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E-mail: fehmitabak@yahoo.com
ORCID ID: orcid.org/0000-0001-8632-2825

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E-mail: esragulakinci@yahoo.com
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E-mail: imran.solak@gmail.com
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Address for Correspondence:
Viral Hepatitis Prevention Society
Sağlık Mahallesi, Süleyman Sırrı Caddesi No: 2/15
Sıhhıye, Ankara, Turkey
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Viral Hepatitis Journal (Formerly Viral Hepatit Dergisi) is the regular publishing organ of the Viral Hepatitis Society. This periodical journal covers diagnosis, treatment, epidemiology, prevention and information of hepatitis.

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

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

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

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E-mail: info@viralhepatitdergisi.org

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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.

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

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

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

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

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

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

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

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

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

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

Short Announcements

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

• Author number for review articles should not exceed three.

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

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

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

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

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

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

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

ARTICLE SECTIONS

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

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

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

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

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

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

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

Keywords:

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

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

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

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

Original researches should have the following sections;

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

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

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

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

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

Conclusion: The conclusion of the study should be highlighted.

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

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

Case Reports

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

Review Articles

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

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

Letters to the Editor

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

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

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

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

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

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

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

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

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

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

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

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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

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Research Article

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Viral Hepatitis Journal 2019;25(3):84-87

An Evaluation of Hepatitis B Virus and Hepatitis C Virus Frequency and the Anti-hepatitis B Surface Seropositivity of Syrian Refugees in the Karabük Province

Karabük İlindeki Suriyeli Sığınmacılarda Hepatit B Virüs, Hepatit C Virüs Sıklığının ve Anti-hepatit B Yüzey Seropozitifliğinin Araştırılması

Nergis Aşgın, Şerife Satılmış

Karabük University Training and Research Hospital, Clinic of Medical Microbiology, Karabük, Turkey

ABSTRACT

Objectives: To investigate the frequency of hepatitis B Virus (HBV) and HCV with anti- hepatitis B Surface (HBs) seropositivity using serological and molecular methods in Syrian refugees in the Karabük.
Materials and Methods: The study included the HBs Antigen (HBsAg), anti-HBs, anti-HCV, HCV-RNA, HBV-DNA, and HCV genotyping results of Syrian refugees who presented at the Karabük University Training and Research Hospital between January 2016 and March 2019.
Results: The 809 patients were comprised of 536 (66.3%) females and 273 (33.7%) males with a mean age of 34 years. HBsAg was found to be positive in 2.3% of patients and in all HBV-DNA were positive. The anti-HBs seropositivity rate was determined as 21.6% and a significant difference was determined between age groups (p=0.001). The anti-HCV was positive in 8 (1%) patients and the HCV-RNA viral load was determined in 7/8. In these 7 patients, the HCV genotype was determined as genotype 1b in 3, genotype 3 in 2, and genotype 4 in 2.
Conclusion: This study showed that while the frequency of HBV and HCV in Syrian refugees was similar to the data for Turkey, anti-HBs seropositivity was extremely low. Hepatitis B vaccination programs for Syrian refugees should be implemented and regularly followed up.
Keywords: Syria, hepatitis, virus

ÖZ

Amaç: Bu çalışmada amacımız Karabük ilindeki Suriyeli sığınmacılarda serolojik ve moleküler yöntemlerle hepatit B Virüs (HBV) ve HCV sıklığı ile anti-hepatit B yüzey (HBs) seropozitifliğini araştırmaktır.
Gereç ve Yöntemler: Bu çalışmaya Ocak 2016-Mart 2019 tarihleri arasında Karabük Üniversitesi Eğitim ve Araştırma Hastanesi'ne başvuran toplam 809 Suriyeli sığınmacının HBs antijen (HBsAg), anti-HBs, anti-HCV, HCV-RNA, HBV-DNA ve HCV genotipleme sonuçları dahil edildi.
Bulgular: Toplam 809 hastanın 536'sı (%66,3) kadın, 273'ü (%33,7) erkekti. Hastaların yaş ortalaması 34 idi. Hastaların %2,3'ünde HBsAg pozitif bulundu ve bunların tümünde HBV-DNA pozitif idi. Anti-HBs seropozitiflik oranı ise %21,6 olup, yaş grupları arasında anlamlı farklılık gözlendi (p=0,001). Anti-HCV, hastaların %1'inde (n=8) pozitifti. Anti-HCV pozitif sekiz hastanın 7'sinde HCV-RNA viral yük saptandı. Yedi hastanın 3'ü HCV genotip 1b, 2'si genotip 3 ve 2'si de genotip 4 olarak tiplendirildi.
Sonuç: Bu çalışmada Suriyeli sığınmacılarda HBV ve HCV sıklığı Türkiye verilerine benzer oranlarda iken, anti-HBs seropozitifliği oldukça düşük bulunmuştur. Suriyeli sığınmacılara yönelik hepatit B aşılama programları düzenli olarak takip edilmelidir.
Anahtar Kelimeler: Suriye, hepatit, virüs

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Address for Correspondence: *Şerife Satılmış MD, Karabük University Training and Research Hospital, Clinic of Medical Microbiology, Karabük, Turkey*
E-mail: serifesatilmis89@gmail.com ORCID ID: orcid.org/0000-0001-5310-3933 **Received:** *10.09.2019* **Accepted:** *30.10.2019*

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Introduction

The 2011 outbreak of civil war in Syria led to the greatest refugee crisis since World War II, with 4.8 million of Syrians fleeing to neighboring countries, which included Egypt, Jordan, Lebanon, Turkey and Iran. In September 2019, it was reported that there are 3.658 million Syrian refugees in Turkey. This has led to various health problems, one of which is viral hepatitis (1,2). Viral hepatitis can lead to severe health problems such as cirrhosis of the liver and hepatocellular carcinoma (HCC) (3,4). The hepatitis B virus (HBV) and hepatitis C virus (HCV) are transmitted sexually and from infected mothers to newborns vertically, through parenteral contact with infected blood and body fluids (5).

HBV is the most common cause of chronic viral hepatitis worldwide. Approximately 250 million people throughout the world are infected with chronic HBV and it is estimated that on average 887.000 die each year due to cirrhosis or HCC (6). Vaccination against HBV is the most effective method of protection. In 1998, hepatitis B vaccination was included in the national vaccination program in Turkey, and from that time, has been administered in three doses. In Syria, a three-dose vaccination for HBV has been recommended since 1993 and the vaccination rates, especially in young people, increased (7). However, vaccination programs cannot be fully implemented due to the civil war in Syria.

A vaccine for HCV has not yet been developed. More than 71 million people worldwide are infected with HCV, and it is estimated that 399.000 deaths per year are due to HCV infection-related cirrhosis or liver cancer (8). The prevalence of HCV has been reported as <1% in Syria (9).

The aim of this study was to investigate the frequency of HBV and HCV and anti-hepatitis B surface antigen (HBsAg) seropositivity using serological and molecular methods in Syrian refugees in the Karabük province of the western Black Sea region of Turkey.

Materials and Methods

This study was approved by the Non-invasive Clinical Research Ethics Board of Karabük University (approval number: 77192459-050.99-E.41215). The study included the HBsAg, anti-HBs, anti-HCV, HCV-RNA, HBV-DNA and HCV genotyping results of 809 Syrian refugees who were admitted at the Karabük University Training and Research Hospital between January 2016 and March 2019. Results of repetitive patient results were excluded from the study and the first serological parameters of each patient were evaluated.

The patients were separated into 4 age groups of 0-20 years, 21-40 years, 41-60 years, and 61+ years. The test results of patients were obtained from the hospital laboratory information system.

In the serum samples sent to the microbiology Laboratory, the HBsAg, anti-HBs, and anti-HCV tests were performed using the chemiluminescence method with the ARCHITECT i2000 SR device (Abbott Diagnostics, USA). For HBV-DNA and HCV-RNA, first viral nucleic acid isolation was performed by the quantitative polymerase chain reaction (qPCR) method with a Magnesia 16 device (Anatolia Genework, Turkey). Then, the HBV-DNA and HCV-RNA quantitative tests were performed using the qPCR method with a Montania 4896 device (Anatolia Genework, Turkey) according to the manufacturer's instructions. In HCV-RNA positive patients, the Bosphore HCV genotype kit (Anatolia Geneworks, Turkey) was used to determine 8 different genotypes of HCV (1, 1a, 1b, 2, 3, 4, 5, and 6). The HCV genotyping test was also applied using the qPCR method with the Montania 4896 device.

Statistical Analysis

The data obtained in the study were analyzed statistically using SPSS version 22.0 software (SPSS Inc, Armonk, NY, USA). Conformity of the data to normal distribution was assessed using the Kolmogorov-Smirnov test. Descriptive statistics were stated as number (n), percentage (%) and median values. For group comparisons, the Pearson's chi-square test was used. A value of $p<0.05$ was accepted as statistically significant.

Results

The 809 patients were comprised of 536 (66.3%) females and 273 (33.7%) males with a mean age of 34 years (range: 2-94 years). The presence of HBsAg was detected in 19 (2.3%) patients, 10 females and 9 males. In all the HBsAg positive patients HBV-DNA viral load was detected. Anti-HBs seropositivity was determined in 175 (21.6%) patients and a significant difference was determined between age groups in respect to this parameter ($p=0.001$) (Table 1).

No significant relationship was determined between the genders in respect to HBsAg and anti-HBs seropositivity ($p=0.200$). Of the 10 female HBsAg positive patients, eight were of child-bearing age (15-49 years). Anti-HCV was positive in 8 (1%) patients, with HCV-RNA positivity determined in 7. In these 7 patients, the HCV genotype was determined as genotype 1b in 3, genotype 3 in 2, and genotype 4 in 2. The distribution of the serological parameters according to age and gender were shown in Table 2.

Discussion

Chronic hepatitis B is a global health threat causing high rates of mortality and morbidity. Of patients, 20%-40% are at risk of developing cirrhosis and HCC. Based on the HBV carrier rate, the world can be divided into 3 regions of high, medium, and low endemicity. Turkey is a middle endemic country (2%-8%) for HBV with a prevalence of 2.3% (10,11). Vaccination coverage has increased in Turkey in recent years with vaccination rates increasing from 64% in 1999 to 98% in 2016 (12). While the vaccination rate in Syria was 83% in 2008, it has fallen to 69% due to the outbreak of war (5,7). Also, Syria is one of the middle endemic countries

Table 1. Distribution of anti-HBs results between age groups

Age	Anti-HBs negative n (%)	Anti-HBs positive n (%)
0-20 years	62 (62.6)	37 (37.4)
21-40 years	387 (80.8)	92 (19.2)
41-60 years	130 (80.2)	32 (19.8)
>61 years	55 (79.7)	14 (20.3)
Total	634 (78.4)	175 (21.6)

HBs: Hepatitis B surface

Table 2. The distribution of the serological parameters of Syrian patients according to age and gender

		HBsAg		Anti-HBs		Anti-HCV	
		%	n	%	n	%	n
Gender	Female (n=536)	10	0.02	116	21.6	5	0.009
	Male (n=273)	9	0.03	59	21.6	3	0.01
Age groups	0-20 (n=99)	2	0.02	37	37.3	-	-
	21-40 (n=479)	12	0.02	92	19.2	1	0.002
	41-60 (n=162)	3	0.02	32	19.7	4	0.02
	>61 (n=69)	2	0.03	14	20.2	3	0.04

HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C Virus

for HBV (13). The results of this study showed that while the rate of anti-HBs seropositivity in Syrian patients in our region was extremely low at 21.6%, HBsAg positivity was determined as 2.3%. All these patients were diagnosed with chronic viral hepatitis and an HBV-DNA viral load was detected in all.

In the current study, HBsAg positivity was not found at a high rate. However, eight of ten HBsAg positive female patients were in the child-bearing age group. İnci et al. (14) also reported HBsAg positivity as 1.1% in 2158 Syrian pregnant women. This constitutes a threat to newborns. The risk of an infant acquiring HBV perinatally from an infected mother is 70%-90%. Therefore active and passive HBV immunoprophylaxis is crucial.

In a study of 140 Syrian children, Köse et al. (5) reported HBsAg positivity at a higher rate (4.2%) than the data for Turkey and anti-HBs seropositivity was determined at a rate of 52.8. This was attributed to Syrian children generally living in poor socio-economic conditions and no follow up vaccinations. In the current study, anti-HBs positivity was found to be 37.4% in the age group <20 years. This rate was higher than that of the other age groups and similar to findings in other studies, indicating that vaccinations were insufficient.

HCV is another important pathogen that leads to viral hepatitis and hepatitis C infections becoming chronic at a rate of 85%. To date, no vaccination has been developed for HCV. In Turkey, the prevalence of HCV has been reported as 1.6% (5,15) and according to Centers for Disease Control and Prevention data, the HCV prevalence in the Syrian population is estimated to be 0.4% (16). The results of this study showed anti-HCV seropositivity in Syrian refugees at a similar rate (1%) to the data for Turkey. In addition to the detection of HCV RNA in anti-HCV positive patients, it is also necessary to identify the HCV genotype to determine the treatment protocol and duration of treatment (17). The most common HCV genotype is genotype 1 worldwide, followed by genotype 3. Studies have reported genotype 1b to be the most frequently seen in Turkey (60%-100%) (18,19,20). However, in a 2015 study by Caliskan et al. (21) of 313 HCV-RNA positive patients, genotype 3 was found at a rate of 46%. This high rate was attributed to the study group being composed of refugees, drug addicts, and prisoners. In Syria, the predominant genotype is genotype 4 (22,23). In the current study, of the seven Syrian refugees found to be HCV-RNA positive, three were determined as genotype 1b, 2 as genotype 3, and 2 as genotype 4, which was similar to the data for Turkey.

Conclusion

Although the results of the current study did not show a high rate of HBV and HCV prevalence in Syrian patients, anti-HBs seropositivity was found to be extremely low.

This indicates that hepatitis B vaccination is not adequate among the Syrian people. Therefore, hepatitis B vaccination programs for Syrian refugees must be followed up regularly. Furthermore, as the majority of HBsAg-positive females were in the child-bearing age group, this constitutes a risk for hepatitis B infection in newborn infants. Pregnant women must be screened for HBV and for those found to be positive, active, and passive immunoprophylaxis must be applied to the infant in the perinatal period.

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Ethics

Ethics Committee Approval: This study was approved by the Non-invasive Clinical Research Ethics Board of Karabük University (approval number: 77192459-050.99-E.41215).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Research Article

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Occult Hepatitis B in Hemodialysis Patients

Hemodiyaliz Hastalarında Okült Hepatit B

Pelin Adar, Şükran Köse, Bengü Tatar

University of Health Sciences, İzmir Tepecik Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, İzmir, Turkey

ABSTRACT

Objectives: We aimed to detect occult hepatitis B (OHB) in hemodialysis patients at a higher-risk for OHB.
Materials and Methods: The study included 567 patients with chronic renal failure aged 18 years and older who underwent hemodialysis in 10 dialysis centers in İzmir province between May 2013 and July 2013. Hepatitis B surface-antigen (HBsAg), anti-hepatitis B core (HBc) immunoglobulin G (IgG) and anti-HBs were detected by ELISA and HBV-DNA levels with polymerase chain reaction (PCR). Detection of HBsAg negativity with HBV-DNA positivity was considered as OHB.
Results: Of 567 patients, 49% were male and the mean age was 62.2 years. All the patients were HBsAg-negative. Isolated anti-HBc IgG positivity was detected in 8 patients while HBV-DNA was negative. Serum HBV-DNA level was 270 IU/mL in only one patient (0.2%) who was anti-HBc IgG-negative.
Conclusion: HBsAg alone is not an adequate serological test to detect HBV infection. HBV-DNA should be tested using molecular diagnostic methods in patients with suspected OHB. Further studies investigating cost-effectiveness and the role of PCR in diagnosis are warranted.
Keywords: Chronic viral hepatitis, hemodialysis, occult hepatitis B

ÖZ

Amaç: Risk grubunda yer alan hemodiyaliz hastalarında okült hepatit B'yi (OHB) saptamayı amaçladık.
Gereç ve Yöntemler: Mayıs 2013-Temmuz 2013 arasında, İzmir ilinde 10 farklı diyaliz merkezinde hemodiyalize giren, 18 yaş ve üzeri, 567 kronik böbrek yetmezliği olan hastalar çalışmaya dahil edildi. Hepatit B yüzey antijeni (HBsAg), anti-hepatit B çekirdeği (HBc) immünoglobulin (IgG) ve anti-HBs ELISA yöntemiyle, HBV-DNA ise polimeraz zincirleme reaksiyonu (PCR) yöntemiyle çalışıldı. HbsAg negatifliği ve HBV-DNA pozitifliği OHB olarak değerlendirildi.
Bulgular: Beş yüz altmış yedi hastanın %49'u erkek, ortalama yaş 62,2 idi. HBsAg tüm hastalarda negatif saptandı. Izole anti-HBc IgG pozitifliği 8 hastada gözlenmiş olup hiçbirinde HBV-DNA saptanamadı. Sadece bir hastada (%0,2) serum HBV-DNA düzeyi 270 IU/mL olarak ölçüldü.
Sonuç: HBsAg tek başına HBV enfeksiyonunu saptamada yeterli bir serolojik test değildir. OHB şüphesi olan hastalarda HBV-DNA düzeyi moleküler tanı yöntemleriyle test edilmelidir. Maliyet etkinliği ve tanıda PCR'nin rolü için daha fazla çalışmalara ihtiyaç olduğu kanaatine varıldı.
Anahtar Kelimeler: Kronik viral hepatit, hemodiyaliz, okült hepatit B

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Introduction

Being one of the most important causes of chronic liver disease, hepatitis B virus (HBV) is a significant cause of morbidity and mortality all over the world. HBV infection is a major health problem with 400-500 million people chronically infected worldwide. It is a known fact that 5% of people with acute hepatitis B develop chronic infection and a substantial number of these cases develop cirrhosis associated with higher risk of, hepatocellular carcinoma (HCC) (1).

HBV infection is diagnosed by detection of various antigens belonging to this virus or the antibodies developed by the host against these antigens with specific serological tests. Hepatitis B surface antigen (HBsAg) is one of the most important markers of HBV infection. HBsAg positivity in serum for more than six months indicates chronic HBV infection (2,3). Antibody to anti-HBs appears

Address for Correspondence: *Pelin Adar MD, University of Health Sciences, İzmir Tepecik Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, İzmir, Turkey*
Phone: +90 533 637 69 20 E-mail: pelinadar@gmail.com ORCID ID: orcid.org/0000-0003-4251-8396 **Received:** *14.05.2018* **Accepted:** *06.12.2018*
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following the disappearance of HBsAg. Anti-HBs shows recovery and immunity. Anti-hepatitis B core (HBc) immunoglobulin G (IgG) positivity, which is detected together with anti-HBs, is defined as natural immunity (2,4,5). Presence of HBV-DNA is the most sensitive indicator of viral replication.

Detection of serological markers is important in the identification of infection but has little clinical importance. Polymerase chain reaction (PCR) detects low levels of viral DNA in serum or liver in some patients whose HBsAg levels are undetectable or in patients who have HBsAg disappearance with HBV treatment. Occult hepatitis B (OHB) infection (OBI) is defined as the presence of HBV-DNA in serum or liver in the absence of HBsAg. OBI has also been defined as a serological condition characterized by the presence of isolated hepatitis B core antigen anti-HBc in the absence of HBsAg and anti-HBs. OBI can be classified into 2 groups on the basis of the HBV antibody profile: seropositive OBI (anti-HBc and/or anti-HBs-positive) and seronegative OBI (anti-HBc- and anti-HBs-negative) (6). HBV-DNA level in OBI is generally measured lower than 200 IU/mL (7).

OBI has been reported to be more prevalent in patients with HCC, chronic hepatitis C virus (HCV) infection, cryptogenic cirrhosis, hemodialysis patients, substance users, intravenous drug abusers, patients with human immunodeficiency virus infection, and patients who receive frequent blood transfusions (8). If patients with OBI diagnosis undergo dialysis in the same dialysis machines with HBsAg-negative patients, HBV transmission may occur.

There are not sufficient studies performed on the frequency of OBI in our country and our province. In this study, we aimed to detect OHB in hemodialysis patients who are at a higher risk for OBI.

Materials and Methods

This randomized prospective study included 567 patients with chronic renal failure (CRF) aged 18 and older who underwent hemodialysis in 10 dialysis centers in İzmir province between May 2013 and July 2013. Patients younger than 18 years of age, having a previous diagnosis of HBV infection and undergoing peritoneal dialysis were excluded. Ethic committee approval was taken from the Ethics Committee Board of University of Health Sciences, İzmir Tepecik Training and Research Hospital (approval number: 47/1, date: 24.04.2013).

A form questioning risk factors for HBV contamination including socio-demographic characteristics, medical history such as co-morbidities, IV drug administration, surgery, blood transfusion and time of hepatitis B vaccination was completed by the patients. Written consent form was taken from each patient. Blood samples were taken before dialysis session; serum samples were centrifuged in 3000 rpm for 5 minutes and stored at -80 °C. HBsAg, anti HBc IgG, and anti-Hbs were investigated by the ELISA method (Liaison, Diasorin, Italy) in accordance with the instructions of the manufacturing company. HBV-DNA levels were evaluated by PCR (Roche, Taqman, Switzerland). HBV-DNA levels lower than 20 IU/mL were considered as negative. HBV-DNA presence in serum without HBsAg positivity was defined as OBI.

Results

Forty-nine percent of 567 patients were male and the mean age was 62.2 (range: 24-78) years. The patients were receiving hemodialysis treatment three times a week for four hours. The mean duration of hemodialysis treatment was 60.7 months (range: 4-180 months). The indications for dialysis were diabetes mellitus (42.7%), hypertension (31.3%), glomerulonephritis (12.5%), polycystic kidney disease (4.2%), and others (9.3%) (Table 1). Mean AST: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were 14 (5-42 u/L) and 17 (8-36 u/L), respectively.

Fifty-seven percent of the patients had a history of vaccination with 40 mcg recombinant DNA vaccine in months 0, 1, 2 and 6 according to the standard vaccine schedule. In 1.5% (n=5), insufficient antibody response (anti-HBs <10 IU/mL) was detected despite administration of vaccine twice. Fifteen percent of the patients were considered to have natural immunity due to past infection.

As shown in Table 2, HBsAg was negative in all cases. Isolated anti-HBc IgG positivity was detected in 8 patients while HBV-DNA was negative. Serum HBV-DNA level was 270 IU/mL in only one patient aged 53 years (0.2%) with anti-HBc IgG and anti-Hbs negativity. AST and ALT levels of the patient who was considered as seronegative for OBI, were within the normal range (0-35 IU/L). Hemodialysis duration of the patient was lower than the average (12 months). The patient had a history of diabetes mellitus and hypertension. In terms of chronic hepatitis B, no other risk factors (history of past HBV infection or inactive chronic hepatitis, previous surgery, blood transfusion, suspicious coitus, history of dentist appointment, and family history of hepatitis), beyond hemodialysis and catheterization, were detected.

Table 1. The questionnare including demographics, dialysis reasons and risk factors for hepatitis B virus infection

Socio-demographic characteristics	
Mean age	62.2
Gender	49%
History of hepatitis B virus vaccination	57%
Dialysis reasons	**(%)**
Diabetes mellitus	42.7
Hypertension	31.3
Glomerulonephritis	12.5
Policystic kidney disease	4.2
Others	9.3
Risk factors	**(%)**
IV drug administration	32.6
Operation	13.8
Blood transfusion	26.7
Family history	3.4
Suspicious coitus	2.9

Table 2. The serological parameters of hepatitis B virus in hemodialysis patients

n (%)	HBsAg	Anti-HBc	Anti-HBs	HBV-DNA
8 (1.4%)	(-)	(+)	(-)	(-)
1 (0.1%)	(-)	(-)	(-)	(+)
318 (56%)	(-)	(-)	(+)	(-)
240 (42.3%)	(-)	(-)	(-)	(-)

HBsAg: Hepatitis B surface antigen, HBc: anti-hepatitis B core, HBs: Hepatitis B surface, HBV: Hepatitis B virus

Discussion

HBV infection continues to be a significant issue in hemodialysis units despite vaccine schedules and precautions. Risk of the HBV transmission between hemodialysis patients is explained by the presence of OBI (HBsAg negative but HBV-DNA positive) in addition to the presence of immunosuppression, shared use of dialysis machines, insufficient response to vaccine, blood transfusions and interventions which are performed more frequently for hemodialysis patients than for normal population (9).

The prevalence of OBI in healthy subjects has been reported to vary between 0% and 90% based on the endemicity (10,11,12). In hemodialysis patients, the prevalence of OBI reported in the literature varies greatly, ranging from 0% to 50% (9,13). In a study investigating the prevalence of anti-HBc in hemodialysis patients, HBV-DNA was detected in 1 of 3 anti-HBc positive patients. HBV-DNA was undetectable in 123 anti-HBc negative patients (14). In a study by Ramezani et al. (15), HBV-DNA was detected in 1% of HBsAg negative patients. Similar to that study, Muche et al. (16) found nil prevalence of OHB in chronic hemodialysis patients and a very low prevalence (<1%) in renal transplant patients suggesting that routine screening for HBV-DNA was not required in chronic hemodialysis population in their region.

The frequency of rate of OBI in hemodialysis patients reported in studies performed in our country varies between 0% and 12.4% (17,18,19). The variability in the reported prevalence is related with the regions where the studies were performed, PCR method used, and patient population included. In our study, it was found to be 0.2%. Possible reasons for low frequency of OBI in our study may be regular vaccination and anti-HBs screening of patients, good physical conditions of the hemodialysis units and complete compliance of the study team to the standard precautions. On the other hand, vaccine runaway replication mutants and antiHBs, immune escape mutants in patients who received hepatitis B immune globulin (HBIG), diagnostic escape mutants which could not be detected by some tests used in detecting HBsAg due to change in HBs structure have been reported to be the result of the mutations of the "a" determinant region of HBV S gene in subjects who were immunized with recombinant vaccines (20).

In patients with CRF, the most common reasons for dialysis are diabetes mellitus (22.8%), hypertension (18.1%), and glomerulonephritis (14.2%) (21). CRF etiologies in patients included in this study are consistent with the literature.

Since cellular and humoral immune responses are insufficient in CRF patients, elevations of liver function tests are mild to moderate and, most of the time, the patients have asymptomatic HBV infection. As a result, chronic disease may occur more frequently in patients who undergo hemodialysis compared to normal population (22). Thus, establishing the diagnosis of OBI in hemodialysis patients is difficult. In a study by Yoo et al. (13), there was no significant difference in the liver function tests between HBV-DNA positive and negative hemodialysis patients. Laboratory tests in patient who was considered to have OBI in this study were also normal.

HBV-DNA levels were observed to be low (<200 IU/mL) in cases with OBI in all studies reported in the literature (23,24). Consistent with the literature, the patient with OBI in this study had a HBV-DNA level of 270 IU/mL. This finding suggests that OBI may occur due to low concentrations of undetectable HBsAg rather than presence of mutant HBV. Furthermore, HBV-DNA levels are also very low in these patients; the sensitivity of the test preferred in the diagnosis of OBI is of crucial importance (25).

The possibility of transmission of HBV infection from dialysis patients with OBI to others using same dialysis machine is not clearly known. No detection of OBI in other 26 cases who undergo hemodialysis in the same center with the OBI case suggests that possibility of transmission is low.

Presence of fulminant liver disease, chronic hepatitis, cirrhosis, and HCC has been reported in patients with OBI (26). This suggests a role of OBI in development of cirrhosis and HCC. Carcinogenesis may start with integration of the viral genome into liver cells together with the cytotoxic liver injury due to long-term HBV positivity. Therefore, OHB-seropositive patients without HBV-DNA in serum may also require liver biopsy for further OBI detection. We also detected isolated anti-Hbc IgG positivity in 8 patients and suggested liver biopsy.

Initiation of antiviral treatment should be considered in patients with OBI diagnosis. The patients should be screened for HCC in regular intervals. A favorable response to treatment may be expected in patients with a low viral load (27).

Conclusion

HBsAg alone is not an adequate serological test to detect HBV infection. HBV-DNA should be tested with molecular diagnostic methods in patients with OHB suspicion. For diagnosis of OHB, DNA nucleic acid tests should be performed especially in high-risk patients, those living in endemic regions, individuals with cryptogenic chronic hepatitis, with potential prior exposure before blood or organ donation, transplantation and chemotherapy, and those receiving hemodialysis, even if these patients have anti-HBc and anti-HBs negativity (28). Further studies investigating cost-effectiveness and the role of PCR in diagnosis are warranted.

Ethics

Ethics Committee Approval: Ethic committee approval was taken from the Ethics Committee Board of University of Health

Sciences, İzmir Tepecik Training and Research Hospital (approval number: 47/1, date: 24.04.2013).

Informed Consent: Written consent form was taken from each patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Research Article

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The Evaluation of the Relation Between Drug Compliance and Psychometric Tests in Chronic B Hepatitis Patients Who are Treated with Oral Antivirals

Oral Antiviral Tedavi Alan Kronik Hepatit B Hastalarında İlaç Uyumunun Psikometrik Testlerle İlişkisinin Değerlendirmesi

Esma Kepenek Kurt, Bahar Kandemir

Necmettin Erbakan University Meram Faculty of Medicine, Department of Infectious Diseases And Clinical Microbiology, Konya, Turkey

ABSTRACT

Objectives: Hepatitis B is a viral infection that causes acute and chronic hepatitis and serious complications such as cirrhosis and hepatocellular cancer. Several psychiatric symptoms and depression, particularly anxiety, can be seen in patients. Evaluation of the relation between medication adherence and psychometric tests in patients with Chronic hepatitis B (CHB) who are treated with oral antivirals is aimed in this study.

Materials and Methods: Two hundred and fifty-nine patients were included in this study. Sociodemographic data form, Morisky 8 scale, Beck Anxiety scale (BAS) and Beck Depression scale (BDS) have been applied to the patients.

Results: The age of the patients vary from 17 to 80, average age of the patients is foud as 46.04. A hundred eleven of the patients were women (42.9%). Two hundred and seven patients had no sign of depression (79.9%) and 52 of the patients (20.1%) had depression. The drug compliance was low in patients whose BDS scores were decreased. It has been found out that 195 of the patients had low-grade of anxiety (75.3%), 26 of the patients had midgrade of anxiety (10%), and 38 of the patients had high-grade of anxiety (14.7%). The medication adherence was low in patients whose BAS scores were decreased.

Conclusion: Anxiety and depression are frequent in CHB patients. Therefore, CHB patients should be followed up psychiatrically and should be addressed to the psychiatry specialist when necessary. Depression and anxiety influences the drug compliance. Further studies are required for evaluation the relation between the medication adherence and the level of anxiety and depression in CHB patients.

Keywords: Hepatitis B, Depression, Anxiety, Adherence

ÖZ

Amaç: Hepatit B, akut ve kronik hepatite yol açan siroz ve hepatoselüler kanser gibi ciddi komplikasyonlara neden olan viral bir enfeksiyondur. Kronik hepatit B (KHB) hastalarında birçok psikiyatrik semptom, depresyon ve özellikle anksiyete görülebilir. Bu çalışmada, oral antivirallerle tedavi edilen KHB'li hastalarda ilaç uyumunun psikometrik testlerle ilişkinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya 259 hasta dahil edildi. Hastalara sosyodemografik veri formu, Morisky 8 ölçek, Beck Anksiyete ölçeği (BAÖ) ve Beck Depresyon ölçeği (BDÖ) uygulanmıştır.

Bulgular: Hastaların yaşı 17 ila 80 arasında değişmekte olup, hastaların yaş ortalaması 46,04'tür. Hastaların 111'i (%42,9) kadındı. İki yüz yedi (%79,9) hastada depresyon belirtisi yoktu ve 52 (%2,1) hastada depresyon vardı. Hastalarda BDÖ skorları azaldıkça ilaç uyumu düşüktü. Hastaların 195'inde düşük dereceli anksiyete (%75,3), 26'sının orta dereceli anksiyete (%10) ve 38'inde yüksek anksiyete (%14,7) düzeyine sahipti. Hastaların BAÖ skorları azaldıkça ilaç uyumu düşüktü.

Sonuç: KHB hastalarında anksiyete ve depresyon sıktır. Bu nedenle, KHB hastaları psikiyatrik olarak da izlenmeli ve gerektiğinde psikiyatri uzmanına yönlendirilmelidir. Depresyon ve anksiyete ilaç uyumunu etkiler. KHB hastalarında ilaç uyumu ile anksiyete ve depresyon düzeyi arasındaki ilişkinin değerlendirilmesi için ileri çalışmalar gereklidir.

Anahtar Kelimeler: Hepatit B, Depresyon, Anksiyete, Uyum

Kepenek Kurt E, Kandemir B. The Evaluation of the Relation Between Drug Compliance and Psychometric Tests in Chronic B Hepatitis Patients Who are Treated with Oral Antivirals. Viral Hepat J. 2019;25:92-96.

Address for Correspondence: *Esma Kepenek Kurt MD, Necmettin Erbakan University Meram Faculty of Medicine, Department of Infectious Diseases And Clinical Microbiology, Konya, Turkey*
Phone: 0332 223 77 26 E-mail: esma_kepenek@hotmail.com ORCID ID: orcid.org/0000-0002-0295-1745 **Received:** *31.03.2019* **Accepted:** *26.08.2019*

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Introduction

Hepatitis B virus (HBV) infections are a global public health problem. There is nearly 240 million HBV carrier in the world. Approximately 600.000 people die each year due to cirrhosis and cancer caused by HBV. In a meta-analysis conducted in Turkey, the prevalence of HBsAg was calculated as 4.5% (1).

Especially anxiety, several psychiatric symptoms, distress, difficulty in concentration of the thoughts, muscle strain, sleep disorders and depression may be seen in patients with chronic viral hepatitis (2). Medication adherence has been defined as the use of drugs by the patient in specified dose and time over a certain period of time (3). The symptoms of depression and anxiety may lead to low adherence to treatment, or may be resulted from low adherence to treatment (4).

Growing body of evidence reveals that, the incidence of psychiatric disorders is underestimated in patients with chronic hepatitis. Determination of depression and anxiety in CHB patients, and intervention when necessary are important for the success of treatment. Although there are many studies conducted on this issue, no any study could not find in the literature to investigate medication adherence, and the relationship of medication adherence with anxiety and depression in CHB patients receiving oral antiviral therapy. In this study, we aimed to evaluate medication adherence, and the relationship of medication adherence with anxiety and depression in CHB patients receiving oral antiviral therapy using Beck Anxiety scale (BAS) and Beck Depression scale (BDS).

Materials and Methods

This study was conducted in Infectious Diseases and Clinical Microbiology outpatient clinic between June 2014 and November 2014. Morisky Medication Adherence scale (MMAS-8) survey, BDS and BAS was applied on patients who were followed-up with the diagnosis of CHB and received oral antiviral therapy for ≥6 months. Patients sex and age date were collected. Pregnants, patients with a history of using peg-interferon, a chronic disease leading to impairment in immune system, malignancy and collagen tissue disease, accompanying human immunodeficiency virus, positivity of HCV and HDV, those with the symptoms of decompensated liver cirrhosis, and patients with diagnosed psychiatric diseases were excluded from the study. After receiving approval from Necmettin Erbakan University Meram Faculty of Medicine Ethics Committee (approval number: 2014/687, date: 20.06.2014), the patients were interviewed. Informed consent form was received from patients. Volunteer patients applied sociodemographic data collection form, MMAS-8, BDS, BAS questionnaire.

Sociodemographic data collection form questioned patients' age, sex, duration of being infected with HBV, currently used drugs, duration of using the current drugs, and drugs used for comorbidities.

Morisky Medication Adherence scale-8: MMAS was developed by D.E. Morisky and its validity and reliability were studied by Morisky et al. (5). MMAS is a scale which evaluated drug use behaviours of the patient with 8 questions based on self-report, and involves questions that will be enable a better evaluation of the obstacle which may cause medication nonadherence. It is an easy to fill scale. The questions are responded in the form of "Yes/No". It is scored as 1 for Yes, and 0 for No. MMAS is scored between 0 and 8. A score <6 has been defined as low adherence, 6-<8 as medium adherence, and ≥8 as high adherence. MTUÖ-8 is validity and reliability study for our country (6).

Beck Depression scale: BDS was developed in order to measure severity of depression, to enable follow-up experiences with treatment, and recognize the disease. Each sentence in BDS was numbered between 0 and 3. The scale consists of 21 items that are listed from mild to severe form. It provided four-item Likert type measurement. BDS has been introduced as a self-report scale, which ask the patients evaluating their status for the last one week. For the severity, it is interpreted as 0-9= no/ninimal, 10-16=nild, 17-29=nedium, and 30-63=severe (7). The scale has been translated to Turkish as two distinct forms as BDI and BDS, and its validity and reliability were studied. The cut-off value of the scale was reported as 17 in validity and reliability study of the scale. BDS <17 points was considered as no depression, while BDS >17 indicated depression. In addition, depressive component burden increases with the score obtained from the scale (8).

BAS: BAS was developed by Beck et al. (9) in order to measure levels and severity of anxiety symptoms. BAS is a scale consisting of Likert type 21 items that involve four self-report item and is scored between 0-3. Anxiety levels of patients according to BAS scores have been classified as: 0-17 points low, 18-24 points medium, 25 or higher points as severe anxiety level. High scores of BAS indicate severity of depression and anxiety (10). The validity and reliability study in our country was done by Ulusoy et al. (11).

Statistical Analysis

The data were analyzed using SPSS version 21 software. Chi-square test was used in comparison of categorical variables. Normality of the numerical variables was analyzed with Kolmogorov-Smirnov test. Mann-Whitney U test was used in the comparisons of numerical variables between two group. Whereas Kruskal-Wallis test was used in multiple groups. Spearman's rho correlation was used for deterination of the correlation between numerical variables. Crohnbach alpha coefficient was calculated for reliability analysis of the questionnaire. Frequency tables were prepared for the categorical variables. Descriptive values were visualized with relevant charts. $P<0.05$ values were considered statistically significant.

Results

The mean age of 259 patients included in this study was 46.04±13.13 years. Of all patients, 111 (42.9%) were female and 148 (57.1%) were male.

When CHB duration of 251 patients was evaluated, the mean duration of the disease was found as 10.22±7.39 years. The remaining 8 patients did not know how long they were being infected with HBV. The mean duration of using drugs for hepatitis B was found as 4.55±3.50 years. A total of 255 patients were receiving oral antiviral therapy for mean 2.92±2.50 years. Four patients did not know the duration of using drugs or left this question unanswered.

Of patients, 80 (30.9%) were receiving tenofovir, 79 (30.5%) telbivudine, 50 (19.3%) entecavir, 45 (17.4%) lamivudine, and 5 (1.9%) adefovir.

The mean MMAS-8 score was found as 3.28±1.54 in all patients, 3.23±1.50 in male patients, and 3.34±1.59 in female patients. Medication adherence was statistically significantly higher in female than in male patients (p=0.005). Standard questions used to determine adherence is shown in Table 1.

No significant correlation was found between patients' medication adherence and age distribution (p=0.933), educational status (p=0.419), smoking (p=0.796), alcohol abuse (p=0.096), types of drugs used (p=0.147), comorbidity (p=0.818), and using additional drugs (p=0.579).

The mean BDS was found as 11.16±9.66 in all patients, 9.43±8.48 in male, and 13.45±10.64 in female patients. Fifty-two patients with high BDS, 22 (42.3%) were male, and 30 (57.7%) were female. BDS was statistically significantly higher in female than in male patients (p<0.001), while no statistically significant correlation was found between age and BDS (p=0.086). There was a statistically significant correlation between patients' medication adherence and BDS score (p=0.011). Medical adherence of the patients was higher as BDS decrease. Distribution of the patients' adherence according to BDS is seen in Table 2.

The mean BAS was found as 12.51±11.37 in all patients, 9.51±8.82 in male, and 16.51±13.09 in female patients. Looking to the relationship between sex and anxiety, BAS was statistically significantly higher in female compared with male patients (p<0.001), and no statistically significance correlation was found between age and BAS (p=0.351). There was a statistically significant correlations between patients' medication adherence and BAI score (p<0.001). Medication adherence was found to be lower as BAS decrease. Distribution of the patients' medication adherence according to BAS is seen in Table 3.

There was a statistically significant positive correlation between BDS and BAS of the patients (p<0.001) (r=0.747).

Table 1. Standard questions used to determine adherence

	No, n (%)	Yes, n (%)
1. Do you sometimes forget to take medication?	148 (57.1)	111 (42.9)
2. People sometimes miss taking their medicines for reasons other than forgetting. Thinking over the past 2 weeks, were there any days when you did not take your medicine?	175 (67.6)	84 (32.4)
3. Have you ever cut back or stopped taking your medicine without telling your doctor because you felt worse when you took it?	207 (79.9)	52 (20.1)
4. When you travel or leave home, do you sometimes forget to bring along your medicine?	202 (78)	57 (22)
5. Did you take all your medicines yesterday?	41 (15.8)	218 (84.2)
6. When you feel like your symptoms are under control, do you sometimes stop taking your medicine?	231 (8.2)	28 (10.8)
7. Taking medicine every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your treatment plan?	206 (79.5)	53 (20.5)
8. How often do you have difficulty remembering to take your medicine? A: Never/Almost never, B: Occasionally, C: Sometimes, D: Usually, E: Always	10 (3.9)	249 (96.1)

*MMAS-8: Morisky Medication Adherence scale -8

Table 2. Distribution of adherence according to Beck Depression scale*

	Without depression n (%)	With depression n (%)	Total	p value
Low adherence	193 (82.1)	42 (17.9)	235	0.011
Medium adherence	12 (60)	8 (40)	20	-
High adherence	2 (50)	2 (50)	4	-
Total	207 (79.9)	52 (20.1)	259	-

*BDS: Beck Depression score

Table 3. Distribution of adherence according to BAS*

	Low	Medium	High	Total	p value
Low adherence	183 (77.9)	22 (9.4)	30 (12.8)	235	<0.001
Medium adherence	12 (60)	3 (15)	5 (25)	20	
High adherence	0	1 (25)	3 (75)	4	
Total	195 (75.3)	26 (10)	38 (14.7)	259	

*BAS: Beck Anxiety scale

Discussion

Studies have associated adherence to antiviral therapy with permanent virologic response, and low level of resistance in patients with CHB infection. One of the goals of CHB treatment is to obtain a high adherence with nükleoside analog (NAs). When 1-year medication adherence was evaluated in CHB patients receiving NAs, the mean adherence was reported as 88%±19% (12). Among 259 patients in our study, low adherence was found in 235 (90.7%), medium adherence in 20 (7.7%), and high adherence in 4 (1.5%) patients. In our study, medication adherence rate was quite low. Whereas CHB patients are regularly followed-up and treated in our clinic. Since no significant increase was found in HBV-DNA and liver enzyme levels of our patients at follow-up, we were thinking that these patients were using their drugs regularly. In addition, patients reported at follow-up that sometimes they forgot to take their drugs or forgot at least once in 2 weeks according to Morisky scale, which we used in the present study, and this might caused to evaluate the scale as noncompliant. However, lamivudine, adefovir, entecavir, and tenofovir are well-tolerated. In clinical studies, rate of termination of the treatment due to side effects within the first year is only 3% (12). We did not primarily associate the low rate of adherence in our study with side effects. Another factor, which may affect patients' adherence is the prevalence of disease in the society. Chronic disease such as HT and DM are more common and more symptomatic than CHB in general population. They progress with more serious complications such as stroke if left untreated. These complications are generally known in our society. For these reasons, rate of adherence might be low in majority of patients. Therefore, CHB patients should be emphatically instructed about that they should take their medicines everyday, and should regularly come to follow-ups even if they had no symptoms. Considering all studies in general, marked differences are seen among the methods used, study design, number of patients, treatment regimens administered, duraton of follow-up, definition of adherence, and methods measuring adherence. These differences explain the adherence varying in a such variable range.

Studies investigating medication adherence, and changes of attitudes toward using medication according to sex, have found no correlation between these two variables, while one study has found a correlation in favour of women (13). In our study, medication adherence was higher in female patients, and this might be related to that majority of female patients were housewives and they have more opportunity to attend controls during day.

Among the symptoms of hepatitis and liver diseases, psychiatric disorders may often be seen. Depression may also be seen during course of CHB (14). In a study by İnci (15) 22% of the patients were found to have scores of 17 or higher, which is the cut-off value of depression. In our study also 79.9% had no depression, and 20% had depression, consistently with the study by İnci (15) (22%). BDI is not a psychiatric diagnostic test, and is a prediction test for the existence of depression. Therefore, patients with a high level of BDI were referred to psychiatry specialist for diagnosis and treatment.

Low expectation of sufficiency may lead to depression and anxiety or may be resulted from the symptoms of depression and anxiety. As a result, decrease in adherence level reduces self-efficacy and lead to decrease in medication adherence (16). In a review with 12 studies, patients with depression were found to be 3 times more nonadherent compared to patients without depression, and adherence was shown to be negatively affected by depression. In a study by Bautista et al. (17) with patients aged between 20-70 years in whom antihypertensive therapy was initiated, nonadherence to treatment developed within 3 months was found to be 2.5 folds higher in patients with mild depression, and 1.6 folds in patients with mild anxiety. Screening of the symptoms of depression and anxiety can be used to identify patients at high risk. Intervention targeting these conditions are essential to reveal increased adherence. In our study, patients' medication adherence was lower as BDI scores decreased. This might be resulted from low rate of adherence in vast majority of the patients. In addition, majority of our patients were male (57.1%). Furthermore, medication adherence and BDI level were higher in female patients. Therefore, high medication adherence in women might be resulted from higher level of depression.

Anxiety is the most common symptom among psychiatric symptoms in CHB patients (14). In a study by Chan et al. (18) on 160 Chinese patients with CHB, the rate of anxiety disorders was found as 14%. In our study, patients were evaluated according to BAI, 75.3% had low, 10% medium, and 14.7% high level of anxiety. As is seen, anxiety scores or anxiety rates vary in a wide range among the studies. This difference can be attributed to non-homogeneous groups, different methods used and differences in the number of patients.

In a study by Gois et al. (19) aiming to determine the relationship between adherence and measurements of depression and anxiety, high levels of depression and anxiety were demonstrated to be correlated with medication nonadherence. There are conflicting results about effects of anxiety disorders on medication adherence (20). In a study by Alcántara et al. (21) on 88 patients with uncontrolled HT, investigating anxiety susceptibility index and effects of this on adherence to BP drugs, patients with a high anxiety susceptibility were found to be more noninherent to antihypertensive drugs compared with the patients with a low anxiety susceptibility. In a study about the relationship between emotional problems and treatment adherence of hemodialysis patients, anxiety, cognitive disorder and social support were associated with depression, although these factors were not associated with treatment adherence. The reason for absence of a correlation between treatment adherence and anxiety was attributed to that physical anxiety symptoms (sweating, dizziness, numbness) recorded in surveys are found in kidney disease or the other diseases (22). In this study, looking the results of different surveys anxiety was found in quarter and mild-to-moderate depression in 40% of the samples as in the prevalences of other studies (23). In our study, medication adherence was found to be lower as BAI scores decrease. This is associated with a high level of adherence because of concerns of the patients with anxiety about their health.

Depression may cause anxiety to develop in more than half of patients (24). In a study investigating the relationship between emotional problems and treatment adherence in patients receiving hemodialysis, high levels of depression were found to be associated with high levels of anxiety (25). In our study, a significant

positive correlation was found between BAI and BDI scores. In a study by Cuhadaroğlu (26), it was stated that association of the symptoms of depression and anxiety shows parallelism with the diagnosis of depression from a descriptive perspective, and both conditions may be pathologies intertwined on the same line in terms of the pathogenesis.

Conclusion

Patients should be emphatically instructed by the physician about that should regularly come to follow-ups, and they should take their medicines regularly. Assoociation of anxiety and depression is often seen in CHB patients. Therefore, CHB patients should also be followed up psychiatrically, and should be referred to a psychiatry specialist if deemed necessary. In our study, conditions prone to pathology were found to increase medication adherence. Further studies should be conducted to evaluate the correlation of medication adherence with the levels of anxiety and depression. Further studies with larger groups of patients that will investigate medication adherence and nonadherence, reveal their causes and influencing factors are warranted.

Ethics

Ethics Committee Approval: After receiving approval from Necmettin Erbakan University Meram Faculty of Medicine Ethics Committee (approval number: 2014/687, date: 20.06.2014).

Informed Consent: Informed consent form was received from patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Research Article

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Evaluation of Signal/Cut-off Ratio by Anti-hepatitis Delta Virus Enzyme Immunoassay Method in the Diagnosis of Hepatitis Delta Virus Infection

Hepatit Delta Virüs Enfeksiyonunun Tanısında Anti-Hepatit Delta Virüs Enzim İmmünoassay Yöntemi ile Sinyal/Cut-off Oranının Değerlendirilmesi

Ayfer Bakır, Nuran Karabulut, Sema Alaçam, Murat Yaman, Mustafa Önel, Ali Ağaçfidan

İstanbul University, İstanbul Faculty of Medicine, Department of Medical Microbiology, Division of Virology and Fundamental Immunology, İstanbul, Turkey

ABSTRACT

Objectives: The hepatitis delta virus (HDV) is an enveloped, negative-sense, single-stranded RNA virus in the genus Deltavirus. In this study, it was aimed to evaluate the ratio of anti-HDV signal/cut-off (S/CO) in the diagnosis of HDV infection in patients who were positive for anti-HDV.

Materials and Methods: Between August 2014 and December 2018, 156 patients, who were detected hepatitis B surface antigen (HBsAg) and anti-HDV positivity and were analyzed HDV-RNA, were included in the study. Anti-HDV antibody and HBsAg tests were analyzed by micro-ELISA method in serum samples and HDV-RNA was studied by real-time polymerase chain reaction method in plasma samples.

Results: HDV-RNA was detected in 42.9% (67/156) of the anti-HDV positive patients. The mean S/CO value of anti-HDV in HDV-RNA positive group (8.99±3.53) was significantly higher than HDV-RNA negative group (5.99±3.73) ($p<0.001$). When the S/CO value was determined as 6.13 by receiver operating characteristic (ROC) analysis; sensitivity, specificity, positive, and negative predictive values were 79.8%, 59.7%, 72.4%, and 68.9%, respectively.

Conclusion: In this study, the optimal cut-off value which provides the maximum sum of sensitivity and specificity for the test was found 6.13 by ROC analysis. The reporting with the S/CO ratio of anti-HDV ELISA results and determining each laboratory's optimal cut-off value may be helpful for the diagnosis of HDV infection.

Keywords: HDV, HDV infection, ROC analysis

ÖZ

Amaç: Hepatit delta virüsü (HDV), Deltavirus genusunda, zarflı, negatif polariteli, tek iplikli bir RNA virüsüdür. Bu çalışmada, anti-HDV pozitif olan hastalarda HDV enfeksiyonu tanısında anti-HDV sinyal/cut-off (S/CO) oranının değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Ağustos 2014-Aralık 2018 tarihleri arasında hepatit B yüzey antijeni (HBsAg) ve anti-HDV pozitif saptanan ve HDV-RNA testi çalışılmış toplam 156 hasta çalışmaya dahil edildi. Anti-HDV antikoru ve HBsAg testleri serum örneklerinde mikro-ELISA yöntemi ile, HDV-RNA testi ise plazma örneklerinde real-time polimeraz zincir reaksiyonu yöntemi ile çalışıldı.

Bulgular: Anti-HDV pozitif hastaların %42,9'unda (67/156) HDV-RNA saptandı. HDV-RNA pozitif grubun anti-HDV S/CO ortalaması (8,99±3,53), HDV-RNA negatif gruba (5,99±3,73) göre anlamlı olarak yüksek saptandı ($p<0,001$). Alıcı işletim karakteristiği (ROC) analizi ile S/CO değeri 6,13 belirlendiğinde; duyarlılık, özgüllük, pozitif ve negatif prediktif değerler sırasıyla %79,8, %59,7, %72,4 ve %68,9 idi.

Sonuç: Bu çalışmada, ROC analizi ile test için maksimum duyarlılık ve özgüllük toplamını sağlayan optimal cut-off değeri 6,13 bulundu. Anti-HDV ELISA sonuçlarının S/CO oranı ile bildirilmesi ve her laboratuvarın optimal cut-off değerini belirlemesi, HDV enfeksiyonu tanısında yol gösterici olabilir.

Anahtar Kelimeler: HDV, HDV enfeksiyonu, ROC analizi

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Address for Correspondence: *Ayfer Bakır MD, İstanbul University, İstanbul Faculty of Medicine, Department of Medical Microbiology, Division of Virology and Fundamental Immunology, İstanbul, Turkey*
Phone: +90 546 627 20 18 E-mail: dr.ayfer.bakir@gmail.com ORCID ID: orcid.org/0000-0001-7566-5427 **Received:** *23.06.2019* **Accepted:** *18.11.2019*

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Introduction

The hepatitis delta virus (HDV), which is the only member of the only species in the genus Deltavirus, is an enveloped, negative-sense, single-stranded RNA virus (1,2). HDV requires the simultaneous presence of hepatitis B virus (HBV) to complete its life cycle (3). Although HDV suppresses HBV replication, it causes variable clinical presentation ranging from mild disease to fulminant liver failure (4). It is estimated that 18 million hepatitis B surface antigen (HBsAg) carriers in the world also have anti-HDV antibodies, which is 5% of HBV-infected individuals (5). The Amazon region of South America, the Middle East, the Mediterranean region, West and Central Africa are highly endemic areas for HDV (6). Turkey is considered a moderate endemic area for HDV infection with regional differences (7,8). HDV can cause co-infection with HBV or superinfection in people infected with HBV (9). Diagnosis of coinfection or superinfection with HDV is based on serological and molecular methods. The diagnosis of acute HDV co-infection is based on the detection of HDV-Ag, HDV-RNA and anti-HDV antibodies, together with markers of HBV infection. HBV-DNA, an indicator of HBV replication, could be suppressed during acute HDV infection and could not be detected (10,11). Therefore, all HBsAg positive patients should be analyzed anti-HDV antibody testing and especially should be recommended in case of acute hepatic exacerbation (10). The diagnosis of chronic HDV infection is determined by the absence of immunoglobulin M (IgM) antibody against hepatitis B core antigen and the presence of HBV and HDV infection markers. HDV-RNA is the gold standard method for the diagnosis of HDV infection due to sensitivity and specificity problems in HDV-Ag tests (11).

The first step in the diagnosis of HDV is anti-HDV antibody screening against HDV-Ag in individuals with positive HBsAg (9). Although the sensitivity of ELISA tests is high, populations with low prevalence have lower positive predictive values and higher false positivity rates. The aim of this study was to evaluate the anti-HDV signal/cut-off (S/CO) ratio in the diagnosis of HDV infection in patients with anti-HDV positivity.

Materials and Methods

Study Group

This retrospective study included 156 patients, who were detected HBsAg and anti-HDV positivity and were analyzed HDV-RNA, between August 2014 and December 2018. Ethical approval for this study was obtained by the Ethics Committee of İstanbul University Faculty of Medicine (approval number: 2018/1766/84).

Serological Analysis

Anti-HDV and HBsAg tests in serum samples were performed on Triturus Enzyme Immunoassay Analyzer (Grifols, Spain) using micro-enzyme immunoassay (ELISA) kits (Dia. Pro, Diagnostic Bioprobes, Milano, Italy). The cut-off value was calculated according to the manufacturer's recommendations. Test results were calculated by proportioning the sample absorbance to the cut-off value. If S/CO ratios are ≥1.00, the result of the test is evaluated as positive.

Molecular Analysis

Between August 2014 and October 2016, HDV-RNA extraction was performed using the High Pure Viral Nucleic Acid kit (Roche Applied Science, Basel, Switzerland) in plasma samples. The RNA molecule was transformed into complementary DNA (cDNA) using the Transcriptor First Strand cDNA Synthesis V6 kit (Roche Diagnostics, Mannheim, Germany). The cDNA was amplified by the LightCycler 2.0 instrument (Roche Diagnostics GmbH, Switzerland) using a polymerase chain reaction (PCR) mixture prepared with the TIB Molbiol HDV GmbH kit (Berlin, Germany) and the Light Cycler FastStart Master HybProbe kit (Roche Diagnostics, Germany). Between October 2016 and December 2018, HDV-RNA was obtained using the Qiagen EZ1 virus mini kit V2 nucleic acid extraction kit (Qiagen, Germany). Reverse transcription and amplification of RNA molecules was performed using a PCR mixture prepared with Fluorion HDV QNP 1.0 Real-Time PCR Kit (Iontek, İstanbul, Turkey) by Rotor-Gene Q instrument (Qiagen, Germany).

Statistical Analysis

Data analysis was performed using SPSS 25 (SPSS Inc, Chicago, IL, USA) program. The suitability of the variables to normal distribution was examined by visual methods (histogram and probability graphs) and Kolmogorov-Smirnov test. Variables were compared using Student's t-test or Mann-Whitney U test. Pearson chi-square or Fisher exact tests were used for qualitative variables. Sensitivity, specificity, negative predictive and positive predictive values were investigated by determining the significant cut-off values of the test by receiver operating characteristic (ROC) curve analysis. P value of less than 0.05 was considered statistically significant.

Results

Of the 156 anti-HDV positive patients with a mean age of 48.89±11.65 years (range: 19-90 years), 49.4% were male and 50.6% were female. The mean age of the male and female patients was 47.74±11.93 and 50.01±11.34, respectively (p=0.22). HDV-RNA positivity was detected in 42.9% (67/156) of anti-HDV positive patients.

The mean age of the viremic patients was 49.66±10.20 and the mean age of the non-viremic patients was 48.31±12.67 (p=0.48). The mean S/CO of anti-HDV positive patients was 7.70±3.90 (1.18-19.1). The mean S/CO value of anti-HDV in HDV-RNA positive group (8.99±3.53) was significantly higher than HDV-RNA negative group (5.99±3.73) (p<0.001) (Figure 1). A total of 67 (45.9%) samples with S/CO ≥ 1 by the Dia. Pro anti-HDV assay was false-positive.

When the S/CO value was 6.13 by ROC analysis, a total of 27 (27.6%) samples with S/CO ≥6.13 was false-positive. When S/CO ≥ 6.13, the sensitivity, specificity, positive and negative predictive values were 79.8%, 59.7%, 72.4% and 68.9%, respectively. A total of 24 (28.2%) samples with S/CO ≥ 7.15 was false-positive. When S/CO ≥ 7.15, sensitivity, specificity, positive and negative predictive values were 68.5%, 64.2%, 71.8% and 60.6%, respectively. The area under the curve (AUC) was 0.72 (95% CI: 0.64-0.80), p<0.001 (Figure 2). The performance results of the Dia. Pro anti-HDV test kit at different S/CO values are presented in Table 1.

Discussion

The first approach in the diagnosis of HDV infection is to investigate the antibodies against HDV-Ag in HBsAg-positive individuals. Anti-HDV antibody can be detected in immunocompetent patients with HDV infection (10). While anti-HDV immunoglobulin G (IgG) antibodies are detected in individuals with HDV infection even after clearance of the virus, anti-HDV IgM antibodies can be detected as a serological marker of HDV replication in the majority of patients with acute infection (12). Although anti-HDV IgM antibodies are indicative of acute HDV infection, anti-HDV IgM antibody tests may not be able to detect low antibody titers. Commercial anti-HDV antibody ELISA kits detected both IgM and IgG are preferred for HDV screening in HBsAg positive patients (13,14). However, HDV-RNA is the only reliable parameter of HDV replication.

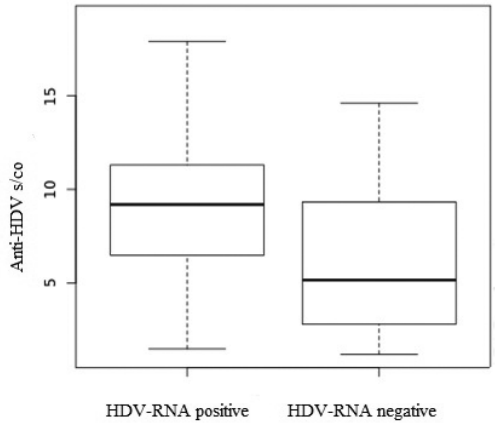

Figure 1. Anti-HDV S/CO ratios in HDV-RNA negative and positive group
HDV: Hepatitis delta virus, S/CO: Signal/cut-off

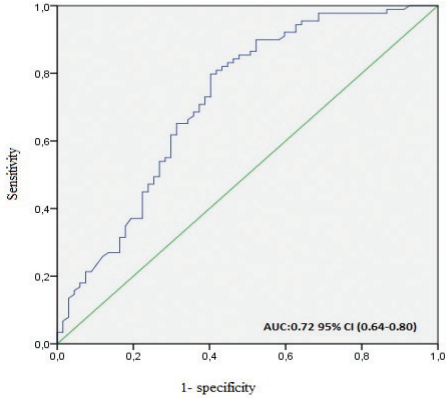

Figure 2. The receiver-operating characteristic curve of anti-HDV S/CO ratio for predicting HDV viremia
HDV: Hepatitis delta virus, S/CO: Signal/cut-off, AUC: Area under the curve, CI: Confidence interval

In this study, HDV-specific total antibodies were investigated and reported semi-quantitatively by determining the S/CO ratio. The mean S/CO of anti-HDV in HDV-RNA positive group (8.99±3.53) was significantly higher than HDV-RNA negative group (5.99±3.73) ($p<0.001$). The results were consistent with rare studies on this subject (15). In a cross-sectional study performed using the anti-HDV radio-immunoassay kit (Abbott Laboratories, Chicago, IL, USA), it was demonstrated that high anti-HDV titers were correlated with HDV viremia (16).

Although studies on prediction of viremia of ELISA S/CO ratio have been performed mostly with the hepatitis C virus (HCV), such studies for HDV are quite limited. Anti-HCV S/CO ratios for prediction of HCV viremia in different studies, differences have shown depending on the size of the sample, HCV prevalence in the studied population, and kit differences (17).

It is of interest to define the best S/CO cutoff before using anti-HDV tests in the clinical routine. Thus, it could be decreased false-positive results. S/CO can vary across kits, and populations with different HDV infection rates. When S/CO ≥ 1 by the recommendation of the manufactory, 45.9% of samples had false-positive by the Dia. Pro anti-HDV assay. When S/CO ≥ 6.13, false-positivity decreased to 27.6%. In the present study, it was observed that the false-positive ratio decreased when the S/CO ratio increased.

In a study performed with the anti-HDV antibody ELISA kit (Hepanostika HDV, Organon Teknika, the Netherlands) in Turkey; when the ELISA index value was 100.10, sensitivity, specificity, negative and positive predictive values were 93%, 80%, 93%, 76% respectively. The AUC was found to be 0.934 (15). In this study, the optimal cut-off value which provides a total of the maximum sensitivity and specificity for the test was found 6.13. The AUC was found 0.72 (95% CI: 0.64-0.80), $p<0.001$ by ROC curve analysis. Results below the optimal cut-off value should be retested with HDV-RNA and another anti-HDV kit. Determination

Table 1. Performance of the Dia. Pro anti-HDV assay according to S/CO ratios

	S/CO[§]		
	≥1	≥6.13	≥7.15
Number of samples	146	98	85
False positives rates	67 (45.9%)	27 (27.6%)	24 (28.2%)
True positive rates	89 (54.1%)	71 (72.4%)	61 (71.8%)
Sensitivity	*	79.8% (69.9%-87.6%)	68.5% (57.8%-78.0%)
Specificity	*	59.7% (47%-71.5%)	64.2% (51.5%-75.5%)
Positive predictive value	*	72.4% (65.9%-78.2%)	71.8% (64.2%-78.3%)
Negative predictive value	*	68.9% (58.5%-77.8%)	60.6% (51.9%-68.7%)

[§]S/CO: Signal/cut-off, *Since HDV-RNA test was not performed to anti-HDV negative samples, it could not be calculated

of cut-off index value in test kits could be clinically important for predicting true HDV viremia.

Conclusion

This study showed that Dia. Pro anti-HDV antibody test kit had a good clinical performance for anti-HDV S/CO value of 6.13. The S/CO ratio in the HDV test could be used in the clinical decision-making process if it can correctly predict the diagnosis of HDV before HDV-RNA is analyzed. In addition to anti-HDV ELISA results, reporting of S/CO ratio and determining each laboratory's optimal cut-off value may be helpful for the diagnosis of HDV infection.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained by the Ethics Committee of İstanbul University Faculty of Medicine (approval number: 2018/1766/84).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.B., A.A., Design: A.B., A.A., Data Collection or Processing: A.B., N.K., S.A., M.Y., M.Ö., Analysis or Interpretation: A.B., N.K., S.A., Literature Search: A.B., M.Y.

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

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Research Article

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Chronic Hepatitis C Prevalence and Physician Awareness in Southeastern Turkey

Türkiye'nin Güneydoğu'sunda Kronik Hepatit C Prevalansı ve Hekim Farkındalığı

Nurettin Tunç

University of Health Sciences, Gazi Yaşargil Training and Research Hospital, Clinic of Gasttroenterology, Diyarbakır, Turkey

ABSTRACT

Objectives: This study aimed to investigate anti-hepatitis C Virus (HCV) positivity and physician-patient awareness in Southeastern Turkey.

Materials and Methods: Age, gender and laboratory data of all patients aged 17 years and above, who referred to University of Health Sciences, Diyarbakır Gazi Yaşargil Training and Research Hospital for any reason between August 2016-April 2018 and underwent anti-HCV testing, were evaluated retrospectively. Dates of anti-HCV positive results and demographic data, such as age and gender of the patients with anti-HCV positivity were retrieved from the hospital's information systems.

Results: In the present study, 120.091 cases were tested for anti-HCV and a total of 855 (0.7%) results were positive, i.e. 553 women (0.61%) and 302 men (0.98%). Anti-HCV was positive in 50 (0.04%) cases under 20 years of age, in 361 (0.48%) cases aged 21-40 years, 237 (1.17%) cases aged 41-60 years, and 207 (1.57%) cases above 61 years of age (p=0.000). The mean delay in diagnosis from the time of anti-HCV identification was 74.88 weeks.

Conclusion: Anti-HCV positivity in our region was found to be consistent with that of regions with low prevalence in Turkey and worldwide. Campaigns to improve awareness among patients and physicians are warranted to prevent delays in HCV diagnosis.

Keywords: Chronic hepatitis C, prevalence, awareness

ÖZ

Amaç: Bu çalışmada amaç, Türkiye'nin Güneydoğu'sunda anti-hepatit C virüs (HCV) pozitifliği ve hekim-hasta farkındalığını saptamaktır.

Gereç ve Yöntemler: Sağlık Bilimleri Üniversitesi, Diyarbakır Gazi Yaşargil Eğitim ve Araştırma Hastanesi'ne Ağustos 2016-Nisan 2018 tarihleri arasında herhangi bir nedenle başvurup anti-HCV bakılan 17 yaş ve üzeri tüm hastaların yaş, cinsiyet ve laboratuvar verileri retrospektif olarak incelendi. Anti-HCV pozitif saptandığı tarih ve anti-HCV pozitifliği olan hastaların yaş ve cinsiyet gibi demografik veriler hastane bilgi sistemlerinden elde edildi.

Bulgular: Çalışmamızda 120,091 olguda anti HCV bakılmış; 553'ü (%0,61) kadın, 302'si (%0,98) erkek, toplam 855'inde (%0,7) pozitif saptanmıştı. Anti-HCV 20 yaş altı 50 (%0,04), 21-40 yaş arası 361 (%0,48), 41-60 yaş arası 237 (%1,17) ve 61 yaş üstü 207 (%1,57) olguda pozitifti. Anti-HCV saptanmasından sonra tanıda gecikme ortalama 74,88 hafta idi.

Sonuç: Bölgemiz anti-HCV pozitifliği Türkiye ve Dünya'da düşük prevalanslı bölgelerle uyumlu saptandı. Hepatit C tanısında gecikmeyi önlemek için hasta ve hekimlerin farkındalığını arttıracak programlara ihtiyaç vardır.

Anahtar Kelimeler: Kronik hepatit C, prevalans, farkındalık

Tunç N. Chronic Hepatitis C Prevalence and Physician Awareness in Southeastern Turkey. Viral Hepat J. 2019;25:101-104.

Introduction

Chronic hepatitis C (HCV) is a major global pathogen and the relevant public health problems are expected be increased in the upcoming years (1,2). Even though chronic HCV is endemic worldwide, its distribution considerably varies by geographic location (3). The highest prevalence rates are reported in Africa and Asia while the regions with low prevalence include the developed countries in North America, Northern and Western Europe, and Australia. Three percentage of world population is chronically infected with chronic HCV, representing an important cause of chronic liver diseases such as cirrhosis, liver fibrosis, hepatocellular

Address for Correspondence: *Nurettin Tunç MD, Health Sciences University Gazi Yasargil Training and Research Hospital, Clinic of Gasttroenterology, Diyarbakır, Turkey*
Phone: +90 412 258 00 60 E-mail: nurettin@firat.edu.tr ORCID ID: orcid.org/0000-0002-9723-4510 **Received:** *08.08.2019* **Accepted:** *15.11.2019*

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carcinoma (HCC) and liver failure (4). HCV is one of the important causes of cirrhosis and HCC. 27% of cirrhosis cases and 25% of HCCs are associated with HCV around the world (5). The role of HCV in the etiology of chronic hepatitis cases in our country has increased in recent years (6). Contribution of HCV to chronic hepatitis cases has increased from 23% to 38.1% for the last 10 years (7), and its contribution to cirrhosis has increased from 25.2% to 45.9% (8).

In a recent study conducted by World Health Organization (WHO) in Europe, HCV prevalence was estimated 2.4% for Western and Central Europe, and 2.9% for Eastern Europe (9).

The rate of anti-HCV positivity has been found to be 0.54% in donor screenings and 1.15% in the general population in our country. Based on these data, the provinces with an anti-HCV positivity rate higher than 1% are Afyon, Düzce, Erzurum, Manisa and Samsun (3). Among the cities with high prevalence in adults, the rate was found to be 1.03-1.75% in Afyon, 1.2% in Erzurum, 1.3% in İzmir and 2.1% in Tokat (10,11).

This study aimed to investigate anti-HCV positivity and physician-patient awareness in Southeastern Turkey.

Materials and Methods

Data of all patients aged 17 years and above, who referred to University of Health Sciences, Diyarbakır Gazi Yaşargil Training and Research Hospital for any reason between August 2016-April 2018 and underwent anti-HCV testing, were evaluated retrospectively. Dates of anti-HCV positive results and demographic data, such as age and gender of the patients with anti-HCV positivity were retrieved from the hospital's information systems. Anti-HCV positive cases were stratified based on age, i.e. younger than 20 years of age, aged 21-40 years, aged 41-60 years and aged 61 years and above. The duration (weeks) from the date of anti-HCV positive result and the date of HCV-RNA testing (delay in diagnosis) was calculated and classified as a delay of 1-12 weeks (three months), 12-52 weeks (3-12 months) or more than 52 weeks (longer than 12 months).

Patients with missing data were not included in the study.

Statistical Analysis

Statistical analysis of all data was performed using the SPSS 21.00 package program. Results are presented as percentage for categorical variables and mean ± standard deviation or median [minimum (min)-maximum (max)] for continuous variables. Chi-square and Fisher's exact test were utilized for the comparison of group rates.

Results

In the present study, 120.091 cases were tested for anti-HCV and a total of 855 (0.7%) results were positive, i.e. 553/89452 women (0.61%) and 302/30639 men (0.98%) (p=0.000). Mean age was 44.48 years for anti-HCV-positive cases (min-max: 17-93). Of 855 cases with anti-HCV positivity, only 248 (29%) were tested for HCV-RNA and received a diagnosis (rate of diagnosis). Among those tested for HCV-RNA, 42/248 (16.9%) were positive and the rate of HCV-RNA positivity in the general population was found to be 0.03% (Table 1).

Table 1. Demographic and laboratory characteristics of Hepatitis C virus

	n (%)	**p (<0.05)**
Age (mean ± SD) (year)	44.8±18.54	-
Gender	**n (%)**	**0.00**
Female	553 (0.61)	-
Male	302 (0.98)	-
Anti-HCV	855 (0.7)	-
HCV-RNA	42/248 (16.9)	-
Delay in diagnosis (weeks)	**n (%)**	**-**
1-12	21 (50)	-
12-52	6 (14.3)	-
>52	15 (35.7)	-
Mean	74.8	-
HCV-RNA + (year)	**-**	**-**
<20	1 (2.4%)	-
21-40	6 (14.3%)	-
41-60	18 (42.9%)	<0.05
>60	17 (40.5%)	
Anti-HCV (year)	**-**	**0.000**
<20	50/11227 (0.04%)	-
21-40	361/745648 (0.48%)	-
41-60	237/20209 (1.17%)	-
>60	207/13152 (1.57%)	

SD: Standard deviation, HCV: Hepatitis C virus

Anti-HCV was positive in 50 (0.04%) cases under 20 years of age, in 361 (0.48%) cases aged 21-40 years, 237 (1.17%) cases aged 41-60 years, and 207 (1.57%) cases above 61 years of age. Increased age was associated with increasing rates of anti-HCV positivity (p=0.000). Of those with anti-HCV positivity, 51.9% were over 40 years of age (Table 1).

Mean age was 56.1 years among the 42 cases with HCV-RNA positivity (min-max: 18-87), with 21 males and 21 females. HCV-RNA was positive in 1 patient (2.4%) under the age of 20 years, 6 patients (14.3%) aged 21-40 years, 18 patients (42.9%) aged 41-60 years and 17 patients (40.5%) aged 61 years or above, and HCV-RNA positivity was more common (83.4%) in those older than 40 years of age (p<0.05) (Table 1).

Mean duration from a positive anti-HCV result to the HCV-RNA testing request (delay in diagnosis) was 74.88 weeks (min-max: 1-308). Three months of delay in diagnosis was noted in 21 patients (50%) while the delay was 3-12 months in 6 patients (14.3%) and more than 12 months in 15 patients (35.7%) (Table 1).

Discussion

Based on recent estimates by WHO, 185 million people are chronically infected with HCV worldwide. Most people infected with HCV are unaware of their infection and most of diagnosed ones cannot still access the treatment (12).

Anti-HCV positivity may indicate a previous and resolved infection, a persistent infection or HCV infection. Anti-HCV may disappear at the end of 10 years in those with resolved acute hepatitis C; therefore, HCV may be considered as an infection which is more common than identified (5).

Prevalence of HCV infection varies around the world. Global prevalence of HCV infection is estimated to be approximately 2.2-3% (13). Stratification of regions based on the incidence of HCV infection is as follows: Egypt and Central Africa (10-20%) (14), Pakistan (8.4%) (15), Central and South Asia, and North Africa/Middle East (12) are the regions/countries with the highest prevalence, while Northern Europe countries such as Germany (0.6%) (16), France (1.1%) (17), Austria (1.8%) (18,19) are countries with the lowest incidence. HCV prevalence has been reported as 3.2% in China (20) and 0.9% in India (21). HCV incidence is 1-2.4% in our country. Studies conducted in our country report that the rate of anti-HCV positivity ranges from 0.05% (blood donors) to 51.6% (hemodialysis patients) across different groups (22). In a study on blood donors in Turkey, anti-HCV positivity was observed at a rate of 2.6% in Şanlıurfa, 2.1% in Tokat (11), 0.4% in İstanbul (23), 1% in Hakkari (24) and 0.62% in Siirt (25) while anti-HCV positivity was observed in 0.7% of the cases in the present study. This rate was consistent with data from Turkey and from regions with low prevalence.

Gregory et al. (26). Showed a significantly higher prevalence of anti-HCV among men compared to women (2.1% vs 1.1%). Consistent with the literature, our study revealed a significantly higher rate of anti-HCV in males compared to females (0.61% in women and 0.98% in men; p=0.000).

In the present study, mean age was 44.48±18.54 years for anti-HCV-positive cases (min-max: 17-93), similar to the mean age (44±9.51 years) reported by Afridi et al. (27).

In a study, anti-HCV prevalence was as follows: 1.0% for 20-29 years, 4.3% for 40-49 years, 1.6% for 50-59 years, and 0.9% for 60 years of age and above (26). Another study reported similar findings with higher HCV prevalence in those aged 40-49 years (28) while the lowest rate of HCV infection was observed in those above 60 and under 19 years of age. A study in Tokat region of our country showed an increasing age-specific prevalence starting from 40 years of age with rates reported as 4.2% for those aged 50-59 years, 3.4% for those aged 60-69 years, and 7.1% for those aged 70-79 years, which is the highest (11). In the present study, 51.9% of the cases were above 40 years of age; however, higher rates of anti-HCV positivity were observed with increasing age, compatible with literature (Table 1) (p=0.000). We believe that it is associated with insufficient awareness among patients and physicians in our population.

In a study which tested HCV-RNA in blood samples of anti-HCV-positive participants, positivity was more common in men compared to women (89.0% vs 63.4%) (26). A higher rate of positivity was noted in those aged 40 years and above (89.6%) compared to individuals younger than 40 years of age (60.2%) (26). Differing from the literature, our study revealed an equal gender distribution of HCV-RNA positivity (21 females, 21 males). HCV-RNA positivity was found to be more common (83.4% in total) in those older than 40 years of age (p<0.05) and this finding was consistent with the literature. The asymptomatic nature of HCV and insufficient awareness lead to delays in diagnosis and treatment.

Timely diagnosis of HCV is of critical importance to implement strategies that aim to reduce the healthcare burden in the future (2). Low rates of diagnosis constitute the main barrier in this regard (2). A trend to higher diagnosis rates is noted in countries such as Austria, Denmark, France, Germany, Sweden and Switzerland (2). Rates of diagnosis vary across Europe from 31% in Czech Republic to 81% in Sweden (2). In the present study, the rate of diagnosis was 29%, which is consistent with regions of low diagnosis rates. On the other hand, the mean duration from anti-HCV positivity to diagnosis was 74.88 weeks (approximately 1.5 years), representing a delay of 1.5 years in diagnosis; delay was three months in 50%, 3-6 months in 15% and more than one year in 35% of the patients. The main focus of a national prevention program is to recommend routine screening tests for those likely to be infected with HCV (29). The low rates of diagnosis in our region reflect the inadequacy of screening strategies and underline the need to revise relevant methods. We believe this issue may be resolved by raising awareness at community level and by informing physicians through an alerting computer program for anti-HCV-positive cases.

Conclusion

Anti-HCV prevalence in our region is consistent with the data from Turkey and other regions with low prevalence. The time from detection of anti-HCV positivity to diagnosis is noted to be too long. This results in delayed diagnosis and complications. Delays in diagnosis should be prevented by raising awareness among patients and through an alerting computer program for physicians concerning anti-HCV-positive cases.

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Ethics

Ethics Committee Approval: Retrospectively study.

Informed Consent: Retrospectively study.

Peer-review: Externally and internally peer-reviewed.

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

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Efficacy of Direct-acting Antivirals in Hemodialysis Patients with Chronic Hepatitis C: A Real-life Retrospective Study

Kronik Hepatit C'li Hemodiyaliz Hastalarında Doğrudan Etkili Antivirallerin Etkinliği: Gerçek Hayatta Retrospektif Bir Çalışma

Nurten Nur Aydın[1], Firdevs Aksoy[1], İlknur Yavuz[2], Serap İskender[3], Arzu Altunçekiç Yıldırım[4], İlknur Esen Yıldız[5], İftihar Köksal[1]

[1]*Karadeniz Technical University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Trabzon, Turkey*
[2]*Giresun University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Giresun, Turkey*
[3]*University of Health Sciences, Kanuni Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Trabzon, Turkey*
[4]*Ordu University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Ordu, Turkey*
[5]*Recep Tayyip Erdoğan University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Rize, Turkey*

ABSTRACT

Objectives: Hepatitis C virus (HCV) infection is common among hemodialysis (HD) patients and is associated with increased morbidity and mortality. New generation direct-acting antiviral (DAA) agents are safe and effective in treatment HCV infection in HD patients. The aim of this a multi-center study was to assess the efficacy of DAAs in HD patients with HCV infection.
Materials and Methods: HD patients with HCV infection followed-up at five centers were included in this retrospective cohort study. Patients demographic and virological characteristics, liver fibrosis status, end of treatment and sustained virologic responses (SVR12) at 12 weeks after treatment were recorded. Treatment of the patients was arranged according to the genotype and drug interactions considering guidelines.
Results: Ninety percent of 20 patients were genotype 1b and were treated for 12 weeks with paritaprevir-ritonavir-ombitasvir-dasabuvir; one patient was genotype 4 and received PrOD + ribavirin (RBV) for 12 weeks; and one patient was genotype 3 and was treated with sofosbuvir + RBV for 24 weeks. HCV-RNA negativity was achieved in all patients at the end of treatment and SVR12 rate was 100%. Significant side effects were not observed in any patients, apart from sleeplessness in one patient and itching in another.
Conclusion: Our real-life data support that new generation DAAs achieve high SVR and are well tolerated in HD patients with HCV. In these patients, intolerance and side effects were not observed, which would otherwise require cessation of the DAA regimen.
Keywords: Hepatitis C, hemodialysis, direct-acting antiviral agent

ÖZ

Amaç: Hepatit C virüsü (HCV) enfeksiyonu hemodiyaliz (HD) hastalarında sık görülür ve artmış morbidite ve mortalite ile ilişkilidir. Yeni nesil direkt etkili antiviral (DAA) ajanları, HD hastalarında HCV enfeksiyonunun tedavisinde güvenli ve etkilidir. Bu çok merkezli çalışmada, HCV enfeksiyonu olan HD hastalarında DAA'nın etkinliğini değerlendirmeyi amaçladık.
Gereç ve Yöntemler: Bu retrospektif kohort çalışmaya beş merkezde HCV enfeksiyonu ile takip edilen HD hastaları dahil edildi. Hastaların demografik ve virolojik özellikleri, karaciğer fibroz durumu, tedavinin sona ermesi ve tedaviden 12 hafta sonra kalıcı virolojik yanıtlar (SVR12) kaydedildi. Hastaların tedavisi kılavuz ilkeleri göz önünde bulundurularak genotip ve ilaç etkileşimlerine göre düzenlendi.
Bulgular: Yirmi hastanın %90'ı genotip 1b idi ve 12 hafta boyunca paritaprevir-ritonavir-ombitasvir-dasabuvir (PrOD) ile tedavi edildi, bir hasta genotip 4 idi ve 12 hafta boyunca PrOD + ribavirin (RBV) aldı, ve bir hasta genotip 3 idi ve 24 hafta boyunca sofosbuvir + RBV ile tedavi edildi. Tedavi sonunda tüm hastalarda HCV-RNA negatifliği sağlandı ve SVR12 oranı %100 idi. Bir hastada uykusuzluk, diğerinde kaşıntı dışında hiçbir hastada önemli yan etkiler gözlenmedi.
Sonuç: Gerçek yaşam verilerimiz, HCV'li HD hastalarında yeni nesil DAA'ların yüksek SVR sağladığını ve iyi tolere edildiğini desteklemektedir. Bu hastalarda, DAA rejiminin kesilmesini gerektiren intolerans ve yan etkiler gözlenmedi.
Anahtar Kelimeler: Hepatit C, hemodiyaliz, direk etkili antiviral ajan

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Address for Correspondence: *Nurten Nur Aydın MD, Karadeniz Technical University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Trabzon, Turkey*
E-mail: nurtennurkenc@hotmail.com ORCID ID: orcid.org/0000-0003-4138-2490 **Received:** *01.08.2019* **Accepted:** *10.10.2019*

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Introduction

Hepatitis C virus (HCV) infection is the most common among hemodialysis (HD) patients and is an important cause of liver disease in this population. The risk of all-causes and liver-related mortality is higher in HD patients with HCV infection (1). The frequency of anti-HCV positivity in HD patients varied from 1.6% to 68% in the world (2).

In Turkey, the prevalence of HCV was ranged from 31.4-51% in HD patients at early 2000s (3). Currently, the prevalence of anti-HCV antibody in HD patients in Turkey is 5.2% (4). Studies have shown that prognosis in HD patients with HCV infection is significantly worse than in HD patients without HCV infection (5,6). Until recently, sustained virologic response (SVR) was achieved in approximately 50% of patients with chronic kidney disease (CKD) infected with HCV using the recommended gold standard therapy of pegylated interferon (peg-IFN) (7). However, severe side-effects and treatment compliance problems were observed in CKD patients using peg-IFN, and drug dosage adjustment and careful close follow-up were required (8). Major progress in the treatment of HCV has been made with the entry into use in recent years of direct-acting antivirals (DAAs), which target viral proteins, leading to increases in SVR and a marked decrease in side-effects (9). However, there is no standard treatment for HD patients with HCV infection. Insufficient data are available for the efficacy and reliability of DAAs in HD patients.

The aim of this study was to evaluate the efficacy of DAAs in HD patients with HCV infection.

Materials and Methods

Treatment-naive or experienced [IFN/pegIFN ± ribavirin (RBV)] 20 HD patients treated with DAAs for HCV infection were included in the retrospective cohort study from five centers in Turkey between June 2016 and May 2018. These centers were the Karadeniz Technical University Faculty of Medicine, Giresun University Faculty of Medicine, Kanuni Training and Research Hospital, Ordu University Faculty of Medicine, and Recep Tayyip Erdoğan University Faculty of Medicine, Department of Infectious Diseases. Demographic characteristics, clinical findings and treatment outcomes of patients were recorded. HCV-RNA, HCV genotype, blood chemistry and blood count were performed before treatment initiation. Ninety percent of patients were non-cirrhotic and 10% were compensated cirrhotic. Two patients were diagnosed with cirrhosis according to the clinical findings, imaging and non-invasive fibrosis score of the treating clinician, and no liver biopsy was performed.

Eighteen patients (90%) were genotype 1b, one each patients were genotype 3 and 4. Genotype 1b patients were treated with a 12-week paritaprevir-ritonavir-ombitasvir-dasabuvir (PrOD) regimen. Genotype 3 and 4 patients were treated with sofosbuvir + RBV for 24 weeks, and PrOD + RBV for 12 weeks, respectively.

Virological, biochemical and serological responses were evaluated 4, 12 and 24 weeks after the start of treatment, and at 36 weeks in the patient receiving 24-week treatment for SVR. This study was approved by Karadeniz Technical University Ethics Committee (approval number: 2019/254, date:20.09.2019). Since our study was retrospective, informed consent was not used.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics (version 23.0) program for Windows. The Wilcoxon signed ranks test was applied to compare differences between pre-treatment and 12th week laboratory values. P values <0.05 were regarded as statistically significant.

Results

All of the 20 patients were completed treatment. The mean age of the patients was 57.8 (±10.5) years. Ninety percent were men and 10% were women. Eleven (55%) patients were treatment-experienced and nine (45%) were treatment-naive. Five of the treatment-experienced patients (45.5%) were non-responders, and 6 patients (54.5%) were relapser. Ninety percent of patients were non-cirrhotic and 10% were compensated cirrhotic. The pre-treatment HCV-RNA median level (log_{10} IU/mL) was 5.2.

Patients' characteristics and basal laboratory values are shown in Table 1.

HCV-RNA was negative in 17 (85%) patients by the 4th week, and with the exception of a patient received sofosbuvir + RBV regimen. In all patients HCV-RNA was negative at the end of treatment and SVR rate was 100% (Table 2). HCV-RNA levels decreased rapidly after patients were started on antiviral therapy (Figures 1,2). Viral responses were independent of previous treatments and liver fibrosis status.

Patients' biochemical markers were also assessed after treatment. Serum alanine aminotransferase (ALT), levels decreased after the start of treatment (Figure 3). Patients' pre-treatment HCV-RNA and ALT values decreased significantly by the 12th week after

Table 1. Patients' characteristics and basal laboratory values

Patients number	**20**
Mean age	57.8 (±10.5)
Male/Female	18/2 (90%/10%)
HCV Genotype (1B/3/4)	(18/1/1) (90%)/(5%)/(5%)
Basal hemoglobin level (g/dL)	12.8 (6.6-17.6)
Basal platelet level (x10^3/µl)	142 (46-271)
Basal ALT level (units)	23.5 (5-56)
Basal HCV-RNA level (log_{10} IU/ml)	5.2 (2.3-7.1)
Liver cirrhosis	2 (10%)
Pre-treatment status (naive/ experienced)	9/11 (45%)/(55%)

HCV: Hepatitis C virus, ALT: Alanine aminotransferase

Table 2. The rates of HCV-RNA negatives during treatment

Virologic response	**n (%)**
4th week	17 (85)
12th week	19 (95)
24th week	20 (100)
End of treatment	20 (100)
SVR12*	20 (100)

HCV: Hepatitis C virus, *SVR12: Sustained virologic responses at 12 weeks

treatment (p<0.001 and p=0.001, respectively). No significant difference was determined in albumin or platelet levels.

Significant side effects were not observed in any patients, apart from sleeplessness in one patient and itching in another, and no complication developed. Both resolved spontaneously without additional treatment during follow-up. None of the patients developed side effects that required treatment interruption or discontinuation.

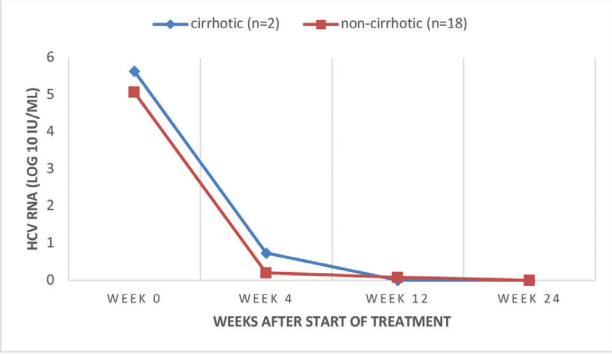

Figure 1. Mean HCV-RNA changes during treatment in cirrhotic and non-cirrhotic patients
HCV: Hepatitis C virus

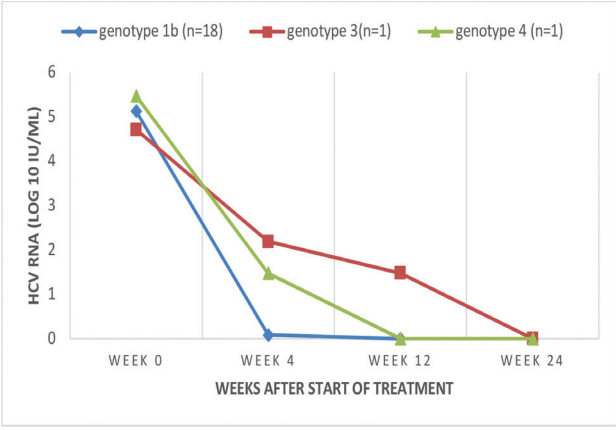

Figure 2. Mean HCV-RNA changes during treatment in genotye 1b, 3 and 4 patients
HCV: Hepatitis C virus

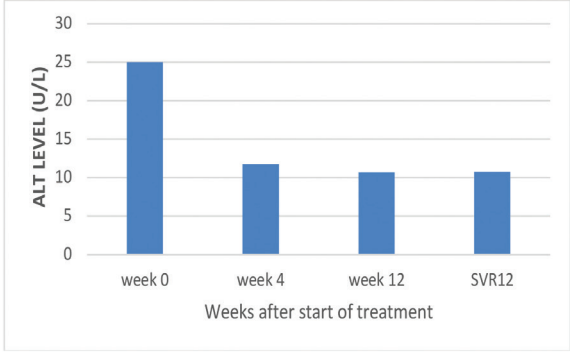

Figure 3. Changes in ALT after treatment
ALT: Alanine aminotransferase

Discussion

HCV infection is frequently seen in patients with CKD, including HD patients. Factors increasing the risk of HCV in HD patients include advanced age, number of blood transfusion, and the prevalence of HCV in the HD unit (10). HCV in CKD patients and pre- and post- kidney transplant patients increases the risk of all-causes and kidney-related mortality (1,10). The treatment of HCV in CKD patients is complex, but prognosis improves in case of treatment. Although HCV is frequently seen in HD units, experience of treatment is still limited (10). Peg-IFN, either alone or in combination with RBV, was until recently recommended as the standard treatment of CKD patients with HCV infection, and this treatment achieved a SVR rate of approximately 50% and has potential toxicity (7). Since RBV cannot be eliminated in patients with HD and CKD, accumulation results in significant side effects, particularly hemolytic anemia. This leads to RBV dose restriction (11). Recent studies have shown that DAAs are extremely well tolerated in CKD with HCV infection and have few side-effects (12,13).

Several clinical studies have confirmed the effectiveness and reliability of non-sofosbuvir containing regimens in patients with advanced kidney failure. In the RUBY-1 study, stage 4 and 5 CKD patients infected with non-cirrhotic genotype 1 were treated with PrOD for 12 weeks, and SVR12 was achieved at a rate of 90%. In the light of these data, it was concluded that the PrOD regimen can be safely used in stage 4 and 5 CKD patients without the need for dose adjustment (14). The EASL 2018 guideline also stated that no dose-adjustment is required for any approved DAA combinations in the treatment of mild and moderate kidney failure (1). The safety of sofosbuvir regimens has been questioned in patients with severe kidney failure. However, the data concerning the safety and efficacy of these regimens are inadequate (15). Sofosbuvir is eliminated by the renal pathway and its use is not recommended in CKD stage 4 or 5 or in patients requiring HD (1,8,15). However, Cox-North et al. (16) reported that treatment with sofosbuvir-based regimens is safe in patients with CKD stage 4 or 5 and infected with HCV if no alternative is available. The HCV-target study evaluated patients with decreased renal functions and determined a SVR rate of 83% for sofosbuvir-containing regimens, concluding that these have no adverse effect on renal functions (17).

In our study, 100% SVR12 was achieved in HD patients with genotype 1b HCV infection receiving PrOD therapy, including elderly patients and two with liver cirrhosis. Genotype 4 HCV infection was treated with PrOD + RBV and HCV-RNA was negative by the end of treatment and SVR12 was achieved. Treatment-experienced patients tolerated PrOD ± RBV better than regimens including peg-IFN. No significant side-effects were observed. One of the patients was non-cirrhotic and genotype 3 and was treated with sofosbuvir + RBV for 24 weeks. This patient's HCV-RNA was negative by the end of treatment, and SVR12 was achieved. No important side-effects other than sleeplessness were observed in our patients at the end of treatment. No suitable therapeutic dose of sofosbuvir for patients with advanced kidney failure has been determined in previous studies (1). Regimens not containing sofosbuvir must be employed for HCV infection in patients with advanced kidney failure or undergo dialysis. In case a sofosbuvir-based regimen has to be used, then close monitoring is required, and treatment must

be promptly modified if kidney functions are impaired or if any side-effect develops (1). Since no alternative was available in our patient infected with genotype 3, sofosbuvir + RBV therapy was initiated. The patient was placed under close observation, and no significant side-effect other than sleeplessness was observed.

Yaraş et al. (18) administered a PrOD regimen to 25 HD patients with HCV. In 92% of patients HCV-RNA was negative by the 4th week, and SVR12 was 100%.

HCV-related liver damage can accelerate immunosuppression. Antiviral therapy must therefore be considered in all HD patients scheduled for kidney transplantation (1). Studies have shown that renal transplanted patients, such as HD patients, have been successfully treated with non-interferon regimens (19,20,21).

Conclusion

Our study shows that DAAs are effective and reliable in HD patients. However, further studies with larger patient numbers examining the efficacy and reliability of these agents in HD patients are now needed. Establishing a course of treatment in HD patients is important for global eradication of HCV.

Ethics

Ethics Committee Approval: This study was approved by Karadeniz Technical University Ethics Committee (approval number: 2019/254, date: 20.09.2019).

Informed Consent: Since our study was retrospective, informed consent was not used.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

Conflict of Interest: Authors declare no conflict of interest.

Financial Disclosure: There was no aid and sponsor for this study.

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The Efficacy of Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir with or without Ribavirin in Patients with Hepatitis C Undergoing Chronic Haemodialysis: A Single Center Experience

Hepatit C Pozitif Kronik Hemodiyaliz Hastalarında Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir ± Ribavirin Tedavisinin Etkinliği: Tek Merkez Deneyimi

Nilay Danış[1], Hüsnü Pullukçu[2], Tansu Yamazhan[2], Galip Ersöz[1], Nalan Ünal[1], Fulya Günşar[1], İlker Turan[1], Zeki Karasu[1], Ulus Salih Akarca[1]

[1]*Ege University Faculty of Medicine, Department of Gastroenterology, İzmir, Turkey*
[2]*Ege University Faculty of Medicine, Department of Infectious Diseases and Clinic Microbiology, İzmir, Turkey*

ABSTRACT

Objectives: Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir (PrOD) seems to be highly effective and safe in chronic haemodialysis (CHD) patients. We presented our experiences of treatment with PrOD in CHD patients.

Materials and Methods: Between July 2016 and September 2018, a total of 25 CHD patients treated with PrOD were enrolled. The patients with hepatitis C virus (HCV) genotype 1a were treated with PrOD plus ribavirin (RBV) (12 or 24 weeks according to whether or not they had compensated cirrhosis), while the patients with genotype 1b were treated with PrOD alone. Liver functions, renal functions, and HCV-RNA levels were measured at baseline, 4, 12, and if applicable, 24 weeks after the initiation of treatment as well as 4 and 12 weeks after therapy.

Results: Nineteen patients received PrOD, while 6 received PrOD plus RBV treatment. Two patients failed to complete the treatment. Two patients with compensated cirrhosis were treated over 24 weeks, while others received 12 weeks. In 23 patients completed 12 weeks, all were HCV-RNA negative at the end of the treatment, and had sustained virologic response (SVR) after the 12 weeks of treatment. The most common side effects were pruritus and anaemia.

Conclusion: The PrOD treatment resulted in a high rate of SVR in HCV-infected CHD.

Keywords: Hepatitis C virus, Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir, haemodialysis

ÖZ

Amaç: Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir (PrOD) rejimi kronik hemodiyaliz (KHD) hastalarında etkili ve güvenlidir. Biz bu çalışmada KHD hastalarında PrOD tedavisi ile ilgili deneyimlerimizi sunmayı amaçladık.

Gereç ve Yöntemler: Temmuz 2016- Eylül 2018 tarihleri arasında, PrOD ile tedavi edilen 25 KHD hastası çalışmaya dahil edildi. Hepatit C virüsü (HCV) genotip 1a olan hastalar PrOD ± ribavirin (RBV) ile tedavi edilirken (tedavi süresi hastanın kompanze sirotik olup olmama durumuna göre 12 veya 24 hafta), genotip 1b olanlar PrOD tedavisi aldılar. Tedavinin başında, 4., 12., varsa 24. haftasında ve tedavi bitimi 4. ve 12. haftalarda hastaların böbrek ve karaciğer testleri ile HCV-RNA düzeyleri kaydedildi.

Bulgular: On dokuz hasta sadece PrOD tedavisi alırken, 6 hasta PrOD ± RBV ile tedavi edildiler. İki hasta tedaviyi tamamlayamadı. İki hasta 24 hafta boyunca tedavi alırken, diğerleri 12 hafta boyunca tedavi aldılar. On iki hafta tedaviyi tamamlayan 23 hasta hem tedavi bitimi hem de tedavi bitiminden 12 hafta sonra HCV-RNA negatiftiler. En sık gözlenen yan etkiler kaşıntı ve anemi idi.

Sonuç: PrOD tedavisi HCV ile enfekte KHD hastalarında kalıcı viral yanıta sahiptir.

Anahtar Kelimeler: Hepatit C virüs, Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir, hemodiyaliz

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Address for Correspondence: *Nilay Danış MD, Ege University Faculty of Medicine, Department of Gastroenterology, İzmir, Turkey*
Phone: +90 232 390 34 75 E-mail: nilaydanis17@gmail.com ORCID ID: orcid.org/0000-0002-3939-3089 **Received:** *8.07.2019* **Accepted:** *13.10.2019*

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Introduction

The estimated prevalence of hepatitis C Virus (HCV) in Turkey is 0.5-1.0% (1). According to the Turkish Society of Nephrology Registry in 2013, the positivity rate of HCV antibody (anti-HCV) was 6.94% in chronic haemodialysis (CHD) patients (2). In addition to liver-related mortality, HCV infection increases all kinds of mortality in CHD patients (3). In order to decrease the mortality, and to eliminate the source of infection, HCV must be treated in patients undergoing CHD. Interferon-based therapies were demanding and less effective in these patients; however, Direct Acting Antivirals (DAAs) seem to be highly effective and safe in patients with chronic renal failure. In this study, we presented our experiences with Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir (PrOD) regime in patients undergoing CHD.

Materials and Methods

This retrospective and medical record review study was performed in HCV-infected CHD patients, who were treated with PrOD regimen between July 2016 and September 2018. Both treatment-naive and those previously treated with pegylated interferon + ribavirin (RBV) patients with or without compensated liver diseases due to chronic HCV (genotype 1a and 1b) were included. Patients with decompensated cirrhosis, presence of hepatitis B virus, human immunodeficiency virus infections, and those who refused the treatment were excluded from the study.

Treatment Modality

The patients with HCV genotype 1a were treated with PrOD plus RBV as follows; the patients having genotype 1a HCV without cirrhosis were treated for 12 weeks, while cirrhotic patients were treated for 24 weeks. On the other hand, the patients infected with HCV genotype 1b were treated with PrOD alone for 12 weeks. Liver function tests, complete blood cell count, and HCV-RNA levels were recorded before treatment, at 4, 12, and if applicable, 24 weeks after the initiation of treatment as well as the 4 and 12 weeks after the end of treatment. Sustained Virologic Response (SVR) was defined as undetectable HCV-RNA at 12 weeks after end of treatment. Virologic failure was defined as virologic breakthrough or detectable HCV-RNA at the end of treatment or during follow-up. HCV-RNA levels were measured via real-time PCR (Abbott Molecular©, Des Moines, IL, USA; with a lower detection limit 12 IU/mL). The clinical trials ethics committee of Ege University Faculty of Medicine approved this study, with the approval number 99166796-050.06.04, in December 2018. Informed consent form was obtained from each patient.

Statistical Analysis

Results were determined via an intent-to-treat analysis with descriptive statistics. Continuous variables were expressed as means with standard deviations and ranges, and categorical variables were expressed as percentages. Categorical variables were analyzed with the chi-square test or Fischer's exact test. For quantitative variables, differences between groups were analyzed by Student's t-test. A p value less than 0.05 was considered as statistically significant.

Results

Thirteen females and 12 males, with a median age of 58 (minimum 37-maximum 74), were included. Two patients had compensated liver cirrhosis and 6 had a history of previous renal transplantation. The patients with prior renal transplantation were not using any immunsupresive drugs at enrollment. Baseline characteristics of the patients are summarized in Table 1. All genotype 1a patients were given RBV 200 mg/day at the beginning of the treatment. Five patients could tolerate 200 mg/day RBV; in one patient, RBV was given 200 mg every other day due to anaemia; but one patient could not tolerate RBV. Nineteen patients received PrOD, while 6 received PrOD + RBV treatment, but total of 23 patients could complete the treatment period. Two patients with liver cirrhosis received therapy over 24 weeks, while all of the other participants received 12 weeks. Treatment modalities and their efficacy are presented in the Table 2. The median HCV-RNA level was 292.759 (914-3.106.349) IU/mL at the initiation of treatment (Table 1).

Table 1. The baseline characteristics of all patients

Variables	n=25
Sex	
Female, n (%)	13 (52%)
Male, n (%)	12 (48%)
Age (years), mean ± SD	55±12
ALT (IU/L mean ± SD	24.6±12.6
HCV-RNA (IU/mL), median (range)	292.759 (914.0-3.106.349)
Genotype	
1a+1b	2 (8%)
1b	18 (72%)
1a	5 (20%)
History of treatment	
Naive	11 (44%)
Treatment-experienced	11 (44%)
No data	3 (12%)

SD: Standard deviation, ALT: Alanine aminotransferase, HCV: Hepatitis C virus

Table 2. The treatment modalities and their efficacy

Planned duration of treatment
Two patients with cirrhosis: 24 weeks
Twenty-three patients without cirrhosis: 12 weeks
Treatment regimen
Nineteen patients treated with PrOD
Six patients treated with PrOD and Ribavirin (200 mg/day starting dose)
Termination of treatments
One patient died at first week
One patient discontinued treatment due to pruritus
SVR12
Twenty-three patients reached to SVR12

PrOD: Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir, SVR: Sustained virologic response

Virologic Response

After 4 weeks of treatment, among 22 patients, HCV-RNA were reported to be negative in 19 (76%) patients and <100 IU/mL (but detectable) in 3 (12%) patients(13, 63, and 16 IU/mL). In 23 patients, who completed 12 weeks of the treatment, SVR at 12 weeks after the end of treatment (SVR12) was 100%. (Figure 1, 2).

Safety and Tolerability of the Treatment

The most common side effect was pruritus, which occurred in 3 (12%) patients. While one patient with genotype 1b discontinued PrOD treatment due to pruritis, she achieved SVR12 after Sofosbuvir + Ledipasvir treatment. The other patient, who could not finish the therapy, because she died at the 4th week due to heart failure (tricuspid valve insufficiency), one patient had grade 3 anaemia, and hence RBV dose was given every other day. Mean alanine aminotransferase (ALT) level was 24.6±12.6 IU/L at the beginning of treatment; 10.9±8.9 IU/L at the week 4, and 11±3.7 IU/L at the end of treatment [(Baseline ALT vs at 4th week, p=0.006), and (at 4th ALT vs at end of the treatment, p=0.772 respectively)]. Compared to reference values (normal values <34 IU/mL), serum mean ALT levels significantly decreased during the treatment.

Discussion

HCV infection in patients with end-stage renal disease (ESRD) may result in more rapid progression in liver disease, and it also increases the rate of liver-related mortality (3). Therefore, it is well known that, HCV infection in CHD patients reduces life expectancy.

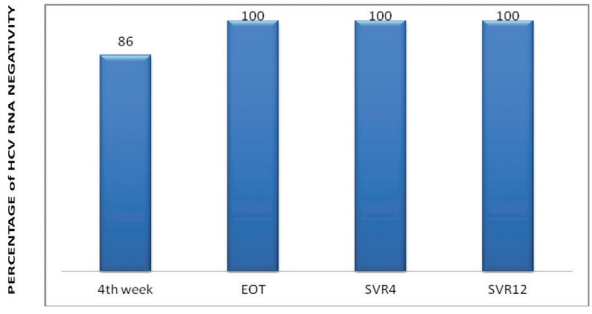

Figure 1. Per protocol analysis for virologic response
EOT: Time of end of the treatment, SVR: Sustained virologic response

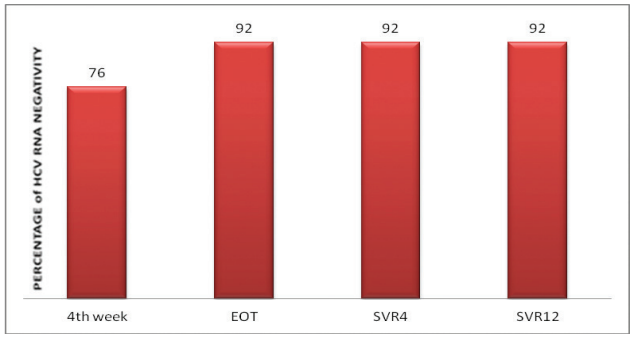

Figure 2. Intent to treat analyses for virologic response
EOT: Time of end of the treatment, SVR: Sustained virologic response, SVR: Sustained virologic response

According to a meta-analysis performed in 11.589 CHD patients, HCV positivity increased the risk of mortality with a HR: 1.34 [95% confidence interval: 1.13-1.59]. Death due to hepatocellular carcinoma and liver cirrhosis were more common in HCV-positive CHD patients than those with HCV-negative (4). Therefore, the treatment of HCV-positive CHD patients is more crucial. Before the use of DAAs for dialysis patients, HCV therapy was a therapeutic challenge during the treatment with interferons (IFNs) and RBV (5,6,7). The most common side effect of IFN/RBV combination was anaemia, especially due to RBV treatment (6,7). Although SVR was better with the combination of pegylated-IFN (PEG-IFN) and RBV than PEG-IFN alone (64% vs 33%, respectively), the Kidney Disease Improving Global Outcomes guidelines do not recommend combination therapy with pegylated-IFN and RBV for the patients having glomerular filtration rate <15 mL/min or undergoing CHD (3,4,5,6,7).

After RUBY-I trial was published, SVR rate >95% was observed and eradication of HCV in ESRD patients became much more possible (8). The real-life experiences published after RUBY-I showed that SVR rates were equal to or greater than those reported in RUBY-I trial (9,10,11,12,13).

The estimated prevalence of HCV infection in haemodialysis patients in Turkey was 6.94% according to Turkish Society of Nephrology registry, in 2013. After DAAs, the prevalence was reduced to 3.94% in the same population at the end of 2017 (14). Turkish Viral Hepatitis Diagnosis and Treatment Guidelines strongly recommend the treatment of ESRD patients with HCV (15). The rate of anti-HCV may be lower than HCV-RNA positivity in CHD patients, because HCV-RNA can be positive in ESRD patients, even if anti-HCV is negative. So it is reasonable to screen CHD patients not with only anti-HCV but also with HCV-RNA levels. Haemodialysis patients should be treated if they have HCV infection (16). PrOD treatment is available in Turkey since June 2016. Real-life data from Turkey has started to be published recently. A multi-center trial from Turkey collected 75 patients with renal failure, 53 of whom were on haemodialysis. Success of the PrOD therapy was 98.6% at the end of therapy and SVR12 rate was 96% (10). Another study published by Yaraş et al. (12) showed that SVR12 was 100% in all (n=25) patients. In our trial, SVR12 was 92% according to ITT analysis and 100% according to per protocol analysis, and also this result was similar to the literature.

In the literature, the most common side effects of PrOD were fatigue and pruritus (9,10,11,12). In our study the most common side effect was pruritus, which was observed in 3 patients. One patient could reach SVR12 by receiving Sofosbuvir/Ledipasvir combination even though could not finish the therapy due to pruritus with PrOD. Indeed, there is a growing evidence on use of Sofosbuvir-based regimens in patients with ESRD. A recent prospective open label observational study assessed the safety and efficacy of Ledipasvir/Sofosbuvir combination in CHD patients with HCV genotype 1 and showed excellent SVR12 rates without any major side effects (17). Other side effect that was observed in only one patient in our study was Grade 3 anaemia. Anaemia was the second (9). These side effects were similar to the real life data of HCV-positive haemodialysis patients treated with DAA and RBV (9,10,11,12).

Study Limitations

The limitations of our study were small number of patients and short follow-up duration compared to the big trials.

Conclusion

The PrOD with or without RBV treatment resulted in a high rate of SVR in HCV-infected patients on haemodialysis. With the success of this treatment, in patients with chronic renal failure on CHD who have a high risk of morbidity and mortality, HCV may no longer be an important comorbidity.

Ethics

Ethics Committee Approval: The clinical trials Ethics Committee of Ege University Faculty of Medicine approved this study, with the approval number 99166796-050.06.04, in December 2018.

Informed Consent: Informed consent form was obtained from each patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.D., H.P., G.E., T.Y., Design: N.D., U.S.A., H.P., Data Collection or Processing: N.D., H.P., T.Y., G.E., İ.T., Z.K., U.S.A., Analysis or Interpretation: N.D., U.S.A., Literature Search: N.D., U.S.A., N.Ü., F.G., Writing: N.D.

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

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Research Article

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microRNA in Patients with Hepatitis B and Hepatitis C Virus Associated Hepatocellular Carcinoma and Cirrhosis

Hepatit B ve Hepatit C İlişkili Hepatosellüler Karsinom ve Siroz Olgularında microRNA

Gökhan Küçükkara[1], Ferhat Gürkan Aslan[1], Bilal Toka[2], Mehmet Köroğlu[1], Mustafa Altındiş[1]

[1]*Sakarya University Faculty of Medicine, Department of Microbiology, Sakarya, Turkey*
[2]*Sakarya University Faculty of Medicine, Department of Internal Medicine and Gastroenterology, Sakarya, Turkey*

ABSTRACT

Objectives: The aim of this study was to determine the potentials of hsa-microRNAs (miRNA)-21-3p, hsa-miRNA-29a-3p, hsa-miRNA-122-3p, hsa-miRNA-192-5p in Hepatitis B (HBV) and HCV related hepatocellular carcinoma (HCC) and liver cirrhosis (LC) cases, being biomarkers by examining their levels.

Materials and Methods: Sixty patients and 26 healthy volunteers were included in the study. Total RNA isolation in the serum samples was performed with the Direct-zol™ RNA MiniPrep (Zymo Research Corp., USA) commercial kit followed by cDNA synthesis and real-time polymerase chain reaction amplification with EPIK miRNA Select Hi/Lo-ROX (Bioline Reagents Ltd., USA). For amplification and analysis, Rotor-Gene Q (QIAGEN, Germany) instrument was used and statistical analyzes were performed with SPSS 21 (IBM, USA) program.

Results: Hsa-miRNA-21-3p and hsa-miRNA-122-3p levels increased 3-4 fold in patients without HCV related LC (HCV-LC) and hsa-miRNA-29a-3p expression in HCV infected patients has significantly decreased ($p<0.05$). hsa-miRNA-192-5p showed a 3-fold increase in HBV related LC (HBV-LC) group ($p<0.05$) but not in other groups. The hsa-miRNA-122-3p value is increased in HCV-LC patients.

Conclusion: In our study, miRNA-21-3p and hsa-miRNA-122 for HBV-HCC and HCV-HCC diseases; hsa-miRNA-21-3p, hsa-miRNA-122 and hsa-miRNA-192-5p for HBV-LC; miRNA-29a-3p test for HCV-LC could be used as diagnostic markers.

Keywords: miRNA, hepatitis, hepatocellular carcinoma and cirrhosis

ÖZ

Amaç: Bu çalışmada hsa-mikroRNA (miRNA)-21-3p, hsa-miRNA-29a-3p, hsa-miRNA-122-3p, hsa-miRNA-192-5p'nin Hepatit B (HBV) ve HCV ilişkili hepatosellüler karsinoma (HCC) ve karaciğer sizrozu (KS) olgularındaki düzeyleri incelenerek, biyobelirteç olma potansiyellerinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya 60 hasta ve 26 sağlıklı gönüllü dahil edilmiştir. Serum örneklerinde total RNA izolasyonu, Direct-zol™ RNA MiniPrep (Zymo Research Corp., USA) ticari kiti ile yapıldıktan sonra cDNA sentezi ve gerçek zamanlı polimeraz zincir reaksiyonu amplifikasyonu EPIK miRNA Select Hi/Lo-ROX (Bioline Reagents Ltd., USA) ile gerçekleştirilmiştir. Amplifikasyon ve analizler için Rotor-Gene Q (QIAGEN, Germany) cihazı kullanılmış, istatistiksel analizler SPSS 21 (IBM, USA) programı ile yapılmıştır.

Bulgular: Hsa-miRNA-21-3p ve hsa-miRNA-122-3p seviyeleri HCV ilişkili KS dışındaki hasta gruplarında 3-4 kat oranında artışlar göstermiş, HCV enfeksiyonlu hastalarda hsa-miRNA-29a-3p ekspresyonunda anlamlı azalmalar saptanmıştır ($p<0.05$). hsa-miRNA-192-5p ise HBV ilişkili KS grubunda 3 kat artış gösterirken ($p<0.05$) diğer gruplarda belirgin farklılıklar göstermemiştir. hsa-miRNA-122-3p değeri, HCV-KS hastalarında artmıştır.

Sonuç: Çalışmamızda, tanı belirteçleri olarak; HBV ilişkili HCC ve HCV ilişkili HCC için hsa-miRNA-21-3p ve hsa-miRNA-122, HBV ilişkili KS için hsa-miRNA-21-3p, hsa-miRNA-122 ve hsa-miRNA-192-5p, HCV-KS için miRNA-29a-3p testlerinin kullanılabileceği belirlenmiştir.

Anahtar Kelimeler: miRNA, hepatit, hepatosellüler karsinom ve siroz

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Address for Correspondence: *Ferhat Gürkan Aslan MD, Sakarya University Faculty of Medicine, Department of Microbiology, Sakarya, Turkey*
Phone: +90 543 291 29 80 E-mail: ferhatgurkan33@hotmail.com ORCID ID: orcid.org/0000-0001-8394-1962 **Received:** *4.07.2019* **Accepted:** *21.09.2019*

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Introduction

Hepatitis B virus (HBV) and HCV viruses are etiologic factors that cause liver damage. It is estimated that approximately 5 percent of the world's population has chronic HBV infection (approximately 350 million people). The prevalence of global HCV is approximately 2% and 180 million people are persistent HCV carriers. However, HBV/HCV infection rates vary from country to country. A significant percentage of chronic HBV and HCV carriers develop necroinflammatory liver diseases of different severity and course patterns such as persistent injury, cirrhosis, hepatic insufficiency and hepatocellular carcinoma (HCC) (1).

High morbidity and mortality rates of HCC require more specific methods and more effective strategies for diagnosis and treatment. Laboratory tests and imaging techniques such as ultrasonography along with histopathology, computed tomography and magnetic resonance are often used to diagnose it (2). All these diagnostic tools are limited due to their cost, availability and reproducibility (3). Therefore, some serum or tissue biomarkers, such as microRNAs (miRNAs), have been developed for clinical applications in recent years (1).

miRNAs are oligonucleotides of small non-coding RNA structure of 18-24 nucleotides (average 22 nt) transcribed from highly conserved DNA regions but not translated into protein. miRNAs play a crucial role in the processing, regulation and similar post-transcriptional levels of intracellular genetic information in all multicellular eukaryotic organisms (4,5,6). miRNAs are involved in numerous pathways that are critical for the cell, and therefore, when they fail to function, they may lead to susceptibility to diseases, particularly cancer (7).

There has been a recent increase in the number of studies that investigate the role of miRNAs in regulating different cellular processes such as energy production, protein synthesis, proliferation, differentiation and apoptosis (8). In the onset and progression of cancer, miRNAs act as tumor suppressors or oncogenes depending on the characters of target genes (4). Disruption of normal miRNA expression patterns has been reported in different liver diseases ranging from chronic hepatitis (CHB) to cirrhosis and HCC (9,10,11). Diagnosing people with HCC at an early stage before clinical signs and symptoms develop is an urgent need for improving prognosis (12).

The aim of this study was to determine hsa-miRNA-21-3p, hsa-miRNA-29a-3p, hsa-miRNA-122-3p and hsa-miRNA-192-5p expression levels in HBV- and HCV-related HCC and liver cirrhosis (LC) cases and their potential to become biomarkers. The miRNAs included in the study were determined by literature review. One miRNA was selected from the well-known change in liver diseases (hsa-miRNA-122-3p), while the other miRNAs were selected from miRNAs, in which studies on this subject have just begun.

Materials and Methods

Sampling Methods

The study sample consisted of 60 patients admitted to gastroenterology for treatment and follow-up and of 26 healthy volunteers. Eighteen participants had HBV-associated HCC, 15 had HBV-related cirrhosis, 8 had HCV-associated HCC and 19 participants had HCV-related cirrhosis. The study was approved by the Ethics Committee of the Sakarya University Faculty of Medicine (approval number: 71522473/050.01.04/132 and date: 28.06.2016). Written informed consent was obtained from patients prior to participation. A study group was established from patients who agreed to participate in the study based on their medical and pathology reports.

Preparation and Analysis of Samples

Blood samples were collected from participants and placed in dry gel tubes. Serums separated from the blood samples were stored at -80 °C until total RNA isolation, which was, then, performed using a Direct-zol™ RNA MiniPrep (ZYMO Research Corp., USA) kit according to the manufacturer's instructions. cDNA synthesis and real-time polymerase chain reaction amplification from the isolate was performed using an EPIK™ miRNA Select Hi/Lo-ROX (Bioline Reagents Ltd.) miRNA amplification kit. Amplification and analysis were performed using a Rotor-Gene Q (QIAGEN, Germany).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (IBM SPSS Statistics 20, USA) at a significance level of 0.05. Recently, it has been proposed that the change in gene expression in a microarray experiment will be best defined by using fold change rather than t-statistic. Fold change is the ratio of two values and measures how much of a variable changes between the two measurements. The difference in the mean obtained by taking the logarithm of the data corresponds to the proportions of the original scale (13,14).

Results

Table 1 shows some demographic and clinical data of the participants. Patients' hsa-miRNA-21-3p, hsa-miRNA-29a-3p, hsa-miRNA-122-3p and hsa-miRNA-192-5p expression levels were evaluated as multiples of those of the control group (Table 2).

Hsa-miRNA-21-3p and hsa-miRNA-122-3p levels of all patient groups, except HCV-LC patients, increased 3-4 fold. hsa-miRNA-

Table 1. Demographic and clinical data on hepatocellular carcinoma and cirrhosis cases

	n	Mean
Age (years)	**60**	**62.15**
Urea (mg/dL)	60	57.48
Uric acid (mg/dL)	60	5.68
Glucose (mg/dL)	60	140.9
Cholesterol (mg/dL)	60	152.92
Triglyceride (mg/dL)	60	119.23
ALT (U/L)	60	37.70
AST (U/L)	60	68.05
AFP Log 10 (ng/mL)	60	3.47
T-Bilirubin (mg/dL)	60	2.21
D-Bilirubin (mg/dL)	60	0.92
HBV-DNA (IU/mL)	33	764621.15
HCV-DNA (IU/mL)	27	165.23

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, AFP: Alpha-fetoprotein, HBV: Hepatitis B Virus, HCV: Hepatitis C virus

Table 2. hsa-miRNA-21-3p, hsa-miRNA-29a-3p, hsa-miRNA-192-5p and hsa-miRNA-122-3p expression levels in HBV and HCV-related hepatocellular carcinoma and cirrhosis cases

	HBV-HCC vs Healthy (fold)	HCV-HCC vs Healthy (fold)	HBV-LC vs Healthy (fold)	HCV-LC vs Healthy (fold)
miRNA-21-3p	3.6744	4.40345	4.705	0.7405
miRNA-29a-3p	1.408	0.40365	1.14	0.536133
miRNA-192-5p	1.4752	1.888	2.978067	1.01925
miRNA-122-3p	2.541438	3.83474	4.204188	0.517475

miRNA: microRNA, HBV-LC: Hepatitis B Virus-liver cirrhosis, HCC: Hepatocellular carcinoma

29a-3p expression significantly decreased in patients with HCV infection ($p<0.05$). hsa-miRNA-192-5p increased 3-fold in HBV-LC patients ($p<0.05$) but showed no significant difference in other groups. hsa-miRNA-122-3p, which is a liver specific miRNA, increased in HCV-LC patients.

Discussion

HCC is an aggressive malignancy with poor prognosis and high mortality rates worldwide. It, however, does not have a reliable and effective and non-invasive biomarker. We, therefore, need new biomarkers to determine HCC early. miRNA expression levels are promising biomarkers, and therefore, are of great interest (15). However, numerous studies on CHB characterize miRNA profiles as controversial and complex.

Ebrahimifard et al. (16) showed that miRNA-122 can be used as a biomarker to detect cirrhosis associated with CHB and HBV before progressing to HCC. We also detected a more significant increase in miRNA 122-3p in the hepatitis B-related HCC and LC groups and in the HCV-related HCC group compared to the healthy control group.

Lin et al. (17) reported that miRNA-29a, miRNA-122 and miRNA-192 synthesis was 2.64, 3.13 and 2.60 times, respectively, higher in patients with HCC than in those diagnosed with CHB. Zhou et al. (18) conducted a study on plasma samples of HBV-related HCC patients and reported a 3.3-, 2- and 2.9-fold increase in miRNA-122, miRNA-21 and miRNA-192, respectively. We also detected 1.4-, 1.48-, 2.54- and 3.67-fold increase in miRNA-29a-3p, miRNA-192-5p, miRNA-122 and miRNA-21, respectively, in HBV-related HCC patients. Tan et al. (19) developed a miRNA panel for HCC diagnosis and reported that miRNA-122 and miRNA-192 synthesis was 0.27 and 0.76 times lower in patients diagnosed with HCC than in healthy volunteers.

Wang et al. (20) conducted a study on 30 patients with HCC and 30 patients with CHB and 30 healthy volunteer participants in Xinxiang, China and reported that patients with HCC had a higher serum miR-21 expression level than CHB or healthy volunteers, which is consistent with miRNA-21 values in our study (20).

Zekri et al. (21) reported that miRNA-122 and miRNA-192 expression was, respectively, 2.2 and 1.88 times higher in HCV-infected patients with HCC than in healthy volunteers. We also detected that miRNA-122 and miRNA-192 upregulation was 3.84 and 1.88 times higher in HCV-related HCC patients than in healthy participants.

Zhou et al. (18) reported that miRNA-122, miRNA-21 and miRNA-192 expression was, respectively, 1.9, 1.2 and 4.6 times higher in patients with HCC than in cirrhosis patients. Tan et al. (19) conducted a study in China and reported that cirrhosis patients had 2 and 1.2 times higher miRNA-122 and miRNA-192 levels, respectively, than patients with HCC. They also reported that miRNA-21 and miRNA-122 synthesis was, respectively, 1.6 and 3.13 times higher in cirrhotic patients than in patients with HCC. We also detected that patients with HCC had higher miRNA-122 and miRNA-192 synthesis than cirrhosis patients. However, miRNA-21 expression was 4.7 times higher in HBV-related LC patients and 0.74 times less in HCV-related LC patients.

Bao et al. (22) conducted a study on serum samples and reported a downregulation in fibrosis-related miRNA-29 and miRNA-21 expression. We also detected a downregulation in miRNA-29a-3p expression in HCV-related HCC and LC patients, a downregulation in miRNA-21-3p expression only in HCV-related LC patients and a 1.14-fold increase in miRNA-29a-3p expression in HBV-related LC patients.

Again, Zekri et al. (21) reported a 0.45- and 1.17-fold change in miRNA-122 and miRNA-192 levels in the serums of cirrhosis patients with HCV infection than in those of healthy control group. We also detected a 0.52-fold downregulation in miRNA-122 and a 1.02-fold upregulation in miRNA-192 in HCV-related LC patients and a 2.98-fold increase in miRNA-192-5p in HBV-related LC patients.

Oksuz et al. (23) reported that miRNA-29a-3p synthesis was 2.95 times lower and miRNA-122 synthesis was 5.22 times higher in HCC patients whereas miRNA-122 synthesis was 1.38 times lower in cirrhosis patients. The results of miRNA-29a reported by Oksuz et al. (23) are different from ours. In our study, MiRNA-29a synthesis was 1.41 and 0.40 times in HBVr-related HCC patients and HCV-related HCC patients, respectively. miRNA-122 synthesis was 2.54 and 3.83 fold in HBV-HCC and HCV-HCC patients, respectively. miRNA-122 synthesis was 0.5-fold in HCV-LC patients.

Having conducted a study on individuals with HCV infection, Waring et al. (24) detected more than 100 miRNA species in serum and found that miRNA-122 level showed the most consistent change in all HCV genotypes in response to treatment. They also reported that miRNA-122 decreased approximately four-fold in two weeks and remained low throughout the treatment in all participants.

Tat Trung et al. (25) stated that mir-21, mir-122 and mir-192 as well as alpha-fetoprotein (AFP) are biomarkers for the diagnosis of HCC in HBV patients, and in particular in HBV-related LC patients with normal AFP levels or in HCC patients with small tumors.

Conclusion

After a quarter century of research, our knowledge of the mechanisms of biosynthesis, effect and function of miRNAs has

been greatly enhanced. Depending on the target mRNA, miRNAs act as tumor suppressors or oncogenes in cancer development. Information on miRNAs should be standardized to be able to use them as biomarkers for cancer development. According to our results, hsa-miRNA-21-3p and hsa-miRNA-122 assays can be used for HBV-HCC and HCV-HCC diseases; Hsa-miRNA-21-3p, hsa-miRNA-122 and hsa-miRNA-192-5p assays can be used for HBV-LC and MiRNA-29a-3p assay can be used for HCV-LC. Further research should be conducted to verify these results to accelerate the applicability of the assays.

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Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of the Sakarya University Faculty of Medicine (approval number: 71522473/050.01.04/132 and date: 28.06.2016).

Informed Consent: Written informed consent was obtained from patients prior to participation.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: M.A., G.K., F.G.A., Design: M.A., F.G.A., B.T., Data Collection or Processing: G.K., F.G.A., B.T., Analysis or Interpretation: G.K., F.G.A., M.A., M.K., Literature Search: G.K., M.A., F.G.A., Writing: G.K., M.A., M.K., F.G.A.

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

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