		

negative patients were lamivudine (n=42), tenofovir (n=8) and entecavir (n=6). The underlying diseases of HBeAg positive patients were hematological malignity (n=4), solid tumor (n=1) and bone marrow transplantation (n=2). The prophylactic therapies of HBeAg positive patients were lamivudine (n=4) and tenofovir (n=3). The underlying diseases of isolated anti-HBc IgG positive patients were hematological malignity (n=3), rheumatological disease (n=2) and bone marrow transplantation (n=1). The prophylactic therapies of isolated anti-HBc IgG positive patients were lamivudine (n=4), entecavir (n=1) and tenofovir (n=1).

The HBVr rate among all our patients was 4.8%, but the figure was 20% among patients in whom prophylaxis was delayed. The rate among the 54 HBsAg-positive patients was 5.6%. Delay time of prophylaxis was 9.5±9.2/month. The time of referral of the patients in group 2 from the clinics treating the underlying disease to our clinic was long. It was thought that this was due to the lack of awareness of the relevant clinics about HBVr. Three of the patients (4.8%) not receiving prophylaxis after being started on IS therapy presented with a manifestation of HBVr. All three patients who developed HBVr were male. IS treatment and underlying disease of

Table 2. The characteristics of the patients who developed hepatitis B virus reactivation

	Patient 1	Patient 2	Patient 3 (Exitus)
Age	25	36	76
Gender	Male	Male	Male
Underlying diseases	Rheumatological disease	Rheumatological disease	Hematological malignity
HBV infection history (HBsAg positivity)	<1 year	<1 year	>5 year
HBV serology	HBsAg positivity	HBsAg positivity	HBsAg positivity
	Anti-HBs negative	Anti-HBs negative	Anti-HBs negative
	HBeAg negative	HBeAg negative	HBeAg negative
	Anti-HBc IgG positivity	Anti-HBc IgG positivity	Anti-HBc IgG positivity
HBV-DNA level (when admitted to hospital)	$1.07x10^3$ IU/mL	$1.07x10^5$ IU/mL	$3.89x10^7$ IU/mL
HBV-DNA level (6. month)	Negative	Negative	-
HBV-DNA level (12. month)	Negative	Negative	-
Immunosuppressive treatment	Infliximab	Rituximab	Azathioprine
Risk group (AGA guideline)	Moderate	High	Low
Prophylactic agents	Lamivudine	Entecavir	Lamivudine
Delay time of prophylaxis (HBVr time)	10/ month	4/ month	30/ month

HBVr: Hepatitis B Virus reactivation, HBsAg: Hepatitis B surface antigen, Ig: Immunoglobulin, AGA: American Gastroenterological Association, HBV: Hepatitis B Virus

the patients were showed in the Table 2. Patient 3 did not receive antiviral prophylaxis during previous IS therapy. The patient received chemotherapy one month before he came to us. When the patient was admitted to the service, Lamivudine prophylaxis was started (because the patient developed respiratory distress and the patient started high-dose steroid therapy). Transaminase levels and HBV-DNA level were increased. HBVr was considered in the patient. Patient 3 died in intensive care unit on the 50th day of hospitalization from fulminant hepatitis (50th Day of Antiviral Prophylaxis). The characteristics of the patients who developed HBVr were shown in the Table 2.

Fifty (79.3%) continued with prophylactic therapy at six-month follow-up. HBV-DNA exceeded 2000 IU/mL in three patients at the end of six months. IS therapy was continuing in two of these patients. Patients had non-compliance to prophylactic therapy. When prophylactic therapy compliance is achieved, the three patients' HBV-DNA became negative at the end of the 12th month. Two of the patients had used lamivudine and one had tenofovir.

The HBV-DNA levels of two patients with negative HBV-DNA at six months rose above 2000 IU/mL at the end of 12 months. Both patients were taking lamivudine. Flare-up occurred at the end of 12 months in one patient not using treatment regularly. While no concrete cause could be identified in the other patient, resistance tests could not be performed in the patients. All the other patients were persisted with HBV-DNA negativity.

Conclusion

Individuals encountering HBV infection are at risk of HBVr when their immunity is suppressed. HBVr may appear with differing clinical manifestations, from asymptomatic disease to a severe and fatal course. This also affects the morbidity and mortality of the underlying disease as a cause of discontinuation of immunosuppression and chemotherapy (11).

Determining serological status and type and duration of immunosuppression by screening patients at risk of reactivation is very important in the management of the antiviral therapy process (1,2,4,7,8,9,10). Patients receiving IS therapy must be scanned in terms of HBsAg, anti-HBs and anti-HBc markers before treatment. In terms of our patients' serological parameters, HBsAg, Anti-HBs and anti-HBc IgG positivity rates were 89.4%, 1.8%, and 100%, respectively. HBVr is more common in patients with HBsAg positivity (1,2,3,4,5,6,7,8,9). Tavakolpour et al. (3) reported a high risk of reactivation in HBsAg- and HbeAg-positive patients. Our three patients with reactivation were HBsAg-positive and HBeAg-negative. Lee et al. (12) reported a 12.3% level of HBVr in 122 HBsAg-positive patients receiving IS therapy due to rheumatological diseases, compared to 5.6% in our study. No previous studies from Turkey, including case reports, have reported this rate.

The type of IS employed and length of use also constitute a risk for reactivation (1,2,3,4,5,6,7,8,9). When drugs that suppress B cells, antracycline derivatives and high-dose corticosteroids are used, the risk of reactivation is above 10%. The risk of reactivation with the use of TNF-alpha, cytokine, integrin, tyrosine kinase inhibitors and low-dose corticosteroids ranges between 1% and 10%. The reactivation risk associated with low-dose or intra-articular corticosteroid or conventional IS drug (azathioprine, 6-merkaptopurin, and methotrexate) use is less than 1% (1,11). In our study, 46.1% (n=29) of patients had received anti-TNF, 38.1% chemotherapy, 7.9% steroids, and 7.9% chemotherapy and steroid therapy. According to the AGA guideline (1), 86.2% of our patients receiving anti-TNF were at moderate risk, and the most commonly used agent was infliximab (n=13). An additional 10.3%

were at high risk, and all had used rituximab, while 3.5% were in the low-risk group. Two of the patients with HBVr had used anti-TNF (infliximab, and rituximab), and the other patient, azathioprine. The IS therapies used by our patients were in the low-, moderate-, and high-risk groups. Although azathioprine involves a low risk (<1% risk of HBVr), HBVr occurred in one of our patients, and that patient died. One previous study reported that a risk of HBVr with azathioprine, but that this was lower compared to other chemotherapeutic agents (13).

The current antivirals of choice in patients receiving IS therapy are tenofovir and entecavir (11,14). Lamivudine therapy was administered to patients with HBVr receiving anti-TNF, and entecavir therapy to a patient using azathioprine.

IS therapies, steroids, and biological agents that have become intensively used in several diseases in recent years constitute a major risk in terms of HBVr, and these cases may be missed in clinical practice. These patients must be evaluated in terms of prophylaxis requirement by means of serological screening before treatment. Prophylactic antiviral therapy prevents HBVr in patients receiving IS therapy, but as seen in our study, delayed treatment can result in morbidity and mortality. HBVr was present in one patient in our low-risk group, and it is impossible to say whether this was associated with the natural course of the disease or else incidental, and further studies involving larger patient numbers on this subject are now needed. It should be remembered that HBVr can also be seen in low-risk patients, and we think that these patients also require close and careful follow-up.

Ethics

Ethics Committee Approval: Retrospective study.
Informed Consent: Retrospective study.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.A., S.K., Concept: F.A., Design: F.A., S.K., Data Collection or Processing: F.A., H.N.K., S.A., İ.K., G.Y., Analysis or Interpretation: F.A., G.Y., Literature Search: S.K., H.N.K., Writing F.A.

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Research Article

55

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Direct-acting Antiviral Therapy for Mixed Genotype Chronic Hepatitis C Infection

Miks Genotip ile Enfekte Kronik Hepatit C Hastalarında Direkt Etkili Antiviral Tedavi

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ABSTRACT

Objectives: Literature data concerning the outcomes of direct acting antiviral (DAA) therapy in mixed genotype hepatitis C virus (HCV) infections are very limited, and the incidence of mixed HCV infection in Turkey is unknown. The aim of this study was to investigate the prevalence of mixed genotype chronic HCV infection, risk factors related to mode of transmission and outcomes of DAA therapy in these patients.

Materials and Methods: Patients with two different HCV genotypes identified in the same blood sample during a 20-month period were analyzed retrospectively in terms of treatment received, adherence and response to treatment and risk factors related to mode of transmission.

Results: During the study period, mix genotypes were detected in 21 (4.2%) out of 495 patients with chronic HCV infection. Fifteen patients (71%) had a history of intravenous drug use. Eleven patients who received DAA treatment was HCV-RNA negative at the end of treatment.

Conclusion: According to our findings, infection with different HCV genotypes is possible in patients with repeated HCV exposure, such as intravenous drug users, but mixed HCV infection can be successfully treated with DAA therapy. In addition, our study may be noteworthy for also including mixed genotypes in the HCV epidemiological shift in our region.

Keywords: hepatitis C virus, HCV genotypes, mixed genotype HCV, direct-acting antivirals, intravenous drug use

ÖZ

Amaç: Miks genotip hepatit C virus (HCV) enfeksiyonlarının direkt etkili antiviral (DEA) ilaçlar ile tedavi sonuçlarına ilişkin çok az literatür verisi bulunmaktadır. Türkiye'de miks HCV enfeksiyonu görülme sıklığı bilinmemektedir. Bu çalışmada, miks genotip ile enfekte kronik hepatit C hasta prevalansının, bu hastalardaki bulaş yoluna ilişkin risk faktörlerinin ve DEA ilaçlar ile tedavi sonuçlarının incelenmesi amaçladı.

Gereç ve Yöntemler: Yirmi aylık çalışma periyodunda, aynı kan örneğinde iki farklı HCV genotipi saptanan hastalar, aldıkları tedavi protokolleri, tedaviye uyumları ve tedaviye yanıtları, bulaş yoluna ilişkin risk faktörleri açılarından retrospektif olarak incelendi.

Bulgular: Çalışma periyodu içinde genotip tayini yapılan 495 hastadan 21'i (%4,2) iki farklı HCV genotipi ile enfekteydi. Bu hastaların 15'inde (%71) damar içi madde kullanımı öyküsü vardı. DEA tedavi alan 11 hastada tedavi sonu HCV-RNA negatif bulundu.

Sonuç: Bulgularımıza göre, damar içi madde kullanımı gibi HCV'ye tekrarlayan maruziyet durumlarında iki farklı HCV genotipi ile enfeksiyon olasıdır, miks HCV enfeksiyonu DEA ile başarılı bir şekilde tedavi edilebilir. Ayrıca çalışmamız, bölgemizdeki HCV epidemiyolojisindeki değişime miks genotiplerin de dahil edilmesi açısından dikkat çekici olabilir.

Anahtar Kelimeler: Hepatitis C virus, HCV genotip, miks genotip, direkt etkili antiviraller, damar içi madde kullanımı

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Introduction

Chronic hepatitis C virus (HCV) infection is a major cause of chronic liver disease. An estimated 71 million people are infected with HCV worldwide (1). In Turkey, the seroprevalence of HCV infection is reported to be 1% (2). Seven genotypes and 67 subgenotypes of HCV have been described to date (3). The distributions of HCV genotypes and subgenotypes vary according to geographical location and mode of transmission. Genotypes 1, 2, and 3 are common globally, while genotypes 4, 5, and 6 are seen in certain geographical locations (4). Genotype 1b is the most common, both globally and in Turkey (4,5).

Because sufficient protective immunity does not develop following primary infection, reinfection is possible upon repeated exposure to HCV after the infection is eliminated either spontaneously or with treatment. Similarly, an individual may be infected with more than one genotype as a result of repeated exposure to HCV. Mixed HCV infection is used to describe patients infected with two or more different HCV genotypes simultaneously (6). Risk groups for repeated exposure to HCV and mixed HCV infection include intravenous drug users (IVDUs), hemodialysis patients, and patients needing frequent transfusion of blood and blood products, such as those with hemophilia (6,7).

With the direct-acting antiviral (DAA) drugs currently used in the treatment of chronic HCV infection, genotyping remains critical for selecting an appropriate treatment protocol and duration, as it was for earlier interferon-based therapies.

Data concerning the outcomes of DAA therapy in mixed HCV infections are very limited (8,9). In this study, we investigated the prevalence of mixed genotype chronic HCV infection, risk factors related to mode of transmission, and outcomes of DAA therapy in these patients.

Materials and Methods

Over a 20-month period from June 2016 and February 2018, HCV genotype analysis was performed for 495 patients diagnosed with chronic hepatitis C infection. Genotyping was done using the HCV Genotype Plus Real-TM kit (Sacace Biotechnologies Caserta, Italy) in the first 14 months and Abbott RealTime HCV genotip 2 kit (USA) in the last 6 months. Patients with two different HCV genotypes identified in the same blood sample were analyzed retrospectively in terms of treatment received, adherence and response to treatment, and risk factors related to mode of transmission. The study was approved by the Ethics Committee of Adana City Training and Research Hospital (approval number: 276, date: 08.29.2018).

Statistical Analysis

Statistical analyses were done using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistical methods were used. Findings were expressed in percent, mean, and standard deviation as appropriate.

Table 1. Treatment protocols and outcomes of the patients

Patient No	Age (Year)	Gender (M/F)	Genotype	HCV-RNA (IU/mL)	Fibrosis	Treatment	SVR[µ]	IVDU[*]
1	79	F	1b+3	79448	6	S/L 24w	Negative	No
2	22	M	1a+3	341415	2	S-R 24w	Negative	Yes
3	39	M	1b+4	2118231	2	PROD-R 12w	Negative	No
4	24	M	1b+4	460617	2	PROD-R 12w	Negative	Yes
5	28	M	2+3	130461	2	S-R 24w	Negative	Yes
6	22	M	1b+4	69329	NA[#]	NA	NA	Yes
7	46	M	1b+4	14008882	NA	NA	NA	No
8	25	M	1b+4	11114825	1	NA	NA	Yes
9	22	M	1b+4	10896696	2	NA	NA	No
10	31	M	1a+3	18662	NA	NA	NA	Yes
11	49	M	3+4	2828190	1	S/L-R 12w	Negative	No
12	26	M	1a+2b	741039	2	S/L-R 12w	Negative	Yes
13	26	M	2+3	25584	NA	NA	NA	Yes
14	32	F	2+4	93060	1	S/L-R 12w	Negative	No
15	25	F	3+4	1011756	2	NA	NA	Yes
16	29	M	1b+3	568124	NA	NA	NA	Yes
17	27	M	2+3	152231	3	S-R 24w	Negative	Yes
18	26	M	2+3	816	1	NA	NA	Yes
19	28	M	2+3	8119	1	S-R 24w	Negative	Yes
20	28	M	1b+4	536827	2	PROD-R 12w	Negative	Yes
21	31	M	2 + 3	3928824	2	NA	NA	Yes

HCV: hepatitis C virüs, SVR[µ]: Sustained virological response, IVDU*: Intravenous drug user, NA[#]: Not-available (Lost to follow- up), S-R: Sofosbuvir + Ribavirin, S/L: Sofosbuvir-Ledipasvir, S/L-R: Sofosbuvir-Ledipasvir + Ribavirin, PROD-R: Ombitasvir/Paritaprevir/Ritonavir + Dasabuvir + Ribavirin, M: Male, F: Female

Results

Of the 495 patients that underwent genotyping during the study period, 21 (4.2%) were infected with two different HCV genotypes. Eighteen (85%) of those patients were males and the mean was 31.7±12.71 (22-79) years. Transmission-related risk factors included IVDU in 15 (71%) patients, posttraumatic multiple blood transfusions in 1 patient, and 2 of the patients were immigrants from Syria. Genotype 1b-4 (7 patients) and genotype 2-3 (6 patients) were the most frequent genotype combinations. Of the 21 patients with mixed HCV infection, 10 were not under follow-up and had not received treatment. Eleven patients who received DAAs completed treatment and tested negative for HCV-RNA at post-treatment. Following treatment, 6 patients attended follow-up for varying periods (2-9 month). The HCV-RNA values of these patients remained negative during follow-up. Treatment protocols and outcomes of the patients are given in Table 1.

Discussion

The prevalence of mixed HCV infection varies with the patient population and the sensitivity of the method used for genotyping. In studies conducted in the general patient population in various countries, the prevalence of mixed HCV infection ranges between 2.2%-7.3% (9-12). In a study in England on groups at risk of repeated exposure to HCV, 9% of hemophilia patients were found to have mixed HCV infection, while the ratio increased to 19% among IVDUs (7). No data are available in Turkey regarding the prevalence of mixed HCV infection in the general population or in specific patient groups. In our study, the ratio of mixed HCV infection was 4.2%. The fact that 71% of these patients had a history of IVDU may be a notable finding regarding the mode of transmission of mixed HCV infection in our region. It has been reported that using more sensitive genotyping methods can reveal higher rates (14-39%) of mixed HCV infection in IVDUs (6). Mixed HCV infection may develop as a result of coinfection (infection with two different genotypes of HCV at the same time) or superinfection (a different genotype of HCV is added to an existing HCV infection) (13,14).

Although sequence analysis is considered the gold standard in HCV genotyping, it is difficult and costly. Therefore, routine diagnostic laboratories utilize the line probe assay method, developed as a commercial kit based on reverse hybridization, or real-time PCR-based methods using genotype-specific primers. Some of these target only the 5'UTR region, while others target the 5'UTR region as well as core or NS5B regions. Targeting multiple regions is reported to increase the sensitivity of accurate genotype and subgenotype identification (15). In our study, we used commercial sets based on one-step, real-time PCR with primers targeting 5'UTR in the first 14 months and 5'UTR and NS5B in the last 6 months.

In many studies on mixed HCV infection, standard commercial genotyping tests are accompanied by sequence analysis. It was emphasized in these studies that standard genotyping methods may effectively identify the dominant genotype, but may not be able to detect minor genotypes that account for less than 20% of the viral population, thus underestimating the actual prevalence of mixed HCV infection. Furthermore, after treatment has successfully eliminated the dominant genotype, it may be superseded by the minor genotype, which may be misinterpreted as relapse/reinfection. Therefore, it is strongly recommended to repeat genotyping in cases of failed treatment (6,10,12).

There are very limited data on the outcomes of DAA therapy in mixed HCV infection (8,9). In a study conducted in Spain, failed DAA therapy was reported in 2 of 6 patients with mixed HCV genotypes (9). In our study, post-treatment HCV-RNA was negative in 11 patients treated with DAA for mixed HCV infection. Of these patients, those still under follow-up were found to have sustained HCV-RNA negativity. Having only recently been licensed, pangenotypic DAA drugs were not covered by medical insurance in Turkey in the time period that we did our study. Treatment protocols for our patients are determined according to the European Association for the Study of the Liver 2016 guidelines and the conditions of reimbursement in our country (16).

Epidemiological studies on HCV infection have demonstrated changes in both patient demographic profile and genotypic distribution in the last 20 years, with genotype 1 gradually being replaced by genotype 3 with the increased use of safe blood and blood products. This shift has been mainly attributed to IVDU becoming a significant mode of transmission (4,17). As in the rest of the world, previous studies have reported similar changes in HCV genotype distribution in Turkey, and it was also suggested that IVDU may be a factor in this shift (18,19).

On the other hand, in 2016 the World Health Organization called for the eradication of HCV (20). HCV eradication seems to be an attainable goal, given that DAA drugs are already in use. However, IVDUs, who constitute a reservoir for HCV, are considered one of the main barriers facing eradication programs (17,21). It has been shown in modeling studies that DAA drugs in this patient group can go beyond treating the infected person and break the chain of transmission at the community level, thus minimizing new cases; this phenomenon has been termed "treatment as prevention".

Although DAA drugs are available and covered by insurance in Turkey, all of the patients in our study with mixed HVC infection who quit follow-up and remained untreated had a history of IVDU, which indicates their unwillingness to receive treatment. These untreated patients can potentially act as a mixed genotype reservoir in the chain of infection. To the best of our knowledge, this is the first study on mixed HCV infection in Turkey, and we believe it contributes to the limited literature data concerning the outcomes of DAA therapy in mixed HCV infection.

Study Limitations

Limitations of this study are that not all patients with mixed HCV infection received treatment and long-term follow-up results were not available for all of the treated patients. Also we conducted a retrospective study of the records.

Conclusion

Our findings indicate that although mixed HCV infection can be successfully treated with DAAs, making these drugs available is not enough to prevent HVC infection. Public health policies should be developed to motivate at-risk groups to receive treatment.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Adana City Training and Research Hospital (approval number: 276, date: 08.29.2018).

Informed Consent: Retrospective study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.M.S., H.K., B.K., Concept: B.M.S., Design: B.M.S., Data Collection or Processing: N.Ü., H.K., H.B.Ş.E., Analysis or Interpretation: B.M.S., H.K., Literature Search: B.M.S., Writing: B.M.S., B.K.

Conflict of Interest: The authors declare no conflict of interest.

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Research Article

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Evaluation of Hepatitis C Virus Genotype Results in İzmir Atatürk Training and Research Hospital

İzmir Atatürk Eğitim ve Araştırma Hastanesine Başvuran Hastalarda Hepatitis C Virüs Genotip Sonuçlarının Değerlendirilmesi

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ABSTRACT

Objectives: The aim was to support both the clinical treatment and management and also to contribute to the epidemiological understanding of the hepatitis C virüs (HCV) genotype in our region.
Materials and Methods: HCV genotyping was performed with suspected HCV infection in İzmir Katip Çelebi University, Atatürk Training and Research Hospital, Medical Microbiology Molecular Laboratory. Genotyping of the samples identified as positive for HCV-RNA was performed by Bosphore HCV Genotyping Kit v3.
Results: HCV genotyping results were obtained in 198 (75.8%) of 261 samples. Genotype 1 was found in 158 (79.8%) of the 198 samples, genotype 3 was found in 23 (11.6%), genotype 4 in 12 (6%) and genotype 2 in 3 (1.5%). Genotype 5 was detected in 2 (1%) individuals and one of whom was found to be a foreign national. Four of the 12 individuals identified as genotype 4 were found to be foreign nationals. Of the 158 genotype 1, 131 (82.9%) were identified as genotype 1b and 21 (13. 3%) 1a.
Conclusion: In our hospital, the most prevalent genotype is 1. This result is compatible with our country.
Keywords: Hepatitis C virus, genotype, epidemiology

ÖZ

Amaç: Bu çalışmada, hepatit V vrüs (HCV)-RNA pozitif hastaların HCV genotip tayinleri retrospektif incelenerek hem klinik tedavi yönetimine destek vermek hem de bölgemizdeki HCV genotip epidemiyolojisine katkı sağlamak amaçlanmıştır.
Gereç ve Yöntemler: İzmir Katip Çelebi Üniversitesi, Atatürk Eğitim ve Araştırma Hastanesi Tıbbi Mikrobiyoloji Moleküler Laboratuvarına HCV genotipleme istenen ve sonuçları çıkanlar çalışmaya alındı. HCV-RNA pozitif olarak tespit edilen örneklerin genotipleme çalışması hepatit C virüsü RNA'sının tüm genotiplerini saptayabilen Bosphore HCV Genotyping Kit v3 ile yapıldı.
Bulgular: HCV genotiplendirme çalışılan 261 örneğin 198 (%75,8) tanesinde sonuç elde edilmiştir. Yüz doksan sekiz örneğin 158'inde (%79,8) genotip 1, 23ünde (%11,6) genotip 3, 12'sinde (%6) genotip 4 ve 3'ünde (%1,5) genotip 2 saptanmıştır. İki (%1) kişide de genotip 5 tespit edilmiş ve bunlardan birinin yabancı uyruklu olduğu görülmüştür. Genotip 4 olarak tespit edilen 12 kişiden 4'ünün yabancı uyruklu olduğu görülmüştür. Genotip 1 olan 158 örneğin 131'i (%82,9) genotip 1b 21'i (%13,3) 1a olarak tespit edilmiştir.
Sonuç: Hastanemizde en sık genotip 1 görülür ve sonuçlar ülkemizle uyumludur.
Anahtar Kelimeler: Hepatit C virüs, genotip, epidemiyoloji

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Introduction

Hepatitis C virus (HCV) is a single-strand positive-directed RNA virus enveloped in the Flaviviridae family. HCV is one of the most important agents of blood-borne diseases such as chronic hepatitis, cirrhosis and hepatocellular carcinoma (1). HCV infects over one hundred million people whole globe including Turkey, where it is a major public health problem that affects large numbers of people (2,3). The rapid replication of the virus and the errors in RNA transcription during this replication play an important role in chronicisation.

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The daily production rate of the HCV is 10^{10}-10^{12} virion and the half-life of the virus is two to three hours (3). Genetic diversity is also important for HCV as in other RNA viruses. Genetic diversity is related to the high rate of replication of HCV and the absence of the function of error repair of RNA dependent RNA polymerase enzyme (4). Molecular epidemiological studies have shown that the distribution and prevalence of subtypes with HCV genotypes are geographically different, and that some genotypes are more dominant in different regions. Some HCV genotypes (genotype 1, 2, 3) are prevalent whole globe while others (genotypes 4, 5, 6) are seen in restricted geographical regions (5).

The genotypic variability of the HCV is a guide in the treatment and follow-up of hepatitis C patients. From the various studies have been carried out in Turkey it was observed that these were mainly on genotype 1b. However, there has also been an increase in the number of new HCV genotypes due to the increase in the number of foreign nationals in our country. In this study, HCV genotypes of HCV-RNA positive patients were analyzed retrospectively with the aim of supporting both clinical management and of contributing to the epidemiological understanding of HCV genotype in our region.

Materials and Methods

The results of HCV genotyping performed on serum samples sent with suspicion of HCV infection at the İzmir Katip Çelebi University, Atatürk Training and Research Hospital Medical Microbiology Molecular Laboratory between January 2013 and December 2017 were included in the study. Demographic data of the patients were obtained from hospital electronic information system and patient files. Ethics committee approval was not required due to the retrospective design of the study. HCV-RNA levels of samples were determined by "real time-polymerase chain reaction (PCR)" method (COBAS Ampli-Prep/COBAS Taqman HCV=Roche Diagnostic, Germany). Genotyping of the samples identified as positive for HCV-RNA was performed in the Microbiology Laboratory of Dokuz Eylül University Faculty of Medicine. Genotyping: viral nucleic acid extraction with EZ-1 virus mini kit (Qiagen) was performed. Bosphore HCV Genotyping Kit v3 was used to detect all genotypes of HCV-RNA (1,1a, 1b, 2, 3, 4, 5, 6) in human serum or plasma samples for HCV genotype determination. The analytical sensitivity of the kit is 100 IU/mL. A portion of the 5 "NS5B region of the HCV genome is amplified and fluorescence detection is performed using FAM, Cy5, and HEX filters. PCR Master Mix 1 in 1, 4, internal control; PCR Master Mix2 1a, 2, internal control; PCR Master Mix3 3 and internal control; PCR Master Mix4 1 and internal control; 5, 6 in PCR Master Mix5, using different labeled probes for internal control detection. The kit contains an internal control to control nucleic acid isolation and PCR inhibition. During RNA isolation or amplification data of the internal control can be added to the PCR reaction mix were visualized with Cy5 filter.

Statistical Analysis

SPSS 25.0 (Chicago, IL, USA) software program was used for data analysis. Mann-Whitney U test and Pearson chi-square test were used to evaluate the data; $p<0.05$ was considered statistically significant.

Results

Between January 2013 and December 2017, the results were obtained in 198 (75.8%) of 261 samples of HCV genotyping conducted by İzmir Katip Çelebi University, Atatürk Training and Research Hospital Microbiology Laboratory. Genotype 1 was found in 158 (79.8%) of the 198 samples, genotype 3 was found in 23 (11.6%), genotype 4 in 12 (6%) and genotype 2 in 3 (1.5%). Genotype 5 was detected in 2 (1%) individuals and one of them was found to be foreign national. Four of the 12 individuals identified as genotype 4 were found to be foreign nationals. Of the 158 genotype 1, 131 (82.9%) were identified as genotype 1b and 21 (13.3%) 1a. The age and sex assessment of patients with HCV infection are given in Table 1. There was no statistically significant difference between men and women in terms of gender in patients with HCV infection ($p>0.05$), but half of them were 55 years of age or older. The mean age of those with genotype 1 (58.1+16.7) was higher than the mean age (45.2+14.8) in patients infected with other genotypes and this was statistically significant ($p=0.01$).

Discussion

The nucleotide sequences of HCV genotypes differ by 31-34% from each other, the differences of subtypes are 20-23%. Although genotypes have emerged as endemic in geographically distant regions in the long term, most of them are now spread all over the globe. The most prevalent HCV genotype in the globe is 1. Globally, genotype 1 was found in 46% of all HCV infections. Other genotypes were observed in the following ratios: Genotype 3 (22%), genotype 2 and 4 (13% each).Genotype1b is responsible for just 22% of all infections (6). Genotype 1b is the most prevalent and predominant genotype in Turkey and this has been observed again in this study. However, an

Table 1. The age and sex assessment of patients with hepatitis C virus infection

Genotype	<25 age		26-35 age		36-45 age		46-55 age		56-65 age		>65 age		Total	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M
1a	-	1	2	2	1	3	2	3	2	1	2	2	9	12
1b	1	1	3	3	6	11	14	8	16	15	36	17	76	55
2	-	-	-	1	1	-	-	-	-	1	-	-	1	2
3	1	4	3	3	2	2	3	2	1	1	-	1	10	13
4	1	2	1	1	1	1	1	2	1	-	-	1	6	6
5	-	-	-	1	-	1	-	-	-	-	-	-	-	2
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	4	8	9	11	11	18	20	15	20	18	38	21	102	90

M: Male, F: Female

increase in the number of the other genotypes in question is also seen. Although it is seen that a section of these are of foreign origin, it is also significant in Turkish citizens. The findings suggest that the distribution of different HCV genotypes in foreign nationals is important. The events that cause social changes such as war and migration and intensive tourist activity affect the epidemiology of infections (7). İzmir is a city where both domestic and foreign tourism is active. It is also a city that attracts refugees and their migration to the city can explain the HCV genotype differences. In the present study genotypes other than genotype 1 were determined as 15% and genotype 3 was the highest. When we look at the studies published in our country, the highest (40%) ratio of genotype 3, except for our findings, was determined by Kirişçi et al. (8) in Kahramanmaraş and Sağlık et al.(9) also reported the ratio as 11.1% in Antalya. However, Kirişçi et al. (8) did not discuss the possible causes of this higher ratio. On the other hand, they reported that they could be associated with tourists coming from abroad, especially from Russia. Buruk et al. (10), in their study carried out in the Eastern Black Sea Region, emphasized that genotype 1 is dominant in similar way to the present study, but that genotypes other than genotype 1 are higher in the cities where people immigrate to and/or where foreign nationals are living than in the country (11-13).

Genotype 4 is the HCV genotype, which is more prevalent in the Middle East countries worldwide (7). Twelve patients had genotype 4, 4 of which were foreign nationals and were from Middle Eastern countries. The highest rate of Genotype 4 in Turkey has been reported in studies to occur in the cities of Kayseri and Afyon (11,12). Because of the long window period of the anti HCV test used in routine diagnosis of HCV, HCV-RNA examination is used by many laboratories (14,15). In the present study, the mean age of those with genotype was found to be higher than other genotypes. This finding is similar to the findings in Antalya (9).

Study Limitations

We conducted a retrospective study of the records. For this reason, there are some limitations. Some data, including possible transmission routes and risk factors, have not been obtained. Our data supports previous findings showing the dominance of genotype 1b infections in the area. However, there is an increase in the rate of infections caused by other genotypes.

Conclusion

The most prevalant HCV genotype in Turkey is 1b, however, it can be observed that other genotypes are beginning to be seen in both people of foreign origin and in Turkish citizens. A total prevalence of genotype 1 and 4 with poor prognosis in nine out of every ten people suggests that more attention should be paid to treatment and follow-up.

Ethics

Ethics Committee Approval: Retrospective study.
Informed Consent: Retrospective study.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: S.K., Design: A.A.G., A.G.Ş., İ.A., A.S., Data Collection or Processing: S.K., A.S., Analysis or Interpretation: S.K., Literature Search: A.A.G., A.G.Ş., İ.A., A.S., Writing: S.K.

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

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Research Article

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Genotyping Distribution of Hepatitis C Virus in Şanlıurfa Province and Effect of Syrian Patients

Şanlıurfa İlinde Hepatit C Virüsünün Genotip Dağılımı ve Suriyeli Hastaların Etkisi

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ABSTRACT

Objectives: Hepatitis C virus (HCV) is one of the major causes of global mortality and morbidity. The purpose of our study was to characterize the distribution of HCV genotypes in Şanlıurfa, to see the effect of patients from Syria on the distribution of HCV genotypes and to compare our results with other regions in our country.

Materials and Methods: Between January 2011 and December 2015, serum samples of 312 HCV-RNA positive patients (58 Syrian patients) were sent to the laboratory for HCV genotyping. HCV genotype analysis was investigated using a commercial Abbott GT II assay (Abbott, USA).

Results: The most frequent genotype was genotype 1 (69.6%), followed by genotype 2 (14.1%) and genotype 4 (10.3%). Among Syrian patients, the most prevalent genotype was genotype 4 (48.2%), followed by genotype 1 (41.4%) and genotype 5 (8.7%). Our data showed that the prevalence of genotype 1 decreased from 75% to 58.7% and genotype 2 decreased from 21.2% to 12% between 2011 and 2015, while genotype 4 increased from 1.9% to 20% and genotype 5 increased from 0% to 1.6% due to the Syrian patients.

Conclusion: Our updated estimates confirm an increase in genotype 4 and genotype 5, particularly in Şanlıurfa due to the Syrian patients.

Keywords: Hepatitis C virus, genotype, migration, molecular microbiology

ÖZ

Amaç: Hepatit C virüsü (HCV), küresel ölüm ve morbiditenin başlıca nedenlerinden biridir. Çalışmamızın amacı Şanlıurfa'da HCV genotiplerinin dağılımını belirlemek, Suriye'den gelen hastaların HCV genotiplerinin dağılımı üzerindeki etkisini görmek ve sonuçları ülkemizdeki diğer bölgelerle karşılaştırmaktır.

Gereç ve Yöntemler: Ocak 2011 ile Aralık 2015 arasında, toplam 312 (58'i Suriyeli hasta) HCV-RNA pozitif hepatit C hastasının serum örnekleri HCV genotiplendirme için laboratuvara gönderildi. HCV genotip analizi, ticari Abbott GT II (Abbott, ABD) kullanılarak araştırıldı.

Bulgular: En sık rastlanan genotip; genotip 1 (%69,6), iken genotip 2 (%14,1) ve genotip 4 (%10,3) bunu takip etmekteydi. Suriyeli hastalar arasında en yaygın genotip; genotip 4 (%48,2) iken genotip 1 (%41,4), genotip 5 (%8,7) bunu takip etmekteydi. Verilerimiz 2011-2015 döneminde genotip 1 ve 2 prevalansının sırasıyla %75'ten %58,7'ye ve %21,2'den %12'ye düştüğünü, genotip 4 ve 5'in Suriyeli hastalar nedeniyle sırasıyla %1,9'dan %20'ye ve 0'dan %1,6'ya çıktığını göstermektedir.

Sonuç: Güncellenen tahminlerimiz, özellikle Suriyeli hastalar nedeniyle Şanlıurfa'da genotip 4 ve genotip 5'teki artışı doğrulamaktadır.

Anahtar Kelimeler: Hepatit C virüsü, genotip, göç, moleküler mikrobiyoloji

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Introduction

Hepatitis C virus (HCV) is one of the major globally cause of death and morbidity (1). In 2015, 71 million persons worldwide were living with chronic HCV infection according to the global hepatitis report published by WHO in 2017 (2). Chronic HCV infection is generally associated with the development of liver cirrhosis, hepatocellular carcinoma, liver failure, and death, and is a main indication for liver transplantation (3). HCV has extensive genetic heterogeneity, which phylogenetic analysis categorized into 7 major genotypes and 67 subtypes (4). Genotype 1 (46%) and genotype 3 (22%) are the most common genotypes followed by genotype 2 (13%), genotype 4 (13%), genotype 6 (2%), and genotype 5 (1%) as shown by Gower et al. (5). Genotype 7 has been identified in four individuals originating from the Democratic Republic of Congo (6). Distinction between genotypes remains essential because selection of treatment regimens are mostly still genotype specific (7). The purpose of our study is to characterize the distribution of HCV genotypes in Şanlıurfa, to see the influence of patients from Syria on the the distribution of HCV genotypes and compare our results with other regions in our country.

Materials and Methods

Between January 2011 and December 2015, serum samples of 312 (159 male, 153 female) HCV RNA positive patients were analyzed retrospectively which were sent to Şanlıurfa Mehmet Akif İnan Training and Research Hospital Microbiology laboratory for HCV genotyping. Of the 312 patients, 58 were originated from Syria. HCV antibody was determined by microparticle enzyme immunoassay method (Abbott Laboratories, USA), quantitative HCV-RNA assay was performed by a commercial real-time polymerase chain reaction (PCR) method (Abbott Molecular Inc., USA). HCV genotype analysis was investigated by using a commercial Abbott GT II assay (Abbott, USA). Viral RNA extraction from 500 uL patient serum were completed on the Abbott m2000sp system using the Abbott mSpecimen Preparation System kit (Abbott, USA) according to the manufacturer's instruction. RealTime (RT)-PCR master mixes were prepared using the Abbott m2000sp and the Abbott RT-HCV Genotype II Amplification Reagent Kit (Abbott,USA). According to the manufacturer's recommendation, RT-PCR reactions were performed on the Abbott m2000rt (Abbott, USA) instrument.

Results

The most frequent genotype was genotype 1 (217 patients; 69.6%) followed by genotype 2 (44 patients; 14.1%), genotype 4 (32 patients; 10.3%), genotype 3 (12 patients; 3.8%) and genotype 5 (five patients; 1.6%). Two patients revealed presence of more than one HCV genotype (mixed). In two case (0.6%), genotype 1b was seen to be associated with genotype 3 and genotype 2 (0.3% and 0.3%, respectively). Of the 217 genotype one patients, genotype 1b was detected in 78.8% (n=171), genotype 1a was detected in 7.8% (n=17) and 13.4% (n=29) couldn't subtyped. Among Syrian patients (n=58), the most prevalent genotype was genotype 4 (28 patients; 48.2%) followed by genotype 1 (24 patients; 41.4%), genotype 5 (five patients; 8.7%) and genotype 3 (one patient; 1.7%). Genotype 2 was not detected in any of the Syrian patients. The percent distribution of genotypes both in Turkish and Syrian patients are shown in Table 1. Distribution of patients according to gender and mean age are shown in Table 2. As shown in Figure 1, the prevalence of genotype1 and 2 declined and the prevalence of genotype 4 increased over the years.

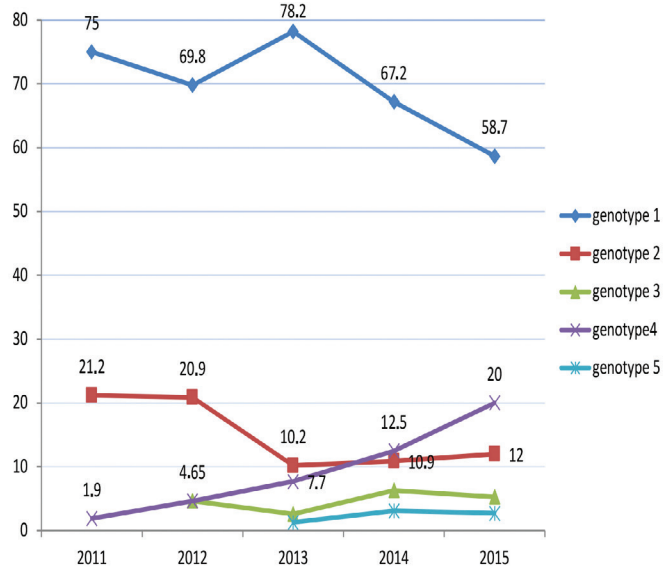

Figure 1. The percent distribution of genotype over years

Table 1. The percent distribution of genotypes both in Turkish patients and Syrian patients

Genotypes	Turkish patients				Syrian patients			
	Male (n)	Female (n)	Total (n)	(%)	Male (n)	Female (n)	Total (n)	(%)
1 (can not subtyped)	15	10	25	(9.8%)	4	-	4	(6.9%)
1a	6	-	6	(2.4%)	6	5	11	(19%)
1b	83	79	162	(63.8%)	4	5	9	(15.5%)
2	18	26	44	(17.3%)	-	-	-	-
3	9	2	11	(4.3%)	1	-	1	(1.7%)
4	1	3	4	(1.6%)	7	21	28	(48.2%)
5	-	-	-	-	3	2	5	(8.7%)
Mixed (1b+2)	1	-	1	(0.4%)	-	-	-	-
Mixed (1b+3)	1	-	1	(0.4%)	-	-	-	-

a: ,b:

Discussion

Knowledge of the geographic distribution of HCV genotypes is still playing an important role for epidemiological studies, treatment and vaccine development (8). The length of the treatment and the opportunity to associate interferon and/or ribavirin with the new direct-acting antiviral therapies still remain dependent on HCV genotype (1).

There are significant variations in global regions alsovary in different regions within countries (9). Gower et al. (5) showed that in North America, Latin America, and Europe genotype 1 (62-71%) was the predominant genotype with genotype 1b accounting for 26%, 39%, and 50% of all cases respectively. North Africa and the Middle East had a large genotype 4 population (71%), which was attributable to the high prevalence of genotype 4 in Egypt. In Asia, genotype 3 dominates followed by genotype 1.

When we check the previous studies which published after 2010 in Turkey, the most representative genotype is genotype 1, ranging between 51.7% and 95.3% (Table 3). The second common genotype distribution shows variability among the regions. Although the second most common genotype in Gaziantep, Antakya and Adıyaman was genotype 2 (7.8%, 9.3% and 11.3%, respectively), the second most common genotype in Kahramanmaraş, Adana and Antalya was reported as genotype 3 (46%, 26% and 11.1% respectively) (11,14,15,16,24). Only few cases of genotype 5, genotype 6 and mixed types are reported (Table 3).

Consistent with the results of other studies in Turkey, in our study genotype 1 was found most common genotype with the prevalance of 69.6%. When Syrian patients were excluded, genotype 1 accounted for 76%.

In the current study, genotype 2 was the second most common genotype (14.1%). This finding was similar to the studies in Adıyaman (11.3%), Antakya (9.3%) and Gaziantep (7.8%) which the cities are geographically close to each other and located at the south and southeast part of Turkey (14,15,24).

In the present study, the third most common genotype was genotype 4 (10.3%), which was attributable to the high prevalence of genotype 4 (48.2%) in Syrian patients. When Syrian patients were excluded, genotype 4 accounted for 1.6%. The frequency of genotype 4 is highest in Central Africa and the Middle East and has increased in prevalence due to migration from the Middle East and Africa (4). In Turkey, genotype 4 distribution does not show high

Table 2. Distribution of patients according to gender and mean age

Genotypes	Gender (%)		Mean age	
	Female	Male	Female	Male
1	45.6	54.4	55	49
2	59	41	59	53
3	16.7	83.3	36	31
4	75	25	49	45
5	40	60	59	48

Table 3. Hepatitis C genotype studies which published after 2010 in Turkey

Researcher (Referance)	Time	Province	Genotypes (%)							Number
			1	2	3	4	5	6	mix	
Tezcan et al. (10)	2010-2012	Mersin	92.3	2.1	4.2	0.8	-	0.4	-	236
Saglik et al. (11)	2009-2013	Antalya	83.4	3.5	11.1	1.6	-	-	0.2	422
Buruk et al. (12)	2009-2012	Trabzon	92.8	1.6	4.9	0.7	-	-	-	304
Kayman et al. (13)	2010-2011	Kayseri	62.4	3.2	1.1	32	-	-	1.3	375
Karslıgil et al. (14)	2011	Gaziantep	88.2	7.8	2	2	-	-	-	51
Öztürk et al. (15)	2010-2012	Adana	58.7	14.6	26	0.6	-	-	-	315
		Antakya	87	9.3	0.9	2.8	-	-	-	324
Caliskan et al. (16)	2010-2014	Kahramanmaraş	51.7	1.3	46	1	-	-	-	313
Altuğlu et al. (17)	2007-2011	İzmir	93.3	1.5	3.7	1.5	-	-	-	535
Us et al. (18)	2009-2014	Eskişehir	94.5	1.5	2	2	-	-	-	203
Tüzüner et al. (19)	2010-2017	Central Anatolia	90	3.8	3.3	2.5	0.2	0.2	-	480
Kirdar et al. (20)	2011-2016	Aydın	90.2	2.1	5.9	1.4	-	-	0.4	286
Duran et al. (21)	2015-2016	Adana	71.4	7.6	16.8	3.4	0.8	-	-	119
Selek et al. (22)	2015-2016	İstanbul	81.2	2.8	16	-	-	-	-	106
Karabulut et al. (23)	2013-2016	İstanbul	82.5	4.6	10.7	2.2	-	-	-	412
Akgun et al. (24)	2013-2016	Adıyaman	84.5	11.3	4.2	-	-	-	-	71
Aktaş et al. (25)	2011-2014	Erzurum	95.3	-	-	3.7	1	-	-	108
Current study	2011-2015	Şanlıurfa	69.6	14.1	3.8	10.3	1.6	-	0.6	312

variability among the regions, ranging between 0.5% and 3.4% (Table 3), while a significant prevalence described only in Kayseri (32%) (13). By using molecular clock analysis, they predicted the introduction of type 4d HCV into the Kayseri region probably 30-75 years ago (26).

The prevalence of genotype 3 is globally higher in the intravenous drug users (9). Studies from Kahramanmaraş and Adana, in which the genotype 3 favored the male gender (95.8%,85% respectively) with a mean age of 25 and 30.5 years (16,21). We also found that genotype 3 was significantly more common in young (mean age: 31) male (83.3%) patients. This findings suggest that intravenous drug use may have become more common among young males (16).

According to the united nations refugee agencies 2017 global report, Turkey hosted the largest number of refugees worldwide, with 3.5 million people (27). Şanlıurfa is located at the southeast part of Turkey near Syrian border, which has a population of 1.985.753 and currently hosts around 420.000 registered Syrian refugees who make up 21% of the overall city population (28).

The finding in this study that the most prevalent genotype was genotype 4 (48.2%) followed by genotype 1 (41.4%), genotype 5 (8.7%) and genotype 3 (1.7%) in 58 Syrian patients. Our results are in concordance with the published data from Syria, in which the most prevalent genotype in 636 HCV-RNA-positive patients from eight medical centres in Syria over a 3-year period was genotype 4 (59%) followed by genotype 1 (28.5%), genotype 5 (10%) and genotype 3 (1.8%) (29).

Of the 64 genotype 5 positive patients, 56 (87%) live in the north of Syria (originated from the northern province around the city of Aleppo), including 21 cases (33%) from Azaz, a small city close to Turkey (29). Similarly, in our study, genotype 5 was found only in patients arriving from Syria. Of the five genotype 5 positive patients, four of them were originated from the city of Aleppo and one was from Kobani. Genotype 5 (35.7%) is the most common genotype in South Africa (1). Antaki et al. (29) finding was the first report describing the presence of genotype 5 in Syria and the Middle East. At that time no cases were reported from neighbouring countries and they were unable to discover an explanation (so far from South Africa, and they consider that there is absolutely no immigration from Africa to Syria) for these unexpected findings. In Turkey, Yildirim et al. (30) presented probably the first finding of genotype 5 identified in three patients in Gaziantep originating from Syria. To the best of our knowledge, this was the first genotype 5 report from Turkey.

Study Limitations

One of the most important limitation of our study was the absence of information about the transmission routes due to the retrospective design of the study.

Conclusion

Migration into Turkey is an emerging phenomenon. Migration has influenced the prevalence of hepatitis C genotype distribution. It's interesting to note that, our data shows that the prevalence of genotype 1 and 2 between the period 2011-2015, has decreased from 75% to 58.7% and from 21.2% to 12% respectively while genotype 4 and 5 has increased from 1.9% to 20% and 0 from 1.6% respectively due to the Syrian patients. Our updated estimations confirm a raise in genotype 4 and genotype 5, in particular in Şanlıurfa due to the Syrian patients. Moreover, further regional and national epidemiological studies are required to see the effect of Syrian patients on genotype distribution.

Ethics

Ethics Committee Approval: Retrospective study.
Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: O.S.C., Design: O.S.C., Data Collection or Processing: A.U.M., Y.V., Analysis or Interpretation: H.H.G., Literature Search: H.Ö., A.B., Writing O.S.C.

Conflict of Interest: The authors declare no conflict of interest.

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Research Article

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Are Liver Transaminases and Hepatitis C Virus-RNA Viral Loads Reliable Markers for Estimating Liver Fibrosis in Patients Recently Diagnosed with Hepatitis C? Evaluation of the Data of Ninety-five Young and Middle-aged Males in a Genotype-1b Prevalent Country

Yeni Hepatit C Tanılı Hastalarda Karaciğer Transaminazları ve Hepatit C Virüs-RNA Viral Yükleri Karaciğer Fibrozunu Öngörmede Güvenilir Belirleyiciler midir? Genotip-1b Baskın Bir Ülkedeki Doksan-beş Genç ve Orta Yaşlı Erkeğe Ait Verilerin Değerlendirilmesi

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ABSTRACT

Objectives: Although liver biopsy is an invasive test, it is still considered as the gold standard method for determining the severity of liver diseases.

Materials and Methods: A total of 95 young and middle-aged male patients were enrolled in this retrospective study. ISHAK scoring-system was used for pathological assessment. The relationships between alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT ratio, hepatitis C virus (HCV)-RNA and histological activity index (HAI) scores in the prediction of fibrosis stages were evaluated.

Results: To predict a F ≥3, age >35 [odds ratio (OR): 3.56; 95% confidence interval (CI): 1.24-10.24; p=0.021)] was considered a significant risk factor. For a F ≥2, ALT and AST values over 40 IU/mL were found to be significant risk factors (OR: 2.97; 95% CI: 1.09-8.06; p=0.03, OR: 2.88; 95% CI: 1.17- 7.09; p=0.020, respectively). All indirect fibrosis parameters except HAI showed low to moderate diagnostic accuracy in the prediction of both F ≥2 and F ≥3 (AUC 0.50 to 0.68). According to the receiver operation characteristic (ROC) analysis results, the optimal cut-off values for predicting F ≥3 for ALT, AST, AST/ALT ratio, Log10 HCV-RNA and HAI were 94 U/L,

ÖZ

Amaç: Karaciğer biyopsisi invaziv bir yöntem olmakla birlikte hala karaciğer hastalıklarının ciddiyetini belirlemek için altın standart yöntem olarak kabul edilir.

Gereç ve Yöntemler: Bu retrospektif çalışmaya toplam 95 genç ve orta yaşlı erkek hasta dahil edildi. Patolojik değerlendirme için ISHAK skorlama sistemi kullanıldı. Fibrozisin evrelerinin öngörülmesinde alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), AST/ALT oranı ile HCV-RNA ve histolojik aktivite indeksi (HAİ) skorları arasındaki ilişkiler araştırıldı.

Bulgular: F3'ü öngörmede, >35 yaşın [olasılık oranı (OR): 3.56; %95 güven aralığı (CI): 1.24-10.24; p=0,021] önemli bir risk faktörü olduğu gösterildi. F ≥2 için, 40 IU/mL'nin üzerindeki ALT ve AST değerlerinin önemli risk faktörleri olduğu ortaya kondu (sırasıyla OR: 2,97; %95 CI: 1,09-8,06; p=0,03 ve OR: 2,88; %95 CI: 1,17-7,09; p=0,020). HAİ dışındaki tüm indirek fibroz göstergeleri, hem F ≥2 hem de F ≥3'ü (AUC 0,50 ila 0,68) öngörmede düşük/orta dereceli tanısal doğruluk gösterdi. Alıcı işletim karakteristiği (ROC) analiz sonuçlarına göre, ALT, AST, AST/ALT oranı, Log10 HCV-RNA ve HAİ için F ≥3'ü öngörmedeki optimum kesme değerleri sırasıyla 94 U/L, 55U/L, 0,78, 6,88 ve 6 idi.

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55 U/L, 0.78, 6.88 and 6, respectively. However, only ALT, AST and HAI had statistically significant results in the ROC analysis ($p<0.05$).
Conclusion: Age, AST, ALT and HAI were considered to be better predictors than AST/ALT ratio and HCV-RNA for estimating liver fibrosis in patients infected with HCV. However, our findings are not sufficient to recommend follow-up of chronic HCV patients based on these parameters only.
Keywords: ALT, AST, Chronic HCV, fibrosis, HCV-RNA, necroinflammation

Bununla birlikte, ROC analizindeki sonuçlar sadece ALT, AST ve HAİ için istatistiksel olarak anlamlı sonuçlara sahipti ($p<0,05$).
Sonuç: Yaş, AST, ALT ve HAİ'nin karaciğerdeki fibrozu öngörmede AST/ALT oranı ve HCV-RNA'dan daha parametreler olduğu düşünülmektedir. Ancak, bulgularımız kronik HCV'li hastaları sadece bu parametrelere dayanarak takibi önermek için yeterli değildir.
Anahtar Kelimeler: ALT, AST, Kronik HCV, Fibroz, HCV-RNA, nekroinflamasyon

Introduction

Hepatitis C virus (HCV) infection remains an important cause of liver cirrhosis and hepatocellular carcinoma (HCC) despite improvement in treatment modalities. It is estimated that there are 185 million people infected with HCV worldwide, 71 million of whom are chronically infected, while about 3-4 million people are considered to be infected annually (1,2). The prevalence of HCV infection differs between 2% and 3.8% in Asian countries, and it is the highest with 15% in Egypt (1). With an approximately 1% infection rate, Turkey is a low endemic country for HCV, and genotype-1b is reported to be predominant with a 91.1% rate (3). Iran, Israel and Cyprus are other genotype-1 prevalent countries in the Middle-East, besides Turkey (4).

In our hospital, we have one of the largest liver histopathology results among young patients with chronic hepatitis C (CHC) infection from genotype-1b prevalent countries in literature. Most of the patients underwent liver biopsy because of military regulations in Turkey. They were mostly newly diagnosed naive, young patients and all had no coinfection or other comorbidities.

Determination of the severity of liver disease is one of the most important stages in the management of patients. However, both patients and clinicians are reluctant to perform liver biopsy due to the invasiveness of the procedure. The aim of present study is to reveal the importance of liver transaminases and HCV-RNA levels to predict the severity of liver diseases.

Materials and Methods

This retrospective study was conducted in a tertiary training and research hospital. The data were gathered and analyzed retrospectively through the patient data management system, liver biopsy and treatment reports. The Clinical Research Ethics Committee of Haydarpaşa Numune Training and Research Hospital on May 22th, 2017 (approval number: HNEAH-KAEK 2017/KK/71) and informed consent was obtained from the patients.

Population

A total of 95 young/middle-aged and male patients, who were all anti-HCV and HCV-RNA positive with a duration of at least six months, enrolled in the study. All of the patients were followed up between January 2008 and January 2017.

Since we work in a military hospital, the vast majority of our cases were new HCV patients diagnosed during the screening process before military service, while the rest were ordinary patients who were candidates for hepatitis C treatment. Turkey has a mandatory military service, and CHC patients with findings of CH in the liver histopathology have the right to be exempted from military service. Furthermore, liver biopsy and histopathologic results were still mandatory until January 2019, for prescribing HCV treatments according to reimbursement regulations of Turkish Ministry of Health. Moreover, treatment options differ according to HCV-genotype and the level of histopathological findings in the liver. Hence, liver biopsies are performed according to health regulations of the Turkish Armed Forces and of the Turkish Ministry of Health, with the consent of patients with HCV.

Follow-up

The study data was gathered retrospectively, however the routine follow-up procedure for patients with HCV is as described below. At the first visit, we checked for patients with a history of HCV infection, who were referred to our hospital from the recruitment offices or other hospitals, for anti-HCV, HCV-RNA, hepatitis B surface antigen, anti-Human Immunodeficiency Virus (HIV), complete blood count, liver transaminases, albumin, total protein, lipid profile, prothrombin time, alpha-fetoprotein, liver ultrasonography and some other additional tests if necessary. If the patient had anti-HCV and HCV-RNA positivity, we followed up the patient for a period of at least six months. At the end of the follow-up period, we performed the same tests and performed liver biopsies on those who still demonstrated positive HCV-RNA results.

Exclusion Criteria

Female patients, children and patients over 45 years old, patients who had a history of HCV treatment with interferon-based or directly acting antiviral therapies, who had symptoms of cirrhosis or who were co-infected with other hepatitis viruses or HIV, or who had other chronic liver diseases or history of drug use, were excluded from the study.

Liver Biopsy and Histopathology

Patients underwent liver biopsy using 16G biopsy needles by the Menghini's aspiration method or subcostal real-time ultrasound-guided liver biopsy by Trucut-style. An adequate biopsy was required to be a minimum of 1.5 cm-long. Histopathologic assessment of liver biopsies was performed according to the Ishak's (5) scoring system for histological grading and staging for CH. Parameters in the study are evaluated in terms of the prediction of fibrosis scores higher than 2 ($F \geq 2$) and 3 ($F \geq 3$).

Laboratory Tests

HCV-RNA viral load quantifications were performed by the Roche® COBAS® AmpliPrep/COBAS® Taqman® HCV Quantitative

Test v2.0 with the lowest detection limit of 15 IU/mL, and the limit of quantification between 15 and 1x108 IU/mL. Anti-HCV tests were performed using chemiluminescence microparticle immunoassay (Abbot®, Architect System; Germany). Serum biochemistry tests for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using biochemical instruments with the upper limit of the normal (ULN) ALT level as 40 U/L.

Statistical Analysis

Statistical analyses were performed using e-picos calculator (www.e-picos.com). Baseline characteristics were presented as means and standard deviations for continuous variables, and as frequencies and percentages for categorical variables. Comparisons of continuous variables were performed by the independent-samples Student's t-test and Mann-Whitney U test according to those distributions. Categorical variables were compared using the chi-squared test and Fisher's exact test. The receiver operation characteristic (ROC) analysis was used to determine optimal cut-off levels of serum ALT, AST, AST/ALT, Log10 HCV-RNA and histological activity indexes (HAI) scores for the study population. Diagnostic performance was analyzed by Medcalc version 18.9 (free trial application). G*Power 3.1.9.2 was used for performing the post-hoc power analysis. The study had a 90 percent power to detect a minimum of 20 percent difference for significant liver histology among patients categorized according to their serum HCV-RNA, ALT and AST levels.

Results

A total of 95 naive, male and young/middle aged patients with chronic HCV infection were included in the study. HCV genotype was confirmed in 36 (37.9%) of the patients; and 28 (93.3%) of them were genotype-1b. Other genotypes were genotype 1a (n=2, 2.1%), genotype 2 (n=2, 2.1%), genotype 3 (n=3, 3.15%) and genotype 4 (n=1, 1.05%). Age of the patients varied between 20 and 45, 75.8% of whom were younger than 35 years-old, while the mean age was 27.9±8.25. The mean ALT, AST, AST/ALT ratio, Log10 HCV-RNA level, histological activity index (HAI) and fibrosis scores of all patients were 83.79±61 U/L, 48.01±25.62 U/L, 0.67±0.26, 6.09±1.11 IU/mL, 5.59±1.82 and 1.89±0.88, respectively. The other main characteristics of the patients are revealed in Table 1. The mean age, ALT, AST, AST/ALT ratio, Log10 HCV-RNA and HAI scores according to fibrosis scores are also presented in Table 2. There were 21 patients with ALT levels ≤40 U/L, and these were divided into two groups as patients with ALT levels ≤30 U/L and patients with ALT levels between 31-40 U/L. Mean age, HAI and fibrosis scores were 23.66±6.77, 4.27±1.85 and 1.5±0.80 in patients with ALT levels ≤30 U/L, respectively. In patients with ALT levels between 31-40 U/L, mean age, HAI and fibrosis scores were also found to be 30.77±9.61, 5.55±1.94 and 1.44±0.88, respectively.

HAI of all patients varied between 2 and 10, while the HAI scores of 4, 5, 6 were found to be the most frequent stages of

Table 1. Baseline characteristics of patients in the study

Total (n=95)	Mean	Median	Minimum	Maximum	Standard deviation
Age	27.9	23	20	45	±8.25
ALT	83.79	72	13	333	±61
AST	48.01	40	13	137	±25.62
AST/ALT	0.67	0.61	0.29	1.61	±0.26
Log10 HCV-RNA	6.09	6.25	2.76	8.6	±1.11
HAI	5.59	5	2	10	±1.82
Fibrosis	1.89	2	0	5	±0.88

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HCV: Hepatitis C virus, HAI: Histological activity index

Table 2. Mean values of variables according to stages of fibrosis

	F0, (n=3)	F1, (n=28)	F2, (n=44)	F3, (n=17)	F4, (n=2)	F5, (n=1)	F6, (n=0)
Age (mean ± SD)	27.33	28.14	25.80	31.53	40.5	29	0
	±10.97	±7.98	±7.06	±9.87	±2.12	-	-
ALT (mean ± SD)	43.66	60.75	84.93	117.76	119	151	0
	±27.43	±31.17	±53.34	±94.60	±100.4	-	-
AST (mean ± SD)	29.33	37.75	48.07	60	86	109	0
	±10.97	±14.91	±22.20	±31.66	±72.12	-	-
AST/ALT (mean ± SD)	0.75	0.69	0.67	0.64	0.725	0.72	0
	±0.26	±0.24	±0.27	±0.26	±0.007	-	-
Log10 HCV-RNA (mean ± SD)	5.02	6.22	5.94	6.24	7.31	7.32	0
	±1.20	±1.22	±1.05	±1.13	±0.57	-	-
HAI-SD	2.33	4.25	5.98	6,76	9	9	0
	±0.58	±1.14	±1.52	±1.35	±1.41	-	-

SD: Standard deviation, F: Fibrosis, HAI: Histological activity index, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HCV: Hepatitis C virus

fibrosis (n=17, 18%; n=22, 23%; n=20, 21%, respectively). Fibrosis scores of patients which varied between 0 and 5; F1 (n=28, 30%), F2 (n=44, 46%) and F3 (n=17, 18%) were reported as the most frequent stages of fibrosis. The distribution of patients according to HAI and fibrosis scores is presented in Figure 1A and 1B.

Risk estimation analysis demonstrated that, ALT >40 IU/mL [odds ratio (OR): 2.97; confidence interval (CI) %95: 1.09-8.06; p=0.037] and AST >40 IU/mL (OR: 2.88; CI %95: 1.17-7.09; p=0.020) were the significant risk factors for F ≥2, while age >35 was the only significant risk factor (OR: 3.56; CI %95: 1.24-10.24; p=0.021) for F ≥3. For other parameters, the ORs were not found to be significant (Table 3).

Cut-off values with the optimum sensitivity and specificity for the prediction of F ≥2 and F ≥3 were determined based on the ROC curve analysis. ROC curves and the diagnostic accuracy results are shown on Figure 2, and 3, and in Table 4,5. The area under the Receiver-Operator-Characteristic curves (AUCs) for ALT, AST, AST/ALT ratio, HCV-RNA and HAI score for the prediction of F ≥2 were 0.65, 0.68, 0.56, 0.50 and 0.86, respectively (Table 4). For F ≥3, AUCs for ALT, AST, AST/ALT ratio, HCV-RNA and HAI score were reported as 0.65, 0.68, 0.53, 0.62 and 0.80, respectively (Table 5).

The optimum values in the ROC analysis for ALT, AST and HAI were found to be significant (p<0.05), while results of Log10 HCV-RNA and AST/ALT ratio were not (p>0.05). The optimum ALT level to predict F ≥2 was 104 U/L, while it was 94 U/L for F ≥3. The optimum AST levels for F ≥2 and F ≥3 were 39 and 55 U/L, respectively. The optimum HAI score to predict F ≥2 was reported as 5, while it was 6 for F ≥3 fibrosis (Table 4,5).

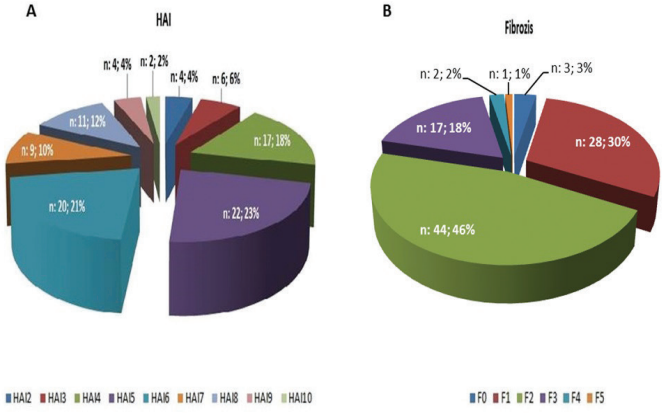

Figure 1. Distribution of the patients in the study according to fibrosis stages and histologic activity index scores. Graphs according to A) Fibrosis stages, B) necroinflammatory grade (histologic activity index score)

Discussion

Severity of hepatic fibrosis is the main indicator of end stage liver diseases (ESLD), HCC and liver related death. Bruden DJT revealed that 1.7% of HCV patients with mild fibrosis developed ESLD during a five years follow-up period. On the other hand, the rate of developing ESLD was 7.9%, 16.4% and 49% among the patients with moderate, severe fibrosis and cirrhosis, respectively (6). He also concluded that treatment in patients with mild fibrosis could be deferred for up to five years, and that early treatment is crucial for patients with other than mild fibrosis. Despite recent efforts to develop alternative non-invasive imaging methods, indirect scoring systems or biomarkers, liver biopsy is still considered as the gold standard method to assess necro-inflammation and fibrosis of liver and also to exclude other concomitant liver diseases (7-9). Additionally, a histopathological result of a liver biopsy was required until January 2019 according to the reimbursement arrangements

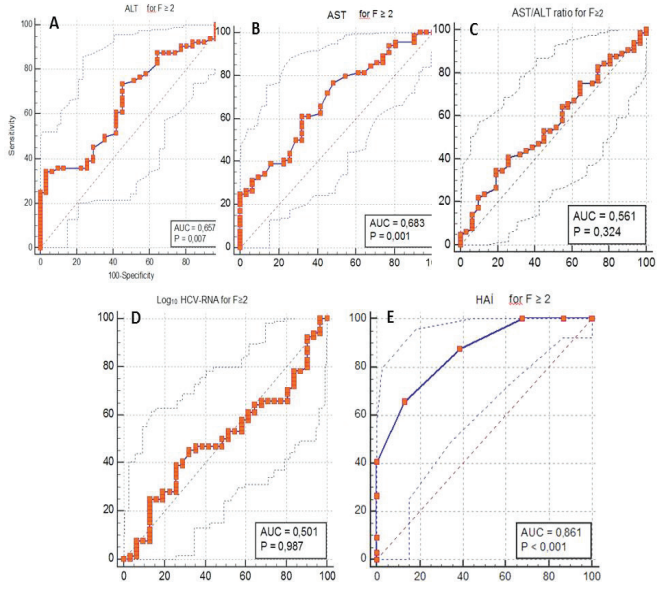

Figure 2. Area under the receiver operating characteristic curves for predicting ≥2 fibrosis stage (F≥2). A) Area under the receiver operating characteristic curves (AUC) for alanine aminotransferase (ALT) B) AUC for aspartate aminotransferase (AST). C) AUC for AST/ALT ratio. D) AUC for Log10 hepatitis C virus-RNA E) AUC for histologic activity index score

Table 3. Risk estimation for F ≥2 and F ≥3 fibrosis

Cut-off levels	F ≥2		F ≥3	
	OR (CI %95)	p	OR (CI %95)	p
Age for >35	1.14 (0.41-3.14)	0.796	3.56 (1.24-10.24)	0.021
ALT for >30 (U/L)*	2.32 (0.68-7.89)	0.197	3.26 (0.39-26.94)	0.45
ALT for >40 (U/L)	2.97 (1.09-8.06)	0.037	3.65 (0.64-14.39)	0.225
AST for >40 (U/L)	2.88 (1.17-7.09	0.020	2.24 (0.80-6.24)	0.118
HCV-RNA for 5 Log10 (IU/mL)	0.71 (0.20-2.45)	0.767	1.88 (0.39-9.15)	0.730

F: Fibrosis; ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, OR: Odds ratio, CI: Confidence interval, HCV: Hepatitis C virus
*Prati criteria; 30 U/L for male, 19 U/L for female for ULN (upper limit of normal) of ALT

Table 4. Diagnostic accuracy estimates (95% CI) of alanine aminotransferase, aspartate aminotransferase, aspartate aminotransferase/alanine aminotransferase ratio, Hepatitis C virus-RNA and histological activity index in theprediction of F ≥2 fibrosis

	ALT (U/L)	AST (U/L)	AST/ALT	Log10 HCV-RNA	HAI
Cut-off value	104	39	0.5	6.78	5
AUC	0.65	0.68	0.56	0.50	0.86
95% CI	0.55-0.75	0.58-0.77	0.45-0.66	0.38-0.60	0.77-0.92
p	0.007	0.001	0.324	0.987	0.001
Sensitivity,	34.38	60.94	34.38	65.62	65.62
95% CI	22.9-47.3	47.9-72.9	22.9-47.3	52.7-77.1	52.7-77.1
Specificity,	96.77	67.74	80.85	19.35	87.10
95% CI	83.3-99.9	48.6-83.3	62.5-92.5	7.5-37.5	70.2-96.4
LR+	10.66	1.89	1.78	0.81	5.09
95% CI	1.5-75.5	1.1-3.3	0.8-3.9	0.6-1.0	2.0-12.9
LR-	0.68	0.58	0.81	1.78	0.39
95% CI	0.6-0.8	0.4-0.9	0.6-1.0	0.8-3.9	0.3-0.6
PPV,	95.7	79.6	78.6	65.62	91.3
95% CI	75.6-99.4	69.3-87.1	62.4-89.0	52.7-77.1	80.5-96.4
NPV	41.7	45.7	37.3	19.35	55.1
95% CI	37.2-46.3	36.2-55.4	31.7-43.3	7.5-37.5	46.0-63.9

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HCV: Hepatitis C virus, HAI: Histological activity index, AUC: Area Under the Receiver-Operator-Characteristic curve, CI: Confidence interval, LR+: Positive diagnostic likelihood ratio, LR-: Negative diagnostic likelihood ratio, PPV: Positive predictive value, NPV: Negative predictive value,

Table 5. Diagnostic accuracy estimates (95% CI) of alanine aminotransferase, aspartate aminotransferase, alanine aminotransferase/aspartate aminotransferase ratio, Hepatitis C virus-RNA and Histological activity index in the prediction of F ≥3 fibrosis

	ALT (U/L)	AST (U/L)	AST/ALT	Log10 HCV-RNA	HAI
Cut-off value,	94	55	0.78	6.88	6
AUC	0.65	0.68	0.53	0.62	0.80
p	0.037	0.010	0.703	0.133	0.001
Sensitivity	50	50	90	45	65
95% CI	27.2 - 72.8	27.2 - 72.8	68.3 - 98.8	23.1 - 68.5	40.8 - 84.6
Specificity	78.67	81.33	26.67	82.67	82.67
95% CI	67.7 - 87.3	70.7 - 89.4	17.1 - 38.1	72.2 - 90.4	72.2 - 90.4
LR+	2.34	2.68	1.23	2.60	3.75
95% CI	1.3 - 4.3	1.4 - 5.1	1.0 - 1.5	1.3 - 5.2	2.1 - 6.8
LR-	0.64	0.61	0.38	0.67	0.42
95% CI	0.4 - 1.0	0.4 - 1.0	0.10 - 1.5	0.4 - 1.0	0.2 - 0.8
PPV	38.5	41.7	24.7	40.9	50
95% CI	25.2 - 53.7	27.3 - 57.6	21.1 - 28.6	25.7 - 58.0	35.7 - 64.3
NPV	85.5	85.9	90.9	84.9	89.9
95% CI	78.9 - 90.3	79.5 - 90.5	71.8 - 97.5	78.9 - 89.5	82.9 - 94.2

ALT, alanine aminotransferase, AST, aspartate aminotransferase, HCV: Hepatitis C virus, HAI: Histological activity index, AUC: Area Under the Receiver-Operator-Characteristic curve, CI: Confidence interval, LR+: Positive Diagnostic Likelihood Ratio, LR-: Negative Diagnostic Likelihood Ratio, PPV: Positive Predictive Value, NPV: Negative Predictive Value

of HCV treatments in Turkey. Efforts on finding indirect indicators and the development of non-invasive scoring systems for the severity of liver diseases are increasingly pursued, and these remain one of the main issues in hepatology. Our goal was to reveal whether or not primary virological and biochemical markers could estimate the stage of fibrosis in the liver; at least, it was to reveal the importance of virological and biochemical markers on determining who needs liver biopsy.

In literature, the male sex and older age is concluded as risk factors for the progression of fibrosis. It is slower in younger ages, and the cumulative probability of cirrhosis is higher in patients older than 45 years (10). Furthermore, our study is based on a naive, male and relatively young population younger than 45 years-old; these baseline characteristics of the study may promote the importance

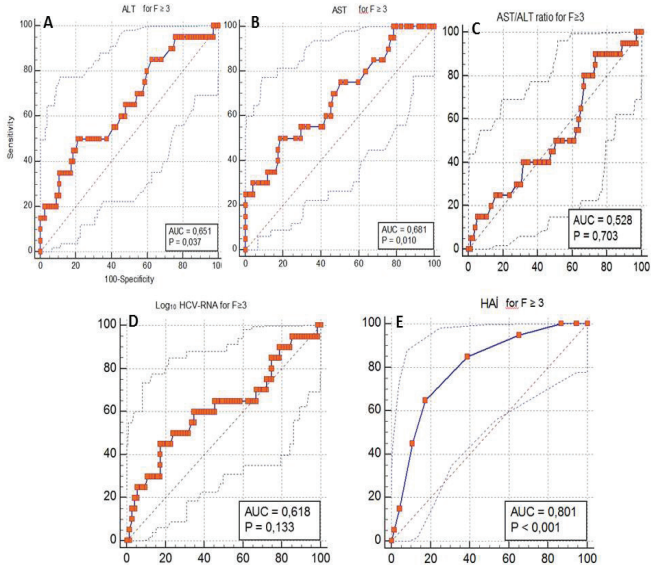

Figure 3. Area under the receiver operating characteristic curves (AUC) for predicting ≥3 fibrosis stage (F≥3). A) Area under the receiver operating characteristic curves (AUC) for alanine aminotransferase (ALT) B) AUC for aspartate aminotransferase (AST). C) AUC for AST/ALT ratio. D) AUC for Log-10 Hepatitis C Virus-RNA E) AUC for histologic activity index score

of the results. Because male sex is considered as population with higher risk, and our study population consisted of one of the youngest population in literature. On the other hand, these characteristics of our study could be considered as limitations of the study, and more attention should be paid to adapting study results to the general population.

Age is one of the predictive factors for the severity of hepatic diseases. We revealed that 35 years could be a suitable cut-off age to determine F ≥3 fibrosis, although our study did not include large number of patients with older age; the mean age raised over 30 years-old in cases with F ≥3. Furthermore, the risk of F ≥3 fibrosis significantly increased over 35 years-old; it was found to be 3.56 times higher in this group. Sanai et al. (11) revealed that age correlated with both fibrosis and necro-inflammation and that age was the only predictor for the severity of necro-inflammation and fibrosis in CHC patients with normal ALT levels. However, age showed no correlation with both of fibrosis and necro-inflammation in patients with elevated ALT levels according to results in the study.

Transaminases are the main predictive factors of liver inflammation and fibrosis in literature. It is reported that higher ALT is associated with faster hepatic disease progression (12). ALT and AST levels in our study increased in parallel with increase in fibrosis scores. F ≥3 fibrosis is likely to occur over three times more in patients with elevated ALT levels in risk estimation analysis. Both ALT and AST showed moderate diagnostic accuracy with AUC values 0.65 and 0.68, respectively. The specificity rates were also found to be considerably higher than the sensitivity rates for both F ≥2 and F ≥3 fibrosis. Similarly to our results, Shahid et al. (13) showed that AST and ALT significantly correlated with the stages of fibrosis. However, there are some studies in literature which demonstrates that AST was better than ALT as a marker of the progression of liver damage, or that both transaminases showed no correlation with fibrosis (11,14,15,16,17,18).

In recent years, there has been an increasing trend to lower the ULN to 30 U/L for ALT for males, which was first revealed by Prati et al. (19,20,21,22). Also in literature, ALT between 25 and 40 U/L is considered as high normal level in which histopathological changes is thought to be more likely to occur in Hepatitis B (23). In our study, although age and HAI scores were higher in patients with ALT between 31 and 40 U/L, mean fibrosis scores in this patients were not higher than in patients with ALT levels ≤30 U/L. Our results did not support the recent literature knowledge; however, the number of patients in our study may be insufficient to comment on this issue.

AST/ALT ratio is one of the important indicators of the stages of fibrosis in literature. In our study, there was no increase in AST/ALT ratio according to the stages of fibrosis, and it had poor diagnostic performance for both F ≥2 and F ≥3 fibrosis. The study results of El-Sayed R et al. (24) were almost the same as ours; they revealed that both AST and the AST/ALT ratio showed a weak correlation with significant fibrosis, and the AUC predicting significant fibrosis was 0.76, which was non-significant. Likewise, Mir IA concluded that the AST/ALT ratio is a non-sensitive marker for liver fibrosis (25). Considering ≥1 ratio, AST/ALT ratio may be useful to determine cirrhosis in HCV (26,27). Of our patients, only 11 had a ratio of ≥1 AST/ALT, and only two of them had F ≥3 fibrosis. We concluded that the AST/ALT ratio has no value in differentiating the severity of liver disease in patients without cirrhosis.

As another indicator of liver fibrosis in literature, HCV-RNA levels did not differ in five different stages of fibrosis. HCV-RNA levels also showed no significance on risk estimation for both F ≥2 and F ≥3 fibrosis considering a HCV-RNA cut-off level of 5 Log10 IU/mL. The diagnostic accuracy of HCV-RNA level was also found to be poor when compared to our results. Although it was correlated with HAI score in the study of Zechini B et al. (18), HCV-RNA was not correlated with fibrosis score. Also similarly to our study results, Gupta et al. (15) revealed that HCV-RNA showed no correlation with both HAI and fibrosis. In summary, we believe that HCV-RNA should not be considered as a good indicator of liver fibrosis.

HAI scores showed an increase parallel with the increase in fibrosis scores. The HAI score of 5 for F ≥2 and HAI score of 6 for F ≥3 fibrosis were also found to be statistically significant cut-off values in the ROC analysis. Although HAI may be considered as a good predictive factor of hepatic fibrosis in hepatitis C infection, it has no value in clinical use. Since, HAI, like fibrosis, requires an invasive procedure and both of them are the results of liver biopsy.

Diagnostic accuracy results of the parameters, except for HAI, in the prediction of F ≥3 fibrosis in our study were poorer than results obtained from the largest study in literature about the diagnostic accuracy of biomarkers (6). In this study, the authors concluded that liver biopsy could still be performed to diagnose stages of fibrosis in patients without cirrhosis. In another study with one of the largest series, AUC for FIB-4 in discriminating F3-4 from F0-2 was 0.83 for HCV (28). In a review study, combination of biomarkers and transient elastography

was concluded to be the most effective strategy to reveal significant fibrosis (29).

Study Limitations

The main limitation of the study is that the study was performed over male and considerable young population which could be considered as a selected population. Second, the results could be more valuable if we could have implemented non-invasive approaches, including serum bio-markers, scoring systems, and imaging techniques, for the assessment of liver fibrosis besides liver biopsy results. So, the results cannot be generalized to all patients, and there is a need for further prospective studies in generalized populations.

Conclusion

In summary, in our study group including young patients, all parameters except HAI showed a poorer diagnostic accuracy than biomarkers in literature like APRI, Fibrotest® or FIB-4 scores. Although age, AST, ALT and HAI revealed relatively better results than AST/ALT ratio and HCV-RNA for predicting F ≥3 fibrosis, our results are not good enough to be relied on in the follow up process of CHC. Including only young and male cases in the study could be considered as the limitation of the study, and more attention should be paid to adapting study results to the general population. Further randomized and controlled studies are needed in general population.

Ethics

Ethics Committee Approval: The study was approved by the Institutional Ethics Committee of on May 22th, 2017 (approval number: HNEAH-KAEK 2017/KK/71)

Informed Consent: Informed consent was obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Y., R.A.Ç., Concept: E.Y., Design: E.Y., R.A.Ç., Data Collection or Processing: E.Y., R.A.Ç., Analysis or Interpretation: E.Y., R.A.Ç., Literature Search: E.Y., R.A.Ç., Writing: E.Y.

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Research Article

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Hepatitis and HIV Seropositivity among Healthcare Workers at Elazığ Oral and Dental Healthcare Center

Elazığ Ağız Diş Sağlığı Merkezi Çalışanlarında Hepatit ve HİV Seropozitifliği

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ABSTRACT

Objectives: In this study, we aimed to explore the prevalence of hepatitis B virus (HBV), HCV and Human immunodeficiency virus (HIV) infection, and anti-HBs and anti-HAV-IgG seropositivity among dentists, supporting healthcare staff and other staff working at Elazığ Oral and Dental Healthcare Center (ODHC).

Materials and Methods: Hepatitis B surface antigen (HBsAg), anti-HBs, anti-HCV, anti-HAV immunoglobulin G (IgG) and anti-HIV seropositivity status of all ODHC employees between January 1, 2016 and December 31, 2016 were analyzed retrospectively. Demographic data of all employees were recorded.

Results: Of 162 ODHC employees, 99 (61.91%) were male and 63 (38.09%) were female, and the mean age was 35.86±8.77 years. Of the employees, 52 were dentists, 36 were nurses, 21 were dental prosthesis technicians and 53 from other various positions. None of the individuals were HBsAg, anti-HCV or anti-HIV positive. All individuals were anti-HBs positive and 96 (80.67%) were anti-HAV IgG positive. The lowest anti-HAV IgG positivity was found in dentists.

Conclusion: In our study, HBsAg positivity was found lower compared to general hospital rates, but comparable to those reported from ODHCs. This may be because our hospital is a local institution where infection prevention measures and training and vaccination activities are actively implemented. With this report, we intended to point out that zero infection and full vaccination is possible by improving training and enhancing awareness.

Keywords: Dental Healthcare Center, hepatitis, HIV, seropositivity

ÖZ

Amaç: Bu çalışma ile Ağız ve Diş Sağlığı Merkezinde (ADSM) çalışan diş hekimleri, yardımcı sağlık personeli ve diğer personelde hepatit B virüs (HBV), HCV, İnsan Bağışıklık Yetmezliği Virüsü (HIV) enfeksiyon sıklığını ve anti-HBs, anti-HAV- immünoglobulin (IgG) seropozitifliğini araştırmayı amaçladık.

Gereç ve Yöntemler: ADSM'de tüm çalışanların hepatit B yüzey antijeni (HBsAg), anti-HBs, anti-HCV, anti-HAV IgG ve anti-HIV seropozitiflik durumları 1 Ocak 2016-31 Aralık 2016 tarihleri arasındaki veriler retrospektif olarak incelendi. ADSM çalışanların demografik verileri kaydedildi.

Bulgular: Çalışmaya alınan 162 ADSM çalışanın 99'u (%61,91) erkek, 63'ü (%38,09) kadın ve yaş ortalaması 35,86±8,77 yıl idi. Bireylerin 52'si diş hekimi, 36'sı hemşire, 21'i diş protez teknisyeni ve 53'ü diğer çalışanlardı. Hiçbir bireyde HBsAg, anti-HCV ve anti-HIV pozitifliği saptanmadı. Çalışan 162 bireyin tamamında anti-HBs pozitifti ve 96'sında (%80,67) anti-HAV IgG pozitifliği saptandı. En düşük anti-HAV IgG pozitifliği diş hekimlerinde saptandı.

Sonuç: Çalışmamızda HBsAg pozitifliği genel hastane taramalarına oranla düşük fakat ağız diş sağlığı merkezlerinden verilen oranlarla benzerdir. Bunun nedeninin lokal bir hastane olması, enfeksiyon kontrol önlemlerinin özenli, eğitim ve aşılama faaliyetlerinin etkin yapılması olabilir. Bu çalışma ile eğitim ve farkındalığın artırılarak sıfır enfeksiyon ve tam aşılamanın mümkün olabileceğini sunmak istedik.

Anahtar Kelimeler: Diş Sağlığı Merkezi, hepatit, HIV, seropozitiflik

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Introduction

Chronic Hepatitis B Virus (HBV) infection is reported to affect 257 million in the worldwide and Approximately 71 million people worldwide are chronically infected with Hepatitis C Virus (HCV) (1,2). HBV and HCV transmission occurs through parenteral contact, sexual contact, horizontal, nosocomial or perinatal transmission (2,3). Prevalence of HBV and HCV among the general population in Turkey are reported to be 4% and 1%, respectively, with variances across the regions (4).

Tens of millions of people are estimated to be infected with Hepatitis A virus (HAV) across the world each year. Hepatitis A prevalence is closely associated with socio-economic development levels, led by indicators such as geographical differences, hygiene and other healthcare conditions (5). Turkey ranks among the countries with intermediate-endemicity (prevalence 8-88%) (6).

Healthcare workers, in their professional routine, often come into contact with infected patient material such as blood and body fluids. This causes them to be more commonly exposed to blood-borne disease factors. HBV alone can also be transmitted by saliva. Depending on the serum level, HBV is identified 1.000 to 10.000 times less in saliva, however, high rates are also seen in saliva in line with higher serum levels (7). According to the World Health Organization (WHO) data more than 85 million healthcare workers across the globe are injured by contaminated medical instruments (8). Hepatitis B vaccine is an effective method to protect from HBV. There is, however, no vaccine available for HCV, therefore, standard precautions come forth as the most effective ways to avoid the risk of transmission among patients and between patients and healthcare workers (9).

In this study we aimed to explore the prevalence of HBV, HCV, Human immunodeficiency virus (HIV) infection and anti- hepatitis B surface (HBs), anti-HAV-immunoglobulin G (IgG) seropositivity among the employees, including the dentists, the supporting healthcare staff and other positions working in Centers for Oral and Dental Healthcare Center (ODHC) outside of general hospitals. We expect that the results of this study will both contribute to the epidemiological data in Turkey and help to determine the efficacy of infection control measures implemented in the involved healthcare institutions.

Materials and Methods

All workers at the state ODHCs active in Elazığ were included in the study, and the data of 162 ODHC workers that were recorded from 1 January to 31 December 2016 for hepatitis B surface antigen (HBsAg), anti-HBs, anti-HCV, anti-HAV-IgG and anti-HIV seropositivity were retrospectively examined. Demographic data were recorded and individuals with recurrent condition were excluded. Approval for the study was taken from Fırat University, Ethics Committee (approval number: 05/04, date: 2019).

Statistical Analysis

The data of the ODHC workers included in the study were reviewed by age, gender, position (dentist, nurse, dental prosthesis technician, clerk, security guard, IT administrator or manager).

Serologic values for HBsAg, anti-HBs, anti-HAV-IgG, anti-HCV, anti-HIV were tested on Architect i2000 SR (Abbott, USA) device with the Chemiluminescent Microparticle Immunoassay method.

Table 1. Distribution of hepatitis B surface antigen, anti-hepatitis B surface, anti-Hepatitis A virus-immunoglobulin G, anti-Hepatitis C Virus and human immunodeficiency virus seropositivity in the Oral and Dental Healthcare Center workers

	Dentist, n (%)	Nurses, n (%)	Prosthesis technicians, n (%)	Others, n (%)	Total, n (%)
Age	33.65±9.27	37.61±8.90	32.33±8.71	38.24±7.30	35.86±8.71
Gender					
Male	29 (55.8)	14 (38.8)	18 (85.8)	38 (71.7)	99 (61.91)
Female	23 (44.2)	22 (61.2)	3 (14.2)	15 (28.3)	63 (38.09)
HBsAg					
Positive	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Negative	52 (100)	36 (100)	21 (100)	53 (100)	162 (100)
Anti-HBs					
Positive	52 (100%)	36 (100%)	21 (100)	53 (100)	162 (100)
Negative	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Anti-HCV					
Positive	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Negative	52(100)	36 (100)	21 (100)	53 (100)	162 (100)
Anti-HIV					
Positive	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Negative	52 (100)	36(100)	21 (100)	53 (100)	162 (100)
Anti-HAV-IGg					
Positive	23 (56.09)	25 (96.15)	9 (81.8)	39 (95.12)	96 (80.67)
Negative	18 (43.91)	1 (3.85)	2 (18.2)	2 (4.88)	23 (19.33)

HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C virüs, HIV: Human immunodeficiency virus, HAV-IGg: Hepatitis A virus-immunoglobulin G

The data were analyzed using the SPSS 22.0 software package. The Pearson's chi-square test was used for intergroup differences and significance limit was set at $p<0.05$.

Results

The study included 162 ODHC workers with a mean age of 35.86±8.77 years of whom 99 (61.91%) were male and 63 (38.09%) were female. Of the 162 workers 52 were dentists, 36 were nurses, 21 were dental prosthesis technicians, and 53 of other positions such as clerk, security guard, IT administrator and manager (Table 1).

The available data included HBsAg, anti-HBsAg, anti-HCV and anti-HIV test results for all 162 workers that were included in the study. None of the individuals were HBsAg, anti-HCV or anti-HIV positive. All 162 workers were anti-HBs positive.

Out of the 162 ODHC workers (43 ODHC workers had no anti-HAV-IgG testing), 119 were tested for anti-HAV-IgG and only 96 (80.67%) were found anti-HAV-IgG positive. Review by profession showed that 25 (96.15%) of the nurses, 39 (95.12%) of the individuals from various other positions, 9 (81.8%) of the dental prosthesis technicians, and 23 (56.09%) of the dentists were anti-HAV-IgG positive. The lowest anti-HAV-IgG positivity rate was found among the dentists, with statistically significant difference between this group and the nurses, dental prosthesis technicians and other positions groups ($p<0.001$). Furthermore, no statistically significant differences were found in terms of anti-HAV-IgG positivity based on age and gender among the ODHC profession groups.

Discussion

In 1992, WHO and the International Labor Organization acknowledged HBV an occupational disease factor (10). In 1996 the Turkish Ministry of Health initiated the program that aims to screen healthcare workers for this virus and vaccinate if seronegative (11). HBV transmission in healthcare workers often occurs through contact with blood. Healthcare workers other than doctors are in direct contact with patients, hence in indirect contact with infected blood and blood products (12).

Even though lumen needles are often held responsible for HCV transmission, blood splash into the conjunctiva and needles without lumen can also cause transmission. Despite these risks, however, the prevalence of HCV infection is not higher among healthcare workers than is in the general population. Out of all needle injuries experienced by healthcare workers, only 1-2% are reported to be associated with needles from HCV infected patients (13).

As is the case across the world, in Turkey, too, improved sanitation and hygiene, and socio-economic development lead to a decline in the number of HAV cases in children, as well as an increase in the number of mindful adults (14). The disease has a severe clinical course in the later years compared to childhood years. In Turkey, risk groups are screened for HAV, and seronegative persons are vaccinated. Furthermore, at the end of 2012 the Turkish Ministry of Health has included Hepatitis A vaccination among routine childhood vaccines (15).

HBsAg and anti-HBs positive rates among healthcare workers in Turkey were reported, each respectively, to be 1.28% and 88.3% by Uludağ Altun et al. (16), 0.9% and 86% by Korkmaz et al. (17), and 0.5% and 88.28% by Keçik Boşnak et al. (11).

In their 1993 survey which explored the approach of dentists to Hepatitis B vaccine, Külekçi and Kartoğlu (18) found a vaccination rate of 10% and the most common reason for non-vaccination to be negligence and indifference. Contrarily, another survey conducted among dentists in the years from 2004 to 2008 reports a vaccination rate of 90%. This favorable change both reflects the increased knowledge and awareness related to protection from Hepatitis B among dentists and indicates the efficacy of the serologic screening and vaccination program put into effect in 1996 in Turkey. In the same survey, dentists ranked HIV as the most feared disease. Interestingly, 31% of those who have been immunized with vaccination indicated Hepatitis B as their most feared disease (19). A 2006 survey conducted with 108 dentists in Italy found HBV transmission to be the most feared outcome (57%) even after they were immunized with vaccination (20).

In a 2017 study that screened dental students, all (100%) were found HBsAg-negative and 93.5% were found anti-HBs positive (21). A study conducted among the workers of the Kırıkkale ODHC in 2012 reports an HBsAg rate of 0.85% and an anti-HBs rate of 89.83% (22). In our study, none of the ODHC workers were found HBsAg-positive, whereas all were anti-BHs-positive. This result may be an outcome of the high vaccination rates seen in the recent years among dentists and ODHC workers.

Although WHO has indicated dentists to be at high risk for HCV transmission, studies show that the prevalence of HCV infection in this group is comparable to (1.2%) (23) or lower than (0.0%) (24) the general population. Anti-HCV positivity among the healthcare workers in Turkey is reported between 0% and 0.34% (11,16,17). Anti-HCV positivity and anti-HIV positivity among Kırıkkale ODHC workers were found to be 1.69% and 0%, respectively. The authors (22), however, report that the positivity value was very close to the threshold value and this could turn out to be false positive in repeated tests. Similar to the results reported from Turkey, in our study, anti-HCV and anti-HIV positivity were not identified in ODHC workers.

WHO recommends implementing Hepatitis A vaccination programs as sanitary conditions improve in intermediate-endemicity regions where the disease has a severe course and the number of mindful adults increase (14). Korkmaz et al. (17) identified an anti-HAV-IgG positivity rate of 71.7% among healthcare workers. In our study, anti-HAV-IgG positivity rate was 80.7% and immunization with vaccine were recommended to healthcare workers who were seronegative for HAV. We believe that the statistically significant low rate of anti-HAV-IgG positivity among dentists, compared to the other positions in the ODHC, may be due to their higher education and socio-cultural levels. A study conducted with dental students, the rate of anti-HAV-IgG positivity was found at 24.9% (21). Considering the mean age of the participants in our study, the rate may be assessed higher compared to the above study. Screening for HAV vaccination should be performed more diligently in the coming years.

Approximately 32.2-38.8 million people worldwide are infected with HIV. Heathcare workers cannot differentiate HIV-positive patients from the patient's history and physical examination. Therefore, all patients should accept blood and other body fluids

potentially infected. Heathcare workers, should work according to standard precautions (25). The first case was reported in 1985 and 14695 cases have been reported until 31 December 2016 in our country (26). In our study, none of the ODHC workers were found HIV positive.

Study Limitations

The limitation of our study was the low number of cases and reflecting local data.

Conclusion

In the recent years, HBsAg positivity rates reported from oral and dental healthcare centers in Turkey are lower than those reported from general hospital screenings, while anti-HBs positivity rates are higher. In our study, too, HBsAg-positivity was found lower than that of the general hospital rates, but comparable to those reported from the ODHCs of these hospitals. In our study, we identified 100% anti-HBs positivity among ODHC workers a rate that has never been reported to date. This may be because our hospital is a local institution where infection prevention measures and training and vaccination activities are actively implemented. Nonetheless, that Hepatitis transmission is one of the most feared conditions among dentists may lead them to take special care personally aside from organized screening and training, hence be the reason for the rise seen in vaccination rates. The first step in preventing and protecting from viral hepatitis is to gain awareness about the condition. With this report we intend to point out that zero infection and full vaccination is possible by improving training and enhancing awareness.

Ethics

Ethics Committee Approval: Approval for the study was taken from Fırat University, Ethics Committee (approval number: 05/04, date: 2019).

Informed Consent: Retrospective study.

Peer-review: External and internal peer-reviewed.

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Research Article

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Viral Hepatitis Prevalence in Two Cognitively Different Risk Groups

Bilişsel Olarak Farklı İki Risk Grubunda Viral Hepatit Sıklığı

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ABSTRACT

Objectives: This study aimed to investigate whether patient awareness of viral hepatitis affected the prevalence of the condition by comparing the awareness levels in two high-risk groups with different cognitive abilities.

Materials and Methods: Hepatitis B surface antigen (HBsAg), anti-HBs, anti-HAV immunoglobulin G (IgG), anti-HCV and Human Immunodeficiency Virus (HIV) seropositivity values of healthcare workers (HCWs) working in a state hospital and of individuals treated in a State Care and Rehabilitation Center for the Mentally Disabled (SCRCMD) between January 1, 2016 and December 31, 2018 were analyzed retrospectively.

Results: One hundred and two HCWs and 110 individuals followed-up by the SCRCMD were included in the study. Of the HCWs, two (2%) were HBsAg positive, 93 (91.2%) were anti-HBs positive and 85 (83.3%) were anti-HAV IgG positive. None of the HCWs were anti-HCV or HIV positive. Of the individuals followed-up by the SCRCMD, eight (7.3%) were HBsAg positive, 61 (55.5%) were anti-HBs positive, 95 (86.4%) were anti-HAV IgG positive and one (0.9%) was anti-HCV positive. None of the individuals followed-up by the SCRCMD were HIV positive. Anti-HBs positivity was found significantly lower in the SCRCMD group compared to the HCW group ($p<0.001$).

Conclusion: Effective infection control measures and more frequent vaccination may reduce hepatitis infection rates in risk groups, especially among individuals with lower cognitive awareness.

Keywords: Hepatitis, seroprevalence, cognitive status, risk group

ÖZ

Amaç: Bu çalışma ile viral hepatitler için yüksek risk grubunda olan fakat bilişsel olarak farklı iki grup arasındaki hastalık farkındalığının hastalık sıklığını etkileyip etkilemediğinin araştırılması amaçlandı.

Gereç ve Yöntemler: Kamuya bağlı devlet hastanesi sağlık çalışanları ile yine kamuya bağlı Ruhsal Engelli Bakım ve Rehabilitasyon Merkezi'nde (REBRM) yaşayan bireylerin 1 Ocak 2016 - 31 Aralık 2018 tarihleri arasındaki hepatit B yüzey antijeni (HBsAg), anti-HBs, anti-HAV immünoglobulin G (IgG), anti-HCV, HIV seropozitiflik durumları retrospektif olarak incelendi.

Bulgular: Çalışmaya 102 sağlık çalışanı ve REBRM'de takip edilen 110 birey dahil edildi. Sağlık çalışanlarının ikisinde (%2) HBsAg pozitif, 93'ünde (%91,2) anti-HBs pozitif ve 85'inde (%83,3) anti-HAV IgG pozitifliği saptanırken, hiçbir sağlık çalışanında anti-HCV ve HIV pozitifliği saptanmadı. REBRM'de takip edilen bireylerin 8'inde (%7,3) HBsAg pozitif, 61'inde (%55,5) anti-HBs pozitif, 95'inde (%86,4) anti-HAV IgG pozitif ve birinde (%0,9) ise anti-HCV pozitifliği saptandı. REBRM bireylerin hiçbirinde HIV pozitifliği saptanmadı. Sağlık çalışanlarına göre REBRM'deki bireylerde anti-HBs pozitifliği anlamlı olarak düşük saptandı ($p<0,001$).

Sonuç: Risk gruplarında özellikle de bilişsel olarak farkındalığı daha düşük bireylerde enfeksiyon kontrol önlemlerinin daha etkin uygulanması ve aşılama oranlarının artırılması, hepatit enfeksiyon oranlarını azaltılabilir.

Anahtar Kelimeler: Hepatit, seroprevalans, bilişsel durum, risk grubu

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Introduction

Globally, about 240 million people are reported to be affected by chronic Hepatitis B Virus (HBV) infection and 185 million people by Hepatitis C Virus (HCV) infection (1,2). Despite the recent global decrease in the prevalence of Hepatitis B, according to a recent analysis the rate of deaths from liver complications associated with hepatitis B has increased by 33% from 1990 to 2013, (3). HCV infection is a major public health problem globally. To be able to establish evidence-based health policies and to wisely use the resources, it is vital to know the epidemiology of HCV infection and its disease burden (4). HBV and HCV is transmitted by infected blood and other body fluids, and acquired through parenteral, sexual, horizontal, nosocomial and perinatal contact (2,5). In Turkey, the prevalence of HBV infection is reported to be 4% and of HCV infection 1%, with variations among regions (6).

Healthcare workers (HCWs) are exposed to numerous infectious agents because of their profession. Individuals working in the operation room, the intensive care unit, the laboratories and the emergency room are in contact with infected patient materials such as blood and other body fluids, which causes them to be more frequently exposed to agents transmitting blood-borne diseases. According to data of the World Health Organization (WHO), more than 85 million people working in hospitals worldwide are injured by contaminated medical instruments (7). Evidence shows that mentally disabled persons are at higher risk of Human Immunodeficiency Virus (HIV), Hepatitis B and Hepatitis C infections than the general population. These patients have less knowledge about how infectious diseases are acquired and which protective measures should be taken. Moreover, they may engage in risky sexual behaviors, and maintain below-average life and hygiene standards. These may constitute the factors that increase transmission risk among mentally disabled individuals (8).

As previous studies report higher rates for the eastern regions of Turkey compared to the western regions, in this study we aimed to investigate whether awareness of patients in an eastern region of Turkey had an effect on the prevalence of the viral hepatitis and compared the awareness levels of two high-risk groups with different cognitive abilities.

Materials and Methods

The study was conducted in the eastern city of Elazığ and included HCWs from the state district hospital and individuals staying in a State Care and Rehabilitation Center for the Mentally Disabled (SCRCMD). Approval was obtained from the Ethics Committee of Fırat University for the study (approval number: 03/20, date: 07.02.2019). The study was designed in line with the Ethical Principles for Medical Research Involving Human Subjects set forth by the World Medical Association in the Declaration of Helsinki.

Hepatitis B surface antigen (HBsAg), anti-HBs, anti-hepatitis A virus (HAV)-immunoglobulin G (IgG), anti-HCV and HIV seropositivity data of the patients recorded from 1 January 2016 to 31 December 2018 were retrospectively examined. Patients with a recurrent condition were excluded. HBsAg, Anti-HBs, anti-HAV-IgG, anti-HCV and HIV serological values were tested using the Chemiluminescent Microparticle Immunoassay method on an Architect i2000 SR (Abbott, USA) device. In the HCV-positive patients, HCV-RNA levels were measured by real time Polymerase chain reaction using Rotor-Gene Q analyser (Qiagene, Hilden, Germany). HBsAg positive patients were divided into two groups, the chronic infection group and the chronic hepatitis group, based on their HBV-DNA levels, HBeAg positivity/negativity, ALT levels, and liver histology, as recommended in the EASL 2017 guidelines (9). Follow-up and treatment processes of the patients were planned as recommended in EASL 2017 guidelines.

Statistical Analysis

The data were analyzed using the SPSS 22.0 package program. The Pearson chi-square test was used for inter-group variables, and a $p<0.05$ value was considered as the limit of significance.

Results

Of the 102 HCWs included in the study 56 (54.9%) were male and 46 (45.09%) were female, with an age range from 21 to 53 (mean: 33.96±8.53 years). All 110 individuals in the SCRCMD group were male with an age range from 20 to 71 (mean: 50.60±11.04 years). The HCWs group included 41 (40.1%) nurses-midwives, 16 (15.6%) doctors, 16 (15.6%) health technicians (anesthesia technician, health officer), 14 (13.7%) laboratory technicians, 12 (11.7%) cleaning personnel, and 3 (2.9%) ambulance drivers.

Two (2%) of the HCWs were HBsAg positive, 93 (91.2%) were anti-HBs-positive, and 85 (83.3%) were anti-HAV-IgG positive, however, none were anti-HCV or HIV-positive. One of the two HCWs diagnosed with chronic Hepatitis B was a nurse and the other a cleaning personnel. No statistically significant differences were identified between HBsAg and anti-HAV-IgG positivity based on occupation groups; however, anti-HBs positivity was found to be significantly lower in the cleaning personnel compared to the other HCWs ($p=0.002$) (Table 1).

Of the individuals in the SCRCMD group, 8 (7.3%) were HBsAg-positive, 61 (55.5%) were anti-HBs-positive, 95 (86.4%) were anti-HAV-IgG-positive, and 1 (0.9%) was anti-HCV positive. None was identified to be HIV positive. Comparison of the HCWs group and the SCRCMD group showed no statistically significant differences for HBsAg, anti-HAV-IgG and anti-HCV positivity, but significantly lower anti-HBs positivity in the SCRCMD group ($p<0.001$) (Table 1).

Of the two HBsAg-positive individuals in the HCWs group, one was receiving treatment with oral antivirals for chronic hepatitis and had negative HBV-DNA, and the other was monitored for chronic infection. Of the eight HBsAg positive individuals in the SCRCMD group, one was receiving antiviral treatment and had negative HBV-DNA. Evaluation of laboratory values showed chronic infection in four patients and follow-up processes were planned. Three patients were evaluated to have chronic hepatitis and started on oral antivirals. One anti-HCV positive patient had previously received treatment and had negative HCV-RNA.

Discussion

In our study, anti-HBs positivity was identified as 91.2% in the HCWs group and 55.5% in the SCRCMD group. The lowest anti-HBs positivity level in the HCWs group was identified in the cleaning personnel (53.1%). Seropositivity rates of the groups are seen to be in line with the education and awareness levels of the individuals.

Table 1. Distribution of hepatitis B surface antigen, anti-hepatitis B surface, anti-hepatitis A virus-immunoglobulin G, anti-hepatitis C Virus and Human Immunodeficiency Virus seropositivity in the healthcare workers group and the State Care and Rehabilitation Center for the Mentally Disabled group

		Seropositivity				
		HBsAg	Anti-HBs	Anti-HCV	Anti-HAV-IgG	Anti-HIV
Healthcare workers (HCWs) (n=102)	Doctors (n=16)	0	15	0	14	0
		0%	93.8%	0%	87.5%	0%
	Nursing (n=41)	1	39	0	32	0
		2.4%	95.1%	0%	78.0%	0%
	Laboratory technicians (n=14)	0	13	0	11	0
		0%	92.9%	0%	78.6%	0%
	Health technicians (n=16)	0	16	0	15	0
		0%	100%	0%	93.8%	0%
	Cleaning personnel (n=12)	1	7	0	11	0
		8.3%	58.3%	0%	91.7%	0%
	Ambulance drivers (n=3)	0	3	0	2	0
		0%	100%	0%	66.7%	0%
	Total	2	93	0	85	0
		2%	91.2%	0%	83.3%	0%
SCRCMD (n=110)		8	61	1	95	0
		7.3%	55.5%	0.9%	86.4%	0%

HBsAg: Hepatitis B surface antigen, HBs: Hepatitis B surface, HCV: Hepatitis C Virus, HAV-IgG: Hepatitis A virus-immunoglobulin G, HIV: Human Immunodeficiency, HCWS: Healthcare workers, SCRCMD: State care and rehabilitation center for the mentally disabled

In 1992 WHO and International Labor Organization have acknowledged HBV as an occupational disease factor (10). In 1996, the Turkish Ministry of Health initiated a practice requiring the examination and, if necessary, the vaccination of HCWs against this virus (11). A study carried out by the European Centre for Disease Prevention and Control in European countries, reported the lowest rate for HBsAg positivity among the general population in the Netherlands and Ireland (0.1%), and the highest in Turkey (9%). The same study reported the lowest rate for anti-HCV positivity in Belgium (0.1%) and the highest in Italy (22.4%) (12).

HBV infection in HCWs occurs by contact with blood rather than by contact with the patients. Supporting HCWs, in particular, not only are in direct contact with patients but often come into contact with infected blood and other similar materials (13). Although lumen needles are often held responsible for documented HCV infection events, such infection can be transmitted via blood splash into conjunctiva or lumenless needles. Despite such risks, the prevalence of HCV infection among HCWs is not higher than that of the general population. Only 1-2% of all needle accidents experienced by the HCWs are reported to be caused by needles used in HCV-infected patients (14). Recent studies have shown that HCV infection could be eliminated in 15-20 years through diagnosis, treatment and strategies toward preventing new cases. To be able to develop the strategies that will eliminate HCV infection, however, it is important to clearly understand the epidemiology of the disease (15,16).

As is the case across the world, improvement in hygiene and sanitation conditions leads to progress in socio-economic conditions, and decrease in HAV circulation among children, as well as an increase in the number of sensitive adults, and the slow progression of the disease in advanced ages (17). Hepatitis A prevalence is closely related to the indicators of socio-economic development levels, particularly to geographical differences, hygiene and other health conditions (18). Turkey has intermediate endemicity with data showing a prevalence of 8-88%. Increase in the mean age for exposure to the virus has raised the number of adolescents and adults who are mindful of the condition in areas of intermediate endemicity (19).

A study conducted in South Korea with 571 HCWs, reported HBsAg positivity to be 2.4% and anti-HBs positivity 76.9%, and that these rates were not different from that of the general population (20). In another study conducted with 601 HCWs, HBsAg positivity was found 1.8%, and anti-HBs positivity 51.4%; and anti-HBs positivity was identified in laboratory technicians (63.6%), doctors (62.7%), nurses (52%) and cleaning personnel (40%) (21).

In a study conducted in Turkey with HCWs, HBsAg positivity of 5.8% was identified among 14.000 HCWs in the years from 1980 to 1990, and of 3.6% from 1990 to 2000; and this decrease was found statistically significant (22). HBsAg and anti-HBs positivity among HCWs in Turkey, one of the risk groups for Hepatitis B infection, was reported 3% and 78.3% by Demir et al. (23); 2.3% and 68.8% by Kutlu et al. (24); 1.28% and 88.3% by Uludağ Altun et al. (25); and 0.5% and 88.28% by Keçik Boşnak et al. (11). Korkmaz et al. (26), on the other hand, reported HBsAg positivity to be 0.9%, anti-HBs positivity 86% and found the lowest anti-HBs positivity values among the cleaning personnel. Another study reported HBsAg positivity to be 1% and anti-HBs positivity 62.7%. This study reported the highest anti-HBs positivity values in doctors (95%) and the lowest anti-HBs positivity values in cleaning personnel (43.86%) (22). In our study, HBsAg positivity among HCWs was found 2% and anti-HBs positivity 91.2%.

The highest anti-HBs positivity was found among nurses (95.3%) and the lowest among cleaning personnel (53.1%). These rates are comparable to the results reported in the literature. That the lowest anti-HBs rate among HCWs was identified in the cleaning personnel may be associated with education and awareness.

In a study conducted with HCWs of five hospitals in the same country, anti-HCV positivity was found to be 2% (21). Anti-HCV positivity among HCWs in Turkey is reported to range from 0% to 0.34% (11,22,25,26). Similarly, no anti-HCV and HIV positivity was identified among HCWs in our study.

Kurugol et al. (27) report anti-HAV-IgG positivity among the by general population in Turkey to be 85.83% for the 20-29 age group, 95.6% for the 30-39 age group, and 99% for the +40-age group. Korkmaz et al. (26) found an anti-HAV-IgG positivity rate of 71.7% among HCWs. In our study, we identified anti-HAV-IgG positivity in 85 out of 102 HCWs (83.3%). WHO recommends Hepatitis A vaccination in regions of intermediate endemicity, where the number of mindful adults with a severely progressing disease is seen to increase parallel to the improvements in sanitation conditions (18). In our study, vaccination was recommended to HCWs who were identified to be seronegative for Hepatitis A.

A study, in which 5.227 mentally disabled individuals were evaluated, HBsAg positivity was reported to be 4.08%, anti-HBs positivity 42.19%, anti-HCV positivity 0.69%, while no anti-HIV positivity was identified (28). In our study, HBsAg positivity was found 7.3%, anti-HBs positivity 55.5%, anti-HCV positivity 0.9% in the SCRCMD group. HIV positivity was not identified. Although proportional difference was found in HBsAg positivity between the HCWs group and in the SCRCMD group, this difference was not statistically significant. This may be a result of the small number of our sample size. We believe that studies with larger-scale samples are needed. No statistically significant differences were identified in anti-HBs positivity between the two groups (p=0.000).

Study Limitations

The limitation of our study was the low number of cases and reflecting local data.

Conclusion

To conclude, that anti-HBs positivity was found lower for the cleaning personnel, individuals possibly of lower educational levels compared to other HCWs, and in the SCRCMD group, emphasizes the importance of differences in cognitive abilities an aspect we have considered when planning and designing our study. Individuals that fall in risk groups should be carefully examined for seropositivity and non-immune individuals should be included in Hepatitis B and Hepatitis A vaccination programs. We believe that hepatitis infection rates can be reduced in risk groups, especially among individuals with lower cognitive awareness, through more effective infection control measures and more frequent vaccination.

Ethics

Ethics Committee Approval: Approval was obtained from the Ethics Committee of Fırat University for the study (approval number: 03/20, date: 07.02.2019).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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