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Address for Correspondence:
Viral Hepatitis Prevention Society
Sağlık Mahallesi, Süleyman Sırrı Caddesi No: 2/15
Sıhhıye, Ankara, Turkey
Phone: +90 312 433 74 26
Fax: +90 312 433 06 54
E-mail: info@viralhepatitisjournal.org

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Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1
34093 İstanbul, Turkey
Phone: +90 (212) 621 99 25 **Fax:** +90 (212) 621 99 27
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
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AIM AND SCOPE

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

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

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

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

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Address for Correspondence

Address: Sağlık Mah, Süleyman Sırrı Cad, No:2/15 Sıhhiye/ANKARA

Phone: +90 (312) 4337426

Fax: +90 (312) 4330654

E-mail: info@viralhepatitdergisi.org

Publishing House

Galenos Yayınevi Tic. Ltd. Şti.

Molla Gürani Mah. Kaçamak Sok. No: 21, 34093, Fındıkzade, Istanbul, Turkey

Phone: +90 212 621 99 25

Fax: +90 212 621 99 27

E-mail: info@galenos.com.tr

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INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

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

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

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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.

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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/),

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

MANUSCRIPT PREPARATION

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

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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).

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

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

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

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

Conclusion: The conclusion of the study should be highlighted.

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

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

Case Reports

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

Review Articles

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

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

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

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

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

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

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• Title Page

• Article sections

• Turkish and English titles

• Abstract (250 words) (Turkish and English)

• Keywords (minimum 3; maximum 6)

• Article divided into appropriate sections

• Complete and accurate references and citations

• List of references styled according to "journal requirements"

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

• "Copyright Form" signed by all authors.

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Address: Sağlık Mah. Süleyman Sırrı Cad, No: 2/15 Sıhhiye/Ankara/Turkey

Phone: +90 312 433 74 26

Fax: +90 312 433 06 54

E-mail: info@viralhepatitdergisi.org

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EDITORIAL

Dear colleagues,

In the last days of 2018, we have interesting publications in the third issue of this journal. This issue includes the research articles titled "The Epidemiology and Trend of Hepatitis C Infection in Hamadan Province: West of Iran. 2011-2016". "Distribution of Hepatitis C Virus Genotypes in Aydın Province". "An Asessment of Sharps Injuries in Healthcare Vorkers", "A Misleading Parameter in the Diagnosis of Chronic Hepatitis B: Persistently Normal Transaminases", "Circulating Glucose-Regulated Protein 78 Levels in Patients with Chronic Hepatitis B Infection", "Does Pegylated-Interferon Still Have High Efficacy Treatment Properties Against Chronic Hepatitis B?" and a case report titled "Failure of Direct-Acting Antiviral Agents Due to Incomplete Hepatitis C Virus Genotyping". Our primary aim is to update the readers with the recent developments. With this purpose in mind, we expect your contributions with original articles. reviews, case reports and letters to the editor. To meet in new issues. This journal is indexed in Emerging Sources Citation

Index (ESCI).

Prof. Dr. Fehmi TABAK

Prof. Dr. Ebubekir Şenateş

Prof. Dr. Rahmet Güner

Prof. Dr. Tansu Yamazhan

Research Article

65

Doi: 10.4274/vhd.2018.0017
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The Epidemiology and Trend of Hepatitis C Infection in Hamadan Province: West of Iran, 2011-2016

Hamadan Bölgesi'nde Hepatit C Enfeksiyonunun Epidemiyolojisi ve Trendi: Batı İran, 2011-2016

Salman KHAZAEI[1], Arash MOFARRAH-ZAT[2], Shahrzad NEMATOLLAHI[3], Ensiyeh JENABI[4], Mohammad MIRZAEI[1], Seyyed Jalal BATHAEI[2], Manoochehr SOLGI[1], Jalaledin AMIRI[2]

[1]*Department of Epidemiology, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran*
[2]*Deputy of Health, Hamadan University of Medical Sciences, Hamadan, Iran*
[3]*Department of Epidemiology & Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[4]*Pediatric Developmental Disorders Research center, Hamadan University of Medical Sciences, Hamadan, Iran*

ABSTRACT

Objectives: Hepatitis C virus (HCV) is a blood-borne virus which is transmitted through the exposure to small amounts of blood. The objective of this study was to investigate trend and epidemiological pattern of hepatitis C infection during seven successive years in Hamadan province during 2011-2016.

Materials and Methods: This cross sectional study was conducted on the new cases of HCV (n=803) who were recorded in the deputy of health of Hamadan University of Medical Sciences. The Cochran-Armitage test was used to investigate the changes in the trend of the disease according to various demographical and clinical characteristics. Incidence rates of HCV were estimated by district for 2016.

Results: Totally, 705 (87.8%) cases were male and 98 (79.7%) were urban dwellers and the highest proportion of the cases (51%) belonged to the age group of 25-44 years. The overall incidence rate of HCV infection in Hamadan province was 5.26 per 100.000 in 2016, while the southern counties had higher incidence rate compared to the northern parts. Only 8.1% of cases were assessed due to clinical symptoms.

Conclusion: Our results showed that HCV has a decreasing trend for both genders in Hamadan province and is most prevalent among males, age group of 25-44 years, married people and urban dwellers. Therefore, educational programs for the transmission and prevention of hepatitis C in the community and for high-risk groups should be conducted with the management of health centers and mass media.

Keywords: Hepatitis C, infection, incidence, trend, Iran

ÖZ

Amaç: Hepatit C virüsü (HCV), küçük miktarda kanla temas yoluyla bulaşan bir virüstür. Bu çalışmada, 2011-2016 yılları arasında, Hamadan bölgesinde ardışık yedi yıl boyunca hepatit C enfeksiyonunun trendi ve epidemiyolojik paterninin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Bu kesitsel çalışma, Hamadan Tıp Bilimleri Üniversitesi sağlık yardımcılığında kayıt altına alınan yeni HCV olguları (n=803) ile yürütülmüştür. Çeşitli demografik ve klinik özelliklerine göre hastalığın trendindeki değişimleri araştırmak için Cochran–Armitage testi kullanılmıştır. HCV insidansı 2016 yılına kadar ilçe bazında değerlendirilmiştir.

Bulgular: Toplamda olguların 705'i (%87,8) erkek, 98'i (%79,7) şehirli olup, olgular (%51) çok yüksek oranda 25-44 yaş grubuna aitti. Hamadan bölgesindeki HCV enfeksiyonunun genel insidans oranı 2016 yılında 100.000'de 5,26 iken, güney bölgeleri kuzey bölgelerine göre daha yüksek insidans oranına sahipti. Olguların sadece %8,1'i klinik semptomlar nedeniyle değerlendirildi.

Sonuç: Elde ettiğimiz sonuçlar, HCV'nin Hamadan bölgesinde her iki cinsiyet için azalan bir trende sahip olduğunu ve en yaygın olarak; erkekler arasında, 25-44 yaş grubunda, evli insanlarda ve şehirlilerde görüldüğünü göstermiştir. Bu nedenle, toplumda hepatit C'nin bulaşmasına ve önlenmesine yönelik ve yüksek riskli gruplar için hazırlanan eğitim programları, sağlık merkezlerinin ve kitle iletişim araçlarının yönetimi ile birlikte yürütülmelidir.

Anahtar Kelimeler: Hepatit C, enfeksiyon, insidans, trend, İran

Khazaei S, Mofarrah-Zat A, Nematollahi S, Jenabi E, Mirzaei M, Bathaei SJ, Solgi M, Amiri J. The Epidemiology and Trend of Hepatitis C Infection in Hamadan Province: West of Iran, 2011-2016. 2018;24:65-69.

Address for Correspondence: *Manoochehr Solgi MD, Department of Epidemiology, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran*
Phone: +98 918 352 08 58 E-mail: m.solgi2121@yahoo.com ORCID ID: orcid.org/0000-0002-7452-331X **Received:** *25.07.2018* **Accepted:** *22.10.2018*

Introduction

Hepatitis C is a liver disease caused by hepatitis C virus (HCV) (1). HCV is a blood-borne virus, usually transmitted through shared drug injection equipment, inadequate sterilization of medical equipment especially syringes and needles in health care settings, and blood transfusion (2). HCV can also be transmitted through sexual contact and can be transmitted from an infected mother to her child, however, this transmission mode is less common (3). The virus can cause either acute or chronic hepatitis, which ranges from a mild illness that lasts a few weeks to a serious and life-threatening illness (4). In total, approximately 71 million people are suffering from chronic hepatitis C infection; and a substantial proportion of people with the infection develop liver cirrhosis or liver cancer. Almost 399.000 people die each year from hepatitis C, cirrhosis and cancer. Despite the favorable effectiveness of antiviral drugs to reduce the risk of liver carcinoma in nearly 95% of people infected with hepatitis C, there is little universal access to diagnosis and treatment and no proved vaccine. Hepatitis C is found throughout the world; however, there are several species (or genotypes) of the HCV and their distribution varies by region (5). The highest annual incidence of HCV infection is observed in the Eastern and European Mediterranean offices of World Health Organization (WHO) (2.3% and 1.5%): while other offices have reported incidences from 0.5% to 1.0%. Depending on the country, HCV infection can be concentrated in some populations (e.g. among people who inject drugs) and/or spreads across general population. Modeling studies have shown that there were 1.75 million new HCV infections (a total of 23.7 new HCV infections per 100.000 people) in 2015 (6). A trends analysis on the incidence rate of HCV from 2008 to 2013 showed that the overall incidence rate (per 100.000 people) varied from 0.55, 0.72, 1.44, 2.69, 1.24 to 1.93 in Iran (7). The prevalence of HCV in one study in 2014 was estimated to be 0.2% (8). Another studies estimated the HCV prevalence ranging from 0.00% to 7.25% depending on the source populations in Iran (9,10). Results of a systematic review also showed that the pooled prevalence of HCV was 0.3% in the general population, 6.2% in the populations at a moderate risk, 32.1% in the high-risk populations, and 4.6% in the specific clinical populations in Iran and according to finding of this study, the prevalence of hepatitis C in Iran in the general population was less than 1% (11). Understanding the epidemiology of HCV is essential for developing cost-effective preventive strategies against HCV. Therefore, the present study was conducted aiming atthe evaluation of epidemiology and trend of hepatitis C infection in Hamadan province during 2011-2016.

Materials and Methods

In this cross-sectional study, we used the information on the reported cases at the provincial level of the National Notifiable Diseases Surveillance System in Hamadan Province from 2011 to 2016.

Hamadan province is located in the west of Iran with an overall surface area of 19.546 square kilometers. According to the National Census, the population of the province was 1.758.268 in 2011.The province consists of 9 districts including Asadabad, Bahar, Hamadan, Famenin, Kabudarahang, Malayer, Nahavand, Tuyserkan and Razan.

According to the national guideline on viral hepatitis management, notification of hepatitis B virüs and HCV infections is mandatory in Iran. Therefore, all public and private laboratories, blood transfusion organizations, hospitals and health centers should report all positive test results of serologic markers of HCV infection to the affiliated district health center on a monthly basis. A "suspected viral hepatitis" was defined based on the criteria of case definition by WHO (6,12). To reach a homogenous and generalizable study sample, those patients who reside in other provinces were excluded. Moreover, cases with previous history of the disease were excluded and only incident cases in this period were enrolled. A complete examination checklist including information on demographic characteristics (age, gender, marital status, and residence), history of high-risk behaviors, and main reason for HCV examination was filled out by healthcare staff. The present study is based on recorded data from disease surveillance system, which was approved by the Deputy Chancellor of Health, and in fact, obtaining informed consent of the patients is not applicable.

Descriptive statistics, such as frequency tables and charts were used for presenting the results. The Cochran-Armitage test for linear trend was used to analyze the possible trends of disease incidence within the study period. Incidence risk of HCV infection (per 100.000 people) estimated by gender, location, marriage status and age group, and incidence risk ratio was computed for comparison of the incidence risks according to demographic characteristics.

Statistical Analysis

Data were analyzed using Stata (version 11.2, StataCorp, and College Station, Texas). A p value of 0.05 was considered statistically significant.

Results

Within this time period, a total of 803 new cases of HCV were registered, of whom705 (87.8%) were male and 640 (79.9%) were lived in urban areas. The mean age of the patients was 43.46±13.82 years (range: 7-92 year), and more than half of them (51%) were in 25-44 years age group. As shown in Figure 1, the incidence of HCV infection ranged from 18.3 in 2011 to 9.38 in 2016 for males (p for trend=0.11) and ranged from 1.6 in 2011 to 1.14 in 2016 for females (p for trend=0.35).

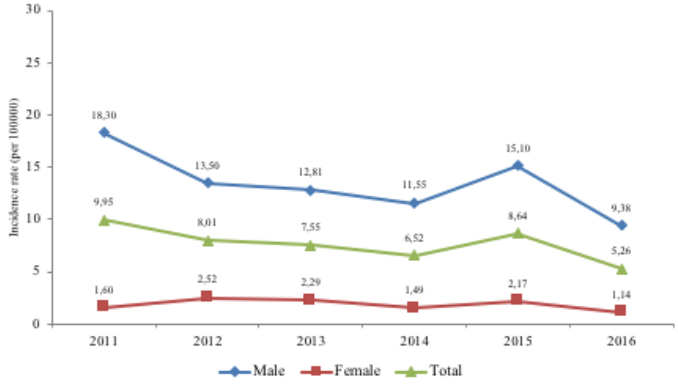

Figure 1. The incidence risk of hepatitis C virus infection by gender and year

Table 1. The incidence risk of hepatitis C virus infection according to demographic variables

Variable	Number (%)	Incidence risk (per 100.000)	Risk ratio	95% CI	p
Gender					
Female	98 (12.2)	2.24	1.00	-	-
Male	705 (87.8)	13.44	7.19	(6.01, 8.62)	p<0.001
Location					
Rural	163 (20.3)	3.88	1.00	-	-
Urban	640 (79.7)	10.17	2.62	(2.22, 3.09)	p<0.001
Marital status					
Single	194 (24.16)	4.13	1.00	-	-
Married	535 (66.63)	9.22	2.23	(1.9, 2.62)	p<0.001
Age group					
25<	37 (4.58)	0.97	1.00	-	-
25-44	409 (51.00)	10.58	10.95	(8.38-14.31)	p<0.001
45-64	291 (36.28)	14.45	14.97	(11.58, 19.35)	p<0.001
65+	65 (8.10)	9.23	9.56	(6.88, 13.29)	p<0.001

CI: Confidence interval

Table 1 shows the incidence risk of hepatitis C according to the demographic characteristics of the patients. The risk of hepatitis C in males was 7.19 times higher than in females (p<0.001). Urban dwellers had 2.62 times higher risk of hepatitis C (p<0.001). Being married was associated with more than two-fold increased risk; while compared to the age group of less than 25 years, those in the age group of 25-44, 45-64 and + 65 had increased risk of HCV by 10.95, 14.97 and 9.56 time, respectively (p<0.001).

The source of notification was clinical laboratory in 55.3%; whereas 13.33% of them were reported by hospitals. The main reason for the examination was one's own free will due to the high-risk behaviors (Figure 2).

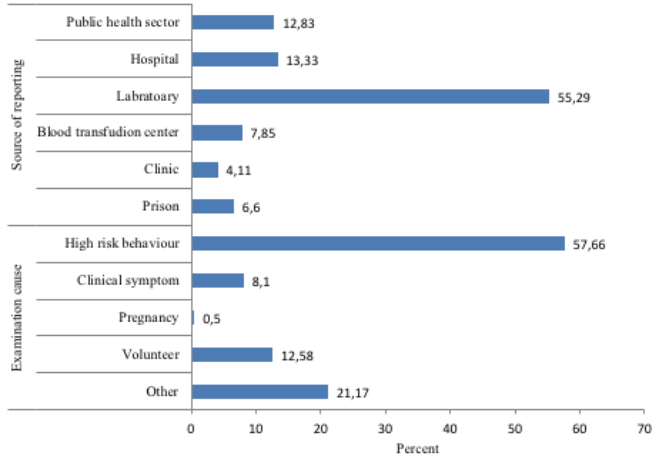

Figure 2. Source of notification and reason of examination of hepatitis C infected patients in Hamadan province (2011-2016)

Discussion

In the present study, demographic and clinical information of 803 HCV-infected patients in Hamadan province were assessed for seven successive years from 2011 to 2016. Our findings indicated that HCV were reported more frequently in males, age group of 25-44 years, married people and urban dwellers. The overall incidence rate of HCV infection in Hamadan province was 5.26 per 100.000 in 2016, while southern counties had the higher incidence rate compared to the northern counties. The source of the notification was clinical laboratory for more than half of the patients. These findings are consistent with those reported in the literature (7,12,13,14,15).

According to our findings, HCV infection is more common in self-employed, jobless and housekeepers. Our findings are similar to those carried out in other areas (7,15). The findings of this study showed that more than 50% of cases of HCV were in the age group of 25-44 years. In addition, the risk of hepatitis C in the age group of 25-44 years was 10 times higher than in those younger than 25 years. The greater risk of HCV in this age group could be due to the higher social activity and thus more exposure to the risk factors.

In this study, the risk of hepatitis C in men was 7 times higher than that in women. Studies performed in Hormozgan, Khuzestan and Hamadan provinces confirmed this finding (16,17,18), which reflect the extent of exposures to HCV risk factors for men. In the present study, the urban areas had the highest number of people with hepatitis which was similar to the results of studies done in Hamadan and Tehran (17,18). Consistent with the results of a study from Kermanshah (19) we also found that most cases of hepatitis

C were married, which could be attributed to the higher proportion of this subgroup and more social activity of these people.

Our findings are consistent with the previous epidemiological studies in other regions of the country (7,20) and suggest a downward trend in the incidence of hepatitis C infection. The incidence of hepatitis C in Hamadan province decreased from 9.95 per 100.000 people in 2011 to 5.26 per 100.000 persons in 2016 in the general population. More specifically, the incidence of hepatitis C decreased from 18.3 in 2011 to 9.38 per 100.000 people in 2016 in men, and decreased from 1.6 to 1.14 in women. In general, due to the implementation of educational programs as well as effective therapeutic strategies on hepatitis C disease by the Ministry of Health, the incidence of hepatitis C in Hamadan province is decreasing. There is currently no effective hepatitis C vaccine. Therefore, awareness programs for the transmission and prevention of hepatitis C in the community and for high-risk groups should be conducted by healthcare centers and mass media.

The present study suffered from a few limitations. Firstly, the collected data are based on the passive surveillance system, therefore, the findings are prone to underreporting. Secondly, HCV infection is usually without symptoms and patients are usually diagnosed accidentally during blood donation or screening during pregnancy, therefore, results strongly prone to underestimation.On the other hand, along with the mentioned limitations of passive surveillance systems, some advantages such as low cost and easier to carry out in comparison with active surveillance data encouraged us for using data from the passive system to monitor the trends of HCV and to provide critical information for monitoring a community's health.

Conclusion

Our results showed that HCV has a decreasing trend for both genders in Hamadan province and is mostly prevalent in males, age group of 25-44 years, married people and urban dwellers. Therefore, educational programs for the transmission and prevention of hepatitis C in the community and for high-risk groups should be conducted with the management of health centers and mass media. Further studies should explore the role of other risk factors in the HCV process and the reporting system.

Acknowledgments: We would like to thank the Deputy of Health of Hamadan University of Medical Sciences which collaborated in the data collection.

Ethics

Ethics Committee Approval: The present study is based on recorded data from disease surveillance system, which was approved by the Deputy Chancellor of Health.

Informed Consent: Hence this study used recorded data of disease surveillance system, informed consent of the patients is not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.M.Z., M.M., S.J.B., J.A., Design: S.K., S.N., J.A., Data Collection or Processing: M.S., M.M., S.J.B., Analysis or Interpretation: S.K., E.J., Literature Search: E.J., S.N., Writing: S.K., A.M.Z., S.N., E.J., M.M., S.J.B., M.S., J.A.

Conflict of Interest: The authors claim that they have no conflict of interest.

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Research Article

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Distribution of Hepatitis C Virus Genotypes in Aydın Province

Aydın İlinde Hepatit C Virus Genotiplerinin Dağılımı

Yasin TİRYAKİ, Alev ÇETİN DURAN, Osman Olcay ÖZÇOLPAN

Aydın State Hospital, Laboratory of Microbiology, Aydın, Turkey

ABSTRACT

Objectives: In this study, we evaulated the distribution and alteration of hepatitis C virus (HCV) genotypes throughout years which has a clinical importance in the treatment and follow-up.

Materials and Methods: Test results obtained from blood samples sent to the molecular microbiology laboratory at Aydın State Hospital for HCV genotype determination were analyzed retrospectively. A total of 182 samples collected between 2014 and 2018 were enrolled in the study. The determination of genotype and viral load of the samples were performed by real time polymerase chain reaction.

Results: 53.8% (98/182) of the samples were collected from male patients and 46.2% (84/182) from female patients. The mean age of the patients was 58.5±15.5 years. 69.2% of the samples were genotype 1b, 18.1% - genotype 1a, 2.2% - genotype 1 (those different from subtype 1a and 1b), 1.7% - genotype 2, 7.2% - genotype 3, and 1.7% of the samples were genotype 4.

Conclusion: In the present study, genotype 1 was the most common genotype (89.5%). Additionally, we have observed a decrease in the frequency of genotype 1b and a slightly increase in the frequency of other genotypes. Determination of HCV genotypes is important for treatment and prognosis of HCV infections.

Keywords: Hepatitis C virus, hepatitis C virus genotypes, epidemiology

ÖZ

Amaç: Çalışmamızda, hepatit C virüs (HCV) enfeksiyonunun tedavi ve takibinde önemli olan, HCV genotiplerinin dağılımı ve yıllar içindeki değişimi incelenmiştir.

Gereç ve Yöntemler: Aydın Devlet Hastanesi Moleküler Mikrobiyoloji Laboratuvarı'na 2014-2018 yılları arasında HCV genotip tayini için gönderilen kan örneklerinden elde edilen test sonuçları retrospektif olarak incelendi. Toplam 182 örneğin viral yükleri ve genotip tayini gerçek zamanlı polimeraz zincir reaksiyonu yöntemi ile belirlendi.

Bulgular: Örneklerin 98'i (%53,8) erkek, 84'ü (%46,2) kadın hastalara ait olup, yaş ortalaması 58,5±15,5 olarak hesaplanmıştır. Örneklerin %69,2'sinde genotip 1b, %18,1'inde genotip 1a, %2,2'sinde genotip 1 (1a, 1b dışı), %1,7'sinde genotip 2, %7,2'sinde genotip 3 ve %1,7'sinde genotip 4 saptanmıştır.

Sonuç: Çalışmamızda, en yüksek oranda genotip 1 (%89,5) rastlanmıştır. Genotip 1b oranında yıllar içinde azalma, diğer genotiplerde ise ılımlı bir artış saptanmıştır. HCV genotiplerinin takibi, tedavi ve prognoz açısından önemli olduğu gibi, epidemiyolojik açıdan da yol göstericidir.

Anahtar Kelimeler: Hepatit C virüs, hepatit C virüs genotipleri, epidemiyoloji

Tiryaki Y. Çetin Duran A. Özçolpan OO. Distribution of Hepatitis C Virus Genotypes in Aydın Province. Viral Hepat J. 2018;24:70-74.

Introduction

Hepatitis C virus (HCV) is an enveloped, single-stranded positive-sense RNA virus classified in the genus *Hepacivirus* of the family *Flaviviridae* (1). HCV infection is an important global public health problem (2). The virus is usually transmitted through blood transfusion, surgical and dental procedures, intravenous drug use and sexual intercourse (3,4,5). HCV infection becomes chronic in approximately 75%-85% of cases. Cirrhosis develops in approximately 20-30% of patients over 20 years of HCV infection. Among these patients, hepatocellular carcinoma develops at a rate of 1%-4% (6). According to the World Health Organization, globally, an estimated 71 million people have chronic hepatitis C infection and approximately 399.000 people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (7). In Turkey, it is estimated that around 1 million people are infected with HCV (8).

Address for Correspondence: *Yasin Tiryaki MD, Aydın State Hospital, Laboratory of Microbiology, Aydın, Turkey*
Phone: +90 506 612 87 20 E-mail: y_tiryaki74@hotmail.com ORCID ID: orcid.org/0000-0002-6494-337X **Received:** *13.08.2018* **Accepted:** *26.10.2018*

HCV has 7 different genotypes and more than 60 subtypes (9,10). HCV genotypes 1, 2 and 3 are the most prevalent globally whereas genotype 4 is common in North Africa and Middle East, genotype 5 in South Africa and genotype 6 is common in Southeast Asia (2,11). Genotype 1 is the most common (46.2%) HCV genotype in the world followed by genotype 3 (30.1%). Genotype 2, 4 and 6 account for 22.8% of cases and genotype 5 is found in less than 1% of cases (11). Determination of geographical distribution of HCV genotypes is critical in the development of rational therapy protocols (12).

In this study, we aimed to determine the distribution of HCV genotypes in a five-year period in our region.

Materials and Methods

The results of HCV genotyping performed between 2014 and 2018 in 182 HCV RNA-positive patients in Aydın State Hospital were retrospectively evaluated. Repeated results from the same patients were excluded. Ethics committee approval was not required due to the retrospective design of the study.

Viral nucleic acid extractions were performed by a Magnesia 16 automated analyzer (Anatolia Geneworks, Turkey) using "Magnesia viral nucleic acid extraction kit EP" (Anatolia Geneworks, Turkey). Quantitation of viral nucleic acids was performed by real time-polymerase chain reaction (RT-PCR) targeting 5' UTR region in a Montania 4896 analyzer (Anatolia Geneworks, Turkey) using "Bosphore HCV Quantification Kit V2" (Anatolia Geneworks, Turkey). This assay had an analytical sensitivity of 25 IU/mL and a linearity range of 10^1-10^9 IU/mL. HCV genotyping [genotypes 1a, 1b, 1 (those different from subtype 1a and 1b), 2,3,4,5,6] was performed by RT-PCR targeting NS5B region in a Montania 4896 analyzer (Anatolia Geneworks, Turkey) using "Bosphore HCV Quantification Kit V3" (Anatolia Geneworks, Turkey). All assays were performed according to the manufacturer's instructions.

Results

53.8% (98/182) of the samples were collected from male patients (mean age: 55.8±15.9 years) and 46.2% (84/182) of the samples were collected from female patients (mean age: 61.7±14.4 years). The mean age of the study population was 58.5±15.5 years. HCV RNA levels ranged between 694 and 31.580.000 IU/mL. 69.2% (126/182) of the samples were genotype 1b, 18.1% (33/182) 1a, 2.2% (4/182) - 1 (those different from subtype 1a and 1b), 1.7% (3/182) - genotype 2, 7.2% (13/182) - genotype 3 and 1.7% (3/182) were genotype 4 (Figure 1).

Distribution of HCV genotypes by years (2014-2018) is presented in Table 1. Gender and age distribution of HCV genotypes between 2014 and 2018 are presented in Table 2.

There were 11 foreign national patients in this study. The nationalities and HCV genotypes of these patients are summarized in Table 3. Genotype 1b (72.7%) was the predominant genotype in this group.

Discussion

Genotype 1b is the most common HCV genotype in Turkey. Recent studies revealed that there has been a decline in the prevalence of HCV genotype 1b infections while an increase in the prevalence of infections caused by other genotypes. This alteration may be due to immigrants, touristic activities and different risk factors for HCV infection, as well as increased safety procedures in medical practice. Table 4 shows the results of some of the HCV genotyping studies in Turkey (13,14,15,16,17,18,19,20,21,22,23,24,25,26,27).

In this study, HCV genotype 1 (89.5%) was found the most common genotype in Aydın. However, we have observed a decrease in the frequency of genotype 1b infection throughout years while a slight increase in genotype 1a infection. Additionally, there has been an increase in the genotype diversity over years particularly in genotype 3 and genotype 4, respectively.

Among foreign nationals (Azerbaijan, Russia, Syria, Ukraine, Romania, Mongolia) enrolled in this study, patients infected with genotype 3 were from Syria and Ukraine while those with

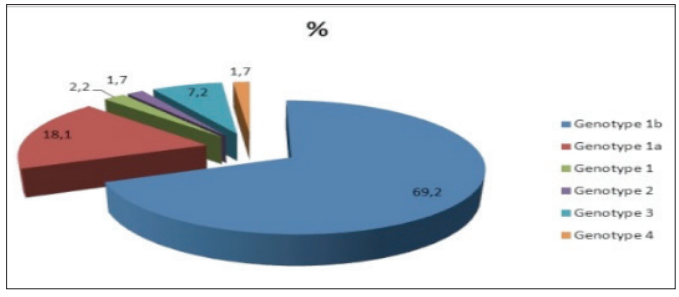

Figure 1. Distribution of hepatitis C virus genotypes

Table 1. Distribution of hepatitis C virus genotypes by years (2014-2018)

Year	HCV genotypes							
	Genotype 1 n (%)				Genotype 2 n (%)	Genotype 3 n (%)	Genotype 4 n (%)	Total n
	1a	1b	1	Genotype 1 Total				
2014	3 (42.9)	4 (57.1)	-	7 (100.0)	-	-	-	7
2015	4 (12.9)	25 (80.6)	-	29 (93.5)	1 (3.2)	1 (3.2)	-	31
2016	7 (19.4)	26 (72.2)	1 (2.8)	34 (94.4)	-	2 (5.6)	-	36
2017	14 (15.2)	61 (66.3)	3 (3.3)	78 (84.8)	2 (2.2)	9 (9.7)	3 (3.3)	92
2018	5 (31.3)	10 (62.5)	-	15 (93.8)	-	1 (6.2)	-	16
Total	33 **(18.1)**	126 **(69.2)**	4 **(2.2)**	163 **(89.5)**	3 **(1.7)**	13 **(7.2)**	3 **(1.7)**	182 **(100.0)**

HCV: Hepatitis C virus

genotype 4 were from Syria (Table 3). Touristic activities in the region and Syrian refugees may be the cause of increase in the diversity of HCV genotypes in Aydın province. Studies performed in various regions of Turkey including Mediterranean, Aegean and southeast Anatolia regions shows that in the areas exposed to immigrant and touristic activities, genotype 1 was still responsible for the vast majority of cases, however, the prevalence of the other genotypes was higher than in the other regions of Turkey (Table 4).

Table 2. Gender and age distribution of hepatitis C virus genotypes between 2014 and 2018

	HCV genotypes n (%)					
	Genotype 1 (total) (n=163)			Genotype 2	Genotype 3	Genotype 4
	1a	1b	1			
Number (n=182)	33 (18.1)	126 (69.2)	4 (2.2)	3 (1.7)	13 (7.2)	3 (1.7)
Gender						
Male n=98 (53.8%)	22 (22.5)	60 (61.2)	3 (3.1)	2 (2.0)	9 (9.2)	2 (2.0)
Female n=84 (46.2%)	11 (13.1)	66 (78.5)	1 (1.2)	1 (1.2)	4 (4.8)	1 (1.2)
Age						
Median (min-max)	56.1 (18-92)	61.7 (24-82)	60.7 (48-72)	41.6 (27-51)	39.5 (17-61)	49.0 (44-54)

HCV: Hepatitis C virus, min: Minimum, max: Maximum

Table 3. Distribution of hepatitis C virus genotypes in foreign nationals

		HCV genotypes n (%)			
Country	Number	Genotype 1b	Genotype 2	Genotype 3	Genotype 4
Azerbaijan	4 (36.3)	4 (36.3)	-	-	-
Russia	2 (18.2)	2 (18.2)	-	-	-
Syria	2 (18.2)	-	-	1 (9.1)	1 (9.1)
Ukraine	1 (9.1)	-	-	1 (9.1)	-
Romania	1 (9.1)	1 (9.1)	-	-	-
Mongolia	1 (9.1)	1 (9.1)	-	-	-
Total	11 (100.0)	8 **(72.7)**	-	2 **(18.2)**	1 (9.1)

Table 4. Results of some of the hepatitis C virus genotyping studies in Turkey

				Genotypes						
Study (Reference)	**Year**	**Province**	**Number (n)**	**Genotype 1 (total) n (%)**	**1a (%)**	**1b (%)**	**2 (%)**	**3 (%)**	**4 (%)**	**5 (%)**
Abacioglu et al. (13)	1995	Izmir	89	84 (94.4)	(19.1)	(75.3)	3 (3.4)	-	2 (2.2)	-
Yarkin and Hafta (14)	2000	Adana/Mersin	62	60 (96.7)	(14.5)	(82.2)	2 (3.3)	-	-	-
Bozdayi et al. (15)	2004	Ankara	365	349 (95.0)	(11.0)	(84.0)	10 (3.0)	3 (1.0)	3 (1.0)	-
Altuglu et al. (16)	2008	Izmir	345	335 (97.1)	(9.9)	(87.2)	3 (0.9)	5 (1.4)	2 (0.6)	-
Gokahmetoglu et al. (17)	2011	Kayseri	146	90 (61.7)	(3.4)	(52.8)	4 (2.7)	-	52 **(35.6)**	-
Buruk et al. (18)	2013	Trabzon	304	282 (92.8)	(5.3)	(87.5)	5 (1.6)	15 (4.9)	2 (0.7)	-
Altuglu et al. (19)	2013	Izmir	535	499 (93.3)	(12.9)	(80.4)	8 (1.5)	20 (3.7)	8 (1.5)	-
Saglik et al. (20)	2014	Antalya	422	352 (83.4)	(14.7)	(63.3)	15 (3.5)	47 **(11.1)**	7 (1.6)	-
Kuscu et al. (21)	2014	Adana	369	289 (78.3)	-	-	23 (6.2)	54 **(14.6)**	3 (0.8)	-
Calıskan et al. (22)	2015	Kahramanmaraş	313	162 (51.7)	-	-	4 (1.3)	144 **(46.0)**	3 (1.0)	-
Kirdar et al. (23)	2015	Aydın	50	48 (96.0)	(18.0)	(72.0)	(2.0)	(2.0)	-	-
Kayman et al. (24)	2015	Kayseri	218	136 (62.4)	(2.3)	(60.1)	10 (4.6)	-	72 **(33.0)**	-
Duran et al. (25)	2016	Adana	119	85 (71.4)	(12.6)	(58.8)	9 (7.6)	20 **(16.8)**	4 (3.4)	1 **(0.8)**
Balin et al. (26)	2017	Elazığ	71	62 (87.3)	-	-	2 (2.8)	7 (9.9)	-	-
Harman et al. (27)	2017	Gaziantep	160	157 (98)	-	(98.0)	1 (0.75)	2 (1.25)	-	-
Presented study	2014-2018	Aydın	182	163 (89.5)	(18.1)	(69.2)	3 (1.7)	13 **(7.2)**	3 (1.7)	-

There are regional differences in the distribution of HCV genotypes in our country. The prevalence of HCV genotype 4 has been reported to be 35% in Kayseri (17,24) while there has been an obvious increase in infections caused by genotype 3 in the cities of southern parts of Turkey, such as Adana and Kahramanmaraş. In this patient population, intravenous drug abuse is relatively frequent (21,22,25). Genotype 5 infections are being reported in Syrian refugees (25).

Even though the frequency of genotype 1, which is associated with poor prognosis, is frequent in Turkey (85-90%), recent studies indicated a decrease in genotype 1 with an increase in other genotypes and regional increase in some genotypes.

Study Limitations

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

Conclusion

As a result, data have to be updated periodically not only for epidemiological purposes but also and more importantly for prognosis and treatment.

Ethics

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

Authorship Contributions

Surgical and Medical Practices: O.O.Ö., Concept: Y.T., Design: A.Ç.D. Data Collection or Processing: O.O.Ö., Analysis or Interpretation: A.Ç.D., Literature Search: Y.T., Writing: Y.T.

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Research Article

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An Assessment of Sharps Injuries in Healthcare Workers

Sağlık Çalışanlarında Delici-Kesici Alet Yaralanmalarının Değerlendirilmesi

Nurten Nur AYDIN, Firdevs AKSOY, Gürdal YILMAZ, İftihar KÖKSAL

Karadeniz Technical University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Trabzon, Turkey

ABSTRACT

Objectives: During the healthcare services, healthcare workers are under the risk of sharps and needle stick injuries. In this study, we evaluated occupational injuries seen in our hospital and emphasized the importance of reducing the risk of exposure to these injuries through compliance with infection control precautions.

Materials and Methods: In this study, we retrospectively evaluated the data of the Infection Control Committee of Karadeniz Technical University Faculty of Medicine on work-related sharp injuries among healthcare workers between 01.01.2016 and 01.02.2018.

Results: This study was carried out with sixty-six healthcare workers. The mean age of the healthcare workers was 29.3(±7.62) years. The injuries were most frequently observed in nurses. Majority of the injuries had occurred in internal medicine clinics and most of them were needle stick injuries. In 99.7% of the injuries, contamination state was known. Forty-nine of 66 employees were immune to the hepatitis B virus. After the injuries, any acute viral infection was not developed. Three out of the 6 employees injured by positive serology of human immunodeficiency virus sources were given prophylaxis for four weeks after contact.

Conclusion: All healthcare workers should be informed about the measures against injuries, the proper use of protective equipment and the importance of vaccination and, they should be subjected to periodic training.

Keywords: Healthcare workers, sharps injuries, hepatitis B, hepatitis C, human immunodeficiency virus

ÖZ

Amaç: Sağlık bakım hizmetleri sırasında sağlık çalışanları delici kesici aletlerle yaralanma tehlikesiyle risk altındadır. Çalışmamızda hastanemizde görülen mesleki yaralanmaların değerlendirilmesi ve enfeksiyon kontrol önlemlerine uyumla maruziyet riskinin azaltılmasının önemine vurgu yapılmıştır.

Gereç ve Yöntemler: Çalışmamızda 01.01.2016-01.02.2018 tarihleri arasında Karadeniz Teknik Üniversitesi Tıp Fakültesi Hastanesi, Enfeksiyon Kontrol Komitesi'nin çalışan yaralanma kayıtları retrospektif olarak değerlendirildi.

Bulgular: Altmış altı sağlık çalışanı formu çalışmaya alındı. Çalışanların yaş ortalaması 29,3±7,62 idi. Yaralanma en sık hemşirelerde görüldü. En fazla yaralanma dahili kliniklerde olmuştu. En fazla yaralanma iğne batması sonrası meydana geldi. Yaralanmaların %99,7'sinde kontaminasyon durumu biliniyordu. Altmış altı çalışanın 49'u hepatit B virüsne karşı bağışıktı. Yaralanma sonrası çalışanlardan hiçbirinde akut viral enfeksiyon gelişmedi. İnsan immün yetmezlik virüsü pozitif kaynakla yaralanan 6 çalışanın 3'ü temas sonrası dört hafta profilaksi aldı.

Sonuç: Tüm sağlık çalışanları, yaralanmalara karşı önlemler, koruyucu ekipmanların uygun kullanımı ve aşılanmanın önemi konusunda bilgilendirilmeli ve periyodik eğitimler almalıdır.

Anahtar Kelimeler: Sağlık çalışanları, delici kesici alet yaralanmaları, hepatitis B, hepatitis C, insan immün yetmezlik virüsü

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This study was presented as a poster abstract at the 14th National Viral Hepatitis Congress on April 26-29, 2018.

Introduction

Healthcare workers are at risk of occupational exposure to infected blood and body fluids contaminated with hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) that can cause significant mortality and morbidity (1,2). Today, although many medical supplies are disposable and infection rates are low, sharps and need lestick injuries (NSI) continue to be the fact that healthcare workers frequently face (3). However, most of the contacts do not result in infection. Infections are mainly caused by mucosal and percutaneous injuries (4). In order to

Address for Correspondence: *Nurten Nur Aydın MD, Karadeniz Technical University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Trabzon, Turkey*
Phone: +90 462 377 56 95 E-mail: nurtennurkenc@hotmail.com ORCID ID: orcid.org/0000-0003-4138-2490 **Received:** *09.07.2018* **Accepted:** *30.10.2018*

reduce the risk of transmission of pathogens from infected blood or body fluids to healthcare workers, hand washing, proper use of protective barriers, adherence to the standard precautions when using needles and other sharp instruments are the most important protection methods for all healthcare workers (1). In this study, it was aimed to determine the occupational injury rates among healthcare workers at high risk for occupational exposure to NSI. In this study, the importance of compliance with infection control precautions in reducing the exposure risk was emphasized.

Materials and Methods

This study was carried out through retrospective examination of the data of the Infection Control Committee of Karadeniz Technical University Faculty of Medicine on work-related sharp injuries between 01.01.2016 and 01.02.2018. The data of sixty-six healthcare workers were evaluated. The healthcare workers were grouped as nurses, doctors, intern students, cleaning staff and technicians. Demographic characteristics of the injured employees, types of injury, use protective equipment, immunization status, and contamination states of the injuries were investigated. The data were recorded in excel format and the results were evaluated.

Results

Sixty-six healthcare workers with a mean age of 29.3±7.62 years were exposed to NSI between 01.01.2016 and 01.02.2018. Twenty-three (34.9%) of the subjects were male, 43 (65.1%) were female. Among the professional groups, the biggest group consisted of nurses (50%) followed by doctors (16.7%), cleaning staff (12.1%), technicians (12.1%), and interns (12.1%), respectively. Most of the injuries were observed in internal medicine clinics (33.3%) followed by those in intensive care units (18.2%), operating room (18.2%), surgical clinics (15.1%), emergency room (13.7%) and in the phlebotomy department (1.5%), respectively. A total of 68.2% of the employees were exposed to needle stick injury, 15.1% to mucosal contact with blood, 9.1% to sharps injury and, 7.6% of them were exposed to contact with other body fluids (Table 1).

During the injuries, 55 of the employees (83.3%) were using gloves as the protective equipment. The contamination status was known in 99.7% of the injuries, whereas it was not known in 0.3% of them. During injuries, 22.7% of the employees were exposed to anti-HCV-positive sources, 13.6% to hepatitis B surface antigen (HBsAg)-positive sources and 9.1% were exposed to the positive HIV-positive sources. Forty-nine of 66 employees were immune to HBV. Because anti-HBs <10 IU was identified in 2 of the 9 employees exposed to HBsAg-positive source, HBV vaccine and immunoglobulin were administered. After the injuries, any acute viral infection was not developed in the employees. Three out of the 6 (9.1%) employees injured by HIV-positive sources were given prophylaxis for four weeks after contact (Table 2). The other 3 employees did not use the recommended drugs for prophylaxis because of side effects, and for having negative HIV RNA testing after 6 months.

Discussion

Healthcare workers face many risks and dangers related to their occupation in the working environment. In terms of occupational exposure, NSI is a serious problem that can often be prevented. Similar injuries also occur in nursing homes, emergency care services, and other health care services, such as private homes. In infections, especially HBV, HCV, and HIV are associated with occupational contagion. However, more than 20 other pathogens can be transmitted by injury (5,6).

Looking at the occupational groups of the 66 healthcare workers investigated in this study, it was observed that, most frequently, nurses had been exposed to injuries (50%). In terms of other occupational groups, following the nurses, doctors (16.7%), cleaning staff (12.1%), technicians (12.1%), and interns (12.1%) had been exposed to injuries, respectively. In the literature, several studies, in which similar evaluations were made, also showed that nurses were the first group being most frequently exposed

Table 1. The data of sharps and needlestick injuries

Mean age	29.3±7.62
Gender	**Number (%)**
Female	43 (65.1%)
Male	23 (34.9%)
Job	**Number (%)**
Nurse	31 (50%)
Doctor	11 (16.7%)
Cleaning staff	8 (12.1%)
Technicians	8 (12.1%)
Intern students	8 (12.1%)
Department	**Number (%)**
Internal clinics	22 (33.3%)
Intensive care units	12 (18.2%)
Operating room	12 (18.2%)
Surgical clinics	10 (15.1%)
Emergency room	9 (13.7%)
Phlebotomy service	1 (1.5%)
Type of injury	**Number (%)**
Pinprick	45 (68.2%)
Mucosal contact with blood	10 (15.1%)
Sharp objects	6 (9.1%)
Contact with other body fluids	5 (7.6%)

Table 2. Healthcare workers exposed to injury and conditions of serology sources

Healthcare workers	66
Anti-HBs >10 IU	49
HBsAg positive	-
Anti-HCV positive	-
Anti-HIV positive	-
Source	
Anti-HCV positive	15 (22.7%)
HBsAg positive	9 (13.6%)
Anti-HIV positive	6 (9.1%)

HBs: Hepatitis B surface, HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C virüs, HIV: Human immunodeficiency virus

to injuries (6,7,8,9). In different studies, the frequency of injuries varies in different occupational groups in several studies. In a study carried out by Merih et al. (10), it was seen that the most frequently injured occupational group was the cleaning staff group with the rate of 71.9%. Another study found that the most frequently injured occupational group was doctors with the rate of 56% (11).

In a study, it was shown that most of the healthcare workers exposed to NSI did not report the injury and did not take the necessary precautions (12). Although the number of NSI cases seems to be low in our study, it is thought that the majority of healthcare workers considers injuries as insignificant and do not report. In a study carried out by Altıok et al. (13), it was indicated that 87.3% of the healthcare workers exposed to SNI did not report the injuries. The Centers for Disease Control and Prevention (CDC) estimates that 385.000 healthcare workers each year are exposed to SNI (6). Due to the missing data, it is not possible to assess the actual size of injuries among healthcare workers. Surveys on healthcare workers showed that at least 50% of occupational injuries were not reported (14,15).

In a study conducted by Kişioğlu et al. (16), it was found that injuries were seen mostly in surgical clinics. In another study conducted with doctors, it was found that the commonest site in which such injuries were sustained was surgical clinics and it was reported that most of the injuries occurred during suturing (17). In contrast to the literature, in the present study, it was determined that the rate of SNI in internal medicine clinics was higher (33.3%); this rate was 18.2%, 18.2%, 15.1%, 13.7% and 1.5% for intensive care units, operating rooms, surgery clinics, emergency clinics and the phlebotomy department, respectively. Studies available in the literature found that needlestick injuries were especially observed during needle recapping (18,19). Similar to the literature, it was also determined in our study that injuries mostly occurred as a result of percutaneous needlestick injuries with the rate of 68.2%.

The CDC recommended standard protection measures in 1982 and updated in the following years. According to these measures, blood and body fluids should be considered potentially infectious (18). In a prospective surveillance of percutaneous, mucous membrane, and cutaneous blood contacts among healthcare workers who provided home infusion therapy or performed procedures using needles or sharp instruments in the home setting, it was determined that masks, gowns and protective glasses or goggles were used in 52%, 5% and 2% of 14.744 procedure visits, respectively (20). Other studies in the literature have shown that the rate of protective equipment use varied between 55% and 68% and that the use of gloves was the most common (18,21). In our study, it was found that 83.3% of healthcare workers used gloves as protective equipment, however, any data related to other protective equipment use was not determined.

In the case of injuries that occur as a result of contact with blood or body fluids, skin regions should be washed with soap and water, and the mucous membranes should only be washed with water (18,21). In terms of potential HBV, HCV and HIV transmission, serologies of the healthcare worker exposed to INS and the source patient should be looked at. Today, prophylaxis is recommended against HBV and HIV after contact and employees should be evaluated in terms of immunization and prophylaxis (18). In this study, anti-HBS >10 IU was found in 49 of the participants. Those who were not immune were enrolled in the vaccination program. For 0.3% of the healthcare workers exposed to NSI, the source causing injury was unknown. In the cases where the source was known, 22.7% of the sources were found to be anti-HCV-positive, 3.6% - HBsAg-positive, and 9.1% were HIV-positive. In the case where the source was HBsAg-positive, because anti-HBs <10 IU was identified in 2 employees, HBV vaccine and immunoglobulin were administered. 3 out of the 6 (9.1%) employees injured by HIV-positive sources were given prophylaxis for four weeks after contact. In a survey study, 3.8% of the injured healthcare workers were reported to have contact-related HBV infection 0.3% had contact-related HCV infection (22). In the present study, the healthcare workers exposed to NSI were followed for 6 months and any acute viral infection was not developed in any of them.

Conclusion

Healthcare workers may experience occupational exposure to NSI during their health care services. This situation creates psychological stress on the employees especially in contaminated injuries. All the healthcare workers should be informed about the measures against injuries, the proper usage of protective equipment and the importance of vaccination. The trainings should be given periodically and the participation of healthcare workers in these trainings should be ensured. It is thought that the transmission frequency of infections will be reduced by full compliance with standard precautions and the correct use of protective equipment.

Ethics

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

Authorship Contributions

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

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Research Article

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A Misleading Parameter in the Diagnosis of Chronic Hepatitis B: Persistently Normal Transaminases

Kronik Hepatit B Tanısında Yanıltıcı Bir Parametre: Sürekli Olarak Normal Transaminazlar

Osman ÖZDOĞAN[1], Serkan YARAŞ[1], Ali Riza KÖKSAL[2], Engin ALTINKAYA[3], Mehmet BAYRAM[2], Banu YILMAZ ÖZGÜVEN[4], Canan ALKIM[2]

[1]*Mersin University Faculty of Medicine, Department of Gastroenterology, Mersin, Turkey*
[2]*Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Gastroenterology, Istanbul, Turkey*
[3]*Cumhuriyet University Faculty of Medicine, Department of Gastroenterology, Sivas, Turkey*
[4]*Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Pathology, Istanbul, Turkey*

ABSTRACT

Objectives: Most of the patients with hepatitis B e (HBe)-negative hepatitis B have persistently normal transaminases (PNALT) levels. Patients, who have higher fibrosis and necroinflammatory activity scores, are at high risk for hepatocellular carcinoma and cirrhosis. Therefore, it is important to distinguish between active and inactive hepatitis in this group.

Materials and Methods: Sixty-six treatment-naïve, non-cirrotic, HBe antigen (HBeAg)-negative and a PNALT and a level of a hepatitis B virus (HBV) DNA level of ≥2000 IU/mL were included in this study. Ishak's scoring system was used for histopathological evaluation. Chronic hepatitis was defined as a fibrosis score of higher than/equal to 2 and/or a histological activity index score of higher than 4.

Results: The percentage of patients diagnosed with advanced fibrosis score and high necroinflammatory activity was 65% and 48%, respectively. Accordingly, 76% of patients were considered to have chronic hepatitis. Level of the HBV DNA was the most significant value for predicting chronic hepatitis. 94.1% of patients with a HBV DNA value over 20000 IU/mL had chronic hepatitis (p<0.001).

Conclusion: As a result of this study, it has been found that the prevalence of chronic hepatitis in our country was high in HBeAg-negative patients with PNALT and a HBV DNA level higher than 2000 IU/mL. We recommend starting treatment in patients with a HBV DNA level higher than 20000 IU/mL without considering any other criteria. Close monitoring or biopsy is recommended in patients with HBV DNA values between 2000 and 20000 IU/mL.

Keywords: Hepatitis B, hepatitis B virus DNA, hepatitis B e antigen-negative chronic hepatitis, inactive hepatitis B virus carrier

ÖZ

Amaç: Hepatit B e (HBe) negatif hepatit B'li hastaların çoğu, sürekli olarak normal transaminazlara (PNALT) sahip olgulardır. Daha yüksek fibrozis ve nekroenflamatuvar aktivite skoru olan hastalar siroz ve hepatosellüler karsinoma için ciddi risk altında olduğundan dolayı bu gruptaki hastalarda aktif/inaktif hepatit ayrım yapmak önemlidir.

Gereç ve Yöntemler: HBe antijen (HBeAg)-negatif olan, sirozu olmayan, hepatit B virüs (HBV) DNA değeri 2000 IU/mL ve üzerinde olan PNALT'li naif 66 hasta çalışmaya dahil edildi. Histopatolojik değerlendirme için İshak skorlama sistemi kullanıldı. Fibrozis skoru 2 ve/veya histolojik aktivite indeksi skoru >4 olan hastalar kronik hepatit olarak kabul edildi.

Bulgular: İleri fibrozis skoru ve yüksek nekroenflamatuvar aktivitesi olan hastaların oranı sırasıyla %65 ve %48 idi. Kronik hepatit olarak kabul edilen hasta oranı %76 idi. HBV DNA seviyeleri, kronik hepatitin öngörme de en önemli değerdi. HBV DNA seviyeleri 20000 IU/m'nin üzerinde olan hastaların %94,1'inde kronik hepatit vardı (p<0,001).

Sonuç: Ülkemizde HBV DNA değerinin 2000 IU/mL üzerinde olduğu HBeAg-negatif PNALT hastalarında kronik hepatit oranı yüksektir. Bu hastalarda HBV DNA'nın 20000 IU/mL'nin üzerinde olması halinde başka kriterlere bakılmaksızın tedaviye başlanmasını öneririz. HBV DNA seviyeleri 2000 ve 20000 IU/mL arasında olan hastalarda yakından izleme veya biyopsi uygun gözükmektedir.

Anahtar Kelimeler: Hepatit B, hepatit B virüs DNA, hepatit B e antijen-negatif kronik hepatit, inaktif hepatit B virüs taşıyıcı

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Address for Correspondence: *Osman Özdogan MD, Mersin University Faculty of Medicine, Department of Gastroenterology, Mersin, Turkey*
Phone: +90 324 241 00 00 E-mail: osmanozdogan2000@gmail.com ORCID ID: orcid.org/0000-0002-8299-5341 **Received:** *18.05.2018* **Accepted:** *10.07.2018*

Introduction

It is estimated that more than 2 billion people, all over the world, have been infected with hepatitis B virus (HBV). On the other hand, there are more than 240 million HBV carriers as well (1). Majority of chronic hepatitis cases (more than half of these cases) are estimated to be hepatitis B e antigen (HBeAg)-negative worldwide (2). In studies conducted in Europe, increased rates of hepatitis B surface antigen (HBsAg)-negative hepatitis have been shown and this ratio has been reported to be 70%-90% in recent years (3,4). Turkey is a country with intermediate endemicty for hepatitis B (2%-8%) and the prevalence of HBeAg-negative hepatitis is 40-85%

Chronic hepatitis B (CHB) can be classically described as presence of positive serum HBsAg for longer than 6 months (5,6). As a dynamic process, CHB infection course can be investigated into five phases. These phases can be defined as follows (7):

i. First phase: HBeAg-positive chronic infection,
ii. Second phase: HBeAg-positive chronic hepatitis,
iii. Third phase: HBeAg-negative chronic infection,
iv. Forth phase: HBeAg-negative chronic hepatitis, and
v. Fifth phase: HBsAg-negative phase.

HBeAg-negative chronic infection is the latent phase that infection enters into non-replicative phase associated with termination of immune response. In this phase, antibody against HBeAg occurs and HBsAg positivity continues, but HBV DNA as indicative of active viral replication is negative or too low (<2000 IU/mL). Formerly, in this period called "inactive HBV carrier" state. HBeAg-negative chronic hepatitis may develop in this phase, therefore, if these patients have not been followed for a long duration, they should not be considered inactive HBV carriers (8). This distinction is important because inactive HBV carriers show a good course while risk of progression to fibrosis, hepatic cirrhosis, and hepatocellular carcinoma (HCC) is more than in patients diagnosed with active disease (9). HBeAg-negative chronic hepatitis is different from inactive carrier state by HBV DNA levels above 2000 IU/mL, continuously or intermittently elevated alanine aminotransferase (ALT) levels, and/or at least moderate fibrosis or liver necroinflammation (10). Approximately in 1-3 of every 100 patients, inactive HBV carrier state progresses to HBeAg-negative chronic hepatitis (11). Guidelines of major professional liver organizations in the world agreed that there is no need to treat or biopsy inactive HBV carrier patients (HBV DNA<2000 IU/mL and ALT levels within the normal range) (7,12,13). These guidelines recommend periodical close monitoring with serum ALT and some parameters including HBV DNA for inactive carrier patients. If ALT and/or HBV DNA levels increase, either direct treatment or treatment according to biopsy results is recommended in these patients. Also, in a small number of patients with a HBV DNA level over 2000 IU/mL, persistently normal ALT (PNALT) is observed and guidelines do not give any clear recommendation for these patients. There are few studies on these patients in the literature and different results are reported from different regions. In our country, there are very few studies in this group of patients. In this context, it was aimed in this study to reveal the prevalence of HBeAg-negative chronic hepatitis in our country and investigate some parameters which indicate HBeAg-negative chronic hepatitis.

Materials and Methods

Patient Enrollment

This was a single-center cross-sectional study. Consecutive HbeAg-negative and HbsAg-positive treatment-naïve patients with a HBV DNA level of >2000 IU/mL, who were admitted to our unit between March 2010 and April 2013, were included in the study. Patients with malignancy, any autoimmune or comorbid disorder and sings of chronic liver disease or cirrhosis were excluded. Additionally, those with hepatitis C and hepatitis D, as well as human immunodeficiency virus-positive patients were also excluded. Clinical and demographic characteristics were noted and body mass index (BMI) was calculated in all patients.

Serological, Biochemical and Hepatitis B Virus DNA Assay

Blood samples of the patients were taken in the morning after a 12-hour overnight fast. Routine examinations were studied in the laboratory of the departments of clinical biochemistry and clinical microbiology. The normal range for ALT values for women was 10-35 U/L and for men, 10-45 U/L. HBsAg, anti-HBs and anti-HCV were studied using an Eti-Max 3000 device with micro-ELISA method. HBeAg, anti-HBe, HAV immunoglobulin (Ig) M and IgG, and anti-HCV were studied on a Liaison device with macro-ELISA method. The level of HBV DNA was studied on a TaqMan 48 analyzer (CTM 48; Roche Molecular Systems, Inc.) using the polymerase chain reaction method and the results were were reported in IU/mL. HBV DNA values were calculated by taking the average of the last two measurements. We investigated a cut-off value to determine the chronic hepatitis using the significant parameters. We have identified 20.000 IU/mL as the cut off value for HBV DNA because HBV DNA is used in many researches (14,15,16,17,18,19,20) and guidelines (7,12,13) to standardize rather than receiver operating characteristics (ROC). For the same reason, the cut off value for ALT was determined as 19 U/L for women and 30 U/L for men (13,14,20,21). We allocated two patient groups with regard to ALT levels: low-normal ALT (LNALT) (for women <19 U/L, for men <30 U/L) and high-normal ALT (HNALT) (for women 19-35, U/L for men 30-45 U/L).

Liver Biopsy and Histopathological Evaluation

Liver biopsies were performed in our clinic by a single physician after an overnight fast by a 16-gauge Hepafix needle using the ultrasound-guided technique. Biopsy samples containing at least 10 portal areas were included in the study and evaluated by an independent pathologist. The pathologist had no information about the patients. Ishak scoring system [fibrosis stage (range, 0-6) and histology activity index (HAI) (range, 0-18)] were used as the classification system (23). Patients with a fibrosis score of ≥2 and /or HAI score of >4 were considered to have HBeAg-negative chronic hepatitis.

This study was approved by the Local Ethics Committee (Şişli Hamidiye Etfal Training and Research Hospital, approval number:12.01.2010, 1119). Written informed consent received.

Statistical Analysis

Statistical analyses were carried out with the use the SPSS program version 21 (Chicago, IL, USA). The variables were examined using analytical techniques (the Shapiro-Wilk test/the Kolmogorov-

Smirnov test) and visual inspection (probability plots, histograms) to find out distribution of the samples. Standard deviations and means were used to carry out descriptive analysis. Ordinal and continuous variables that do not have normal distributions were compared using the Mann-Whitney U test. Student's t-test was used to evaluate differences between the two study subgroups in normally distributed continuous variables. According to the availability of data, the Pearson and Spearman correlation coefficients were used to test correlations among the study variables. A p value of less than 0.05 was considered statistically significant.

Results

Sixty-six patients were enrolled in this study. The mean age of the participants was 40.7±10.5 years. 39 (59.1%) patients were male, 27 (40.9%) were female. The mean ALT level was 31.1±10.1 U/L. The lowest HBV-DNA level was 2.010 IU/mL, the highest was 853.000 IU/mL and the mean HBV DNA value was 101.780±185.426 IU/mL. The number of patients with a HBV DNA value between 2.000 and 20.000 IU/mL was 32 (48%). The characteristics of the patients are shown in Table 1.

The fibrosis score was 2 or over in 65% of the patients (43/66) and the mean score was 1.83±0.83. Only 1 patient had a fibrosis score of 5. In patients with a fibrosis score of 2 or higher, the mean HBV DNA, ALT and aspartate aminotransferase (AST) levels were 141.442±124.089 IU/mL, 32.9±11.6 U/L and 27.9±18.3 U/L, respectively. In patients with a fibrosis score of <2, the mean HBV DNA, AST and ALT levels were 27.630±56.856 IU/mL, 23.1±14.6 U/L and 27.6±12.5U/L, respectively. There was a statistically significant difference in these parameters between the two groups (p<0.001, p=0.037 and p=0.006, respectively). HBV DNA was over the level of 20.000 IU/mL in 30 (69.8%) patients with a fibrosis score of ≥2 and, 27 of these (62.8%) were male.

The mean HAI score was 5.06±2.27 (range: 2-11). HAI score was found to be above 4 in 32 patients (48.5%). The mean HBV DNA, ALT, AST levels in patients with a HAI >4 (192.677±76.645 IU/mL, 35.3±23.1 U/L, 28.8±14.2 U/L, respectively) were considerably higher than in patients with a HAI score of ≤4 (16.231±11.230 IU/mL, 27.03±17.1 U/L, 23.8±16.9 U/L, respectively) (p<0.001, p<0.001, p=0.002, respectively). The HBV DNA level was above 20.000 IU/mL in 26 patients (81.3%), all of whom were male.

Chronic hepatitis was found in 50 HBeAg-negative patients (76%). HBV DNA, ALT and AST values in patients with chronic hepatitis were higher than in those without chronic hepatitis (p<0.001, p=0.03, and p=0.01 respectively). Platelets were found to be significantly lower in chronic hepatitis group. On the other hand, there was no statistically significant difference in other parameters (Table 1).

We evaluated the correlations of other parameters with fibrosis and HAI scores because presence of chronic hepatitis in patients with a HBV DNA level of higher than 2.000 IU/mL was analyzed

Table 1. Characteristics of all patients and those in chronic hepatitis group and non-chronic hepatitis group

	Total (n=66)	Non-chronic hepatitis (n=16)	Chronic hepatitis (n=50)	p
Age (years)	40.7±10.5	38.7±8.1	41.3±11.1	0.472
Disease age (years)	7.5±4.5	6.1±4.7	7.9±4.4	0.07
BMI (kg/m^2)	25.0±3.9	23.8±3.2	25.3±4.0	0.15
Male	39 (59.1%)	8 (50%)	31(62%)	0.43
Fibrosis stage ≥2	43 (65.2%)	0 (0%)	43(86%)	**<0.001**
HAI >4	32 (48.5%)	0 (0%)	32 (64%)	**<0.001**
HBV DNA (10^3 IU/mL)	101.8±185.4	10.8±7.7	130.9±204.9	**<0.001**
ALT (U/L)	31.1±10.1	26.4±9.0	32.60±10.1	**0.03**
AST (U/L)	26.3±6.8	22.7±4.4	27.4±7.1	**0.01**
GGT (U/L)	25.8±15.1	28.5±17.0	24.9±14.6	0.42
ALP (U/L)	73.5±23.4	70.0±16.2	74.6±25.4	0.88
TG (mg/dL)	114.6±56.5	128.0±79.3	110.4±47.2	0.77
TCHOL (mg/dL)	178.2±32.4	175.9±36.9	179.0±31.2	0.74
Alb/Glob ratio	1.6±0.3	1.6±0.3	1.6±0.3	0.96
TSH (mU/L)	2.2±1.4	2.8±2.2	2.0±1.0	0.23
AFP (IU/mL)	4.0±3.5	3.1±2.7	4.3±3.6	0.07
Plt (10^9/L)	213.1±49.4	239.7±59.5	204.1±43.0	**0.03**
MPV (fL)	10.3±0.7	10.3±0.4	10.3±0.8	0.91
Sedimentation (mm/hr)	6.9±5.6	4.7±2.9	7.7±6.1	0.09

Values were given as mean ± standard deviation and categorical variables were given as numbers and percentages in parenthesis. HAI: Histology activity index, ALT: Alanine aminotransaferase, AST: Aspartate aminotransferase, GGT: γ-glutamyl transferase, ALP: Alkaline phosphatase, TG: Triglyceride, TCHOL: Total cholesterol, Alb/Glob ratio: Albumin/globulin ratio, TSH: Thyroidstimulating hormone, AFP: Alpha fetoprotein, Plt: Platelet, MPV: Mean platelet volume, fL: Femtolitre, BMI: Body mass index, HBV: Hepatitis B virus

Table 2. Simple correlation coefficients (r) between histological assessments and clinical and laboratory variables

	Fibrosis		HAI	
	Correlation coefficient	p	Correlation coefficient	p
Age	0.017	0.892	0.119	0.341
Disease age	**0.274**	**0.026**	0.069	0.581
BMI	0.144	0.247	0.139	0.267
Fibrosis	-	-	0.384	**0.001**
HAI	**0.384**	**0.001**	-	-
HBV DNA	**0.535**	**<0.001**	**0.646**	**<0.001**
ALT	**0.319**	**0.009**	**0.398**	**0.001**
AST	**0.414**	**0.001**	**0.427**	**<0.001**
GGT	-0.004	0.975	0.018	0.888
ALP	0.127	0.310	0.110	0.380
TG	-0.035	0.778	0.036	0.776
TCHOL	0.001	0.996	-0.019	0.883
Alb/Glob ratio	-0.060	0.631	0.036	0.776
TSH	-0.045	0.718	**-0.374**	**0.002**
AFP	0.125	0.317	**0.313**	**0.011**
Plt	-0.233	0.060	**-0.382**	**0.002**
MPV	0.108	0.388	0.054	0.668
Sedimentation	0.237	0.055	**0.268**	**0.030**

HAI: Histology activity index, ALT: Alanine aminotransaferase, AST: Aspartate aminotransferase, GGT: γ-glutamyl transferase, ALP: Alkaline phosphatase, TG: Triglyceride, TCHOL: Total cholesterol, Alb/Glob ratio: Albumin/globulin ratio, TSH: Thyroid stimulating hormone, AFP: Alpha fetoprotein, Plt: Platelet, MPV: Mean platelet volume, BMI: Body mass index, HBV: Hepatitis B virus

by HAI and Ishak scoring system. Parameters of HBV DNA, ALT ve AST correlated with fibrosis and HAI scores (p<0.001, p=0.001, p<0.001, respectively). To correlation other parameters with of fibrosis and HAI were either not meaningful or significant (Table 2). The fibrosis score was ≥2 and HAI was >4 in 88% of patients (30/34) and 76% (26/34) of patients with a HBV DNA level of higher than 20.000 IU/mL, respectively. At the same time, a fibrosis score of ≥2 and a HAI score of >4 were present in 40% of patients (13/32) and 19% (6/32) with a HBV DNA value between 2.000 and 20.000 IU/mL (p<0.001). Chronic hepatitis was detected in 94.1% (32/34) of patients with a HBV DNA level of higher than 20.000 IU/mL and in 56.2% (18/32) of patients with a HBV DNA level between 2.000 and 20.000 IU/mL (Figure 1). The specificity and sensitivity of HBV DNA 20.000 IU/mL in the detection of chronic hepatitis were found to be 64% and 87.5%, respectively.

Thirty-three (71.7%) patients had a fibrosis score of ≥2 and 26 patients (56.5%) had a HAI score of >4 in the HNALT group. Ten patients (50%) had a fibrosis score of ≥2 and 5 patients (25%) had a HAI score of >4 in the LNALT group (Figure 2). According to ROC analyses, when the serum ALT cut-off level was 19 U/L for women and 30 U/L for men, sensitivity and specifity in the detection of chronic hepatitis were found to be 75.5% and 47.1%, respectively.

We compared those under and above 40 years of age in order to analyze the relationship between age and chronic hepatitis. Twenty-three of the 31 patients (74.2%) had chronic hepatitis in the group aged below 40 years, while 28 (80%) of 35 patients had chronic

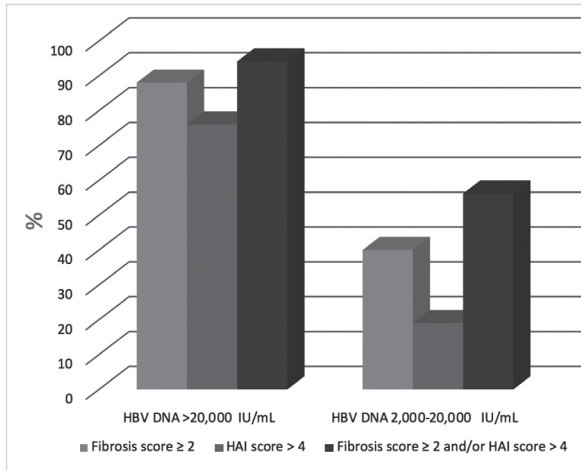

Figure 1. Relationship between HBV DNA levels and rates of chronic hepatitis
HBV: Hepatitis B virüs, HAI: Histology activity index

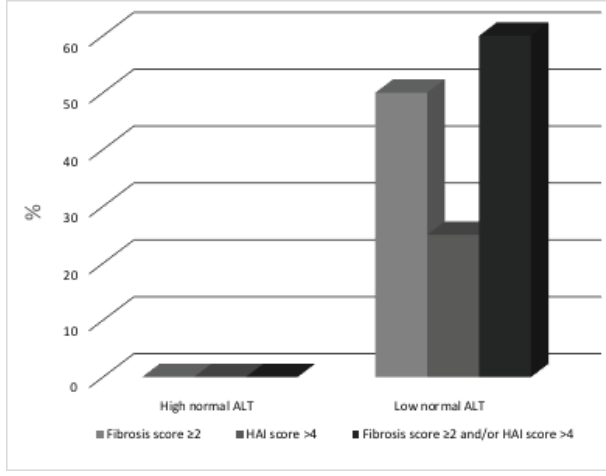

Figure 2. Relationship between serum alanine aminotransaferase levels and rates of chronic hepatitis
ALT: Alanine aminotransaferase, HAI: Histology activity index

hepatitis in the group aged above 40. There was no statistically significant correlation between age and the rate of chronic hepatitis (p>0.05). In addition, there was no statistically significant difference in the rate of chronic hepatitis between females and males [19/27 (70%) vs. 31/39 (79%), respectively p>0.05].

Discussion

It is important to predict chronic hepatitis in patients with HBeAg-negative chronic infection phase (formerly, this period called "inactive HBV carrier" state), because of the fact that while chronic hepatitis patients have a considerably high risk of complications such as HCC and cirrhosis, inactive carriers show a normal course. Liver biopsy is the method which is considered the gold standard test for this distinction (22). We showed in this study that chronic hepatitis rate is considerably higher in HBeAg-negative patients with a HBV DNA level of higher than or equal to the level of 2000 IU/mL and PNALT.

In studies carried out accross Europe**,** the incidence of chronic hepatitis ranges from 0.5% to 4% (13,14,18). Contrary to these

studies, in two studies from Asia, minimal necroinflammation levels (HAI score <4/18 Knodell or Desmet classification system) were detected to be 81% (77/95) and 40% (23/58) (16,17). In a study from United States including192 patients, it was observed that 37% of 59 patients with PNALT had significant fibrosis or inflammation (17). In studies conducted in European countries, fibrosis grade and HAI scores were significantly lower than in studies carried out in other regions.

In our study, the rate of chronic hepatitis was found to be similar to data from Asia. This rate is higher than that in the European populations and it may be due to exclusion of patients with HBV DNA lower than 2000 IU/mL and use of an upper limit of 45 U/L for ALT instead of 40 U/L. In addition, differences in age and genotypes at the time of HBV infection diagnosis between Europe and Asia may have contributed to this result. In the study of Dagtekin et al. (23) from our country, it was reported that need for treatment was determined in 56% of patients according to the biopsy results of 46 patients who had normal ALT, HBeAg-negative and HBV DNA values between 113 and 110.000.000 IU/mL. This result is in agreement with our findings

In our results, patients with a HBV DNA level of higher than 20.000 IU/mL had a higher HAI score, fibrosis level and rate of chronic hepatitis than in those with a HBV DNA value between 2000 and 20.000 IU/mL. In their study including 203 HBeAg-negative patients, Sanai et al. (24) reported that hepatic fibrosis ≥F2 was found in 52.9% (18/34) of patients with a HBV DNA of ≥20.000 IU/mL and PNALT and 18.9% (14/74) of patients with HBV DNA <20.000 IU/mL and PNALT. In a study conducted in Pakistan, the rates of patients with a fibrosis score of ≥2 and a HAI score of >4 were found to be 19% (8/42) and %35.7 (15/42), respectively (25). In a study by Kumar et al. (16), 29 patients with HBV DNA over 20.000 IU/mL were evaluated. Approximately 60% of patients had least moderate levels of necroinflammatory activity but this rate was found to be 15% in patients with HBV DNA between 2.000 and 20.000 IU/mL. In a study by Charatcharoenwitthaya et al. (20) carried out on 142 HBeAg-negative PNALT patients who had a HBV DNA of 2.000-19.999, 20.000-199.999 and ≥200.000 IU/mL, histological indication for treatment (at least grade A2 or stage F2 by METAVIR scoring) was present in 15%, 31%, and 36%, respectively. In the light of this information, it can be stated that level of HBV DNA is the most important parameter. HBV DNA level is an important indicator of disease activity and viral replication. In the literature, increased viral load has been shown to be an important risk factor for HCC and cirrhosis (26,27). We also propose that in the presence of a HBV DNA level over 20.000 IU/mL in HBeAg-negative patients with PNALT, biopsy is required, regardless of any other value. In this study, ALT was measured at least every 3 months and at least 3 times for with a minimum follow-up period of 1 year.

Some studies recommend close follow-up of ALT in patients with HBV DNA values higher than 20.000 IU/mL (14,20). Many studies reported cases of patients with HBV-DNA levels higher than or equal to 20.000 IU/mL and PNALT (15,16,20,23,24). Even though ALT levels correlate with liver cell necrosis and fibrosis, we assume that ALT cannot always correlate with chronic HBV infection like hepatitis C. Geographical origin, race, BMI, gender, abnormal lipid and carbohydrate metabolism, and alcohol use can affect the levels of ALT. Maybe it is important to set an upper limit for ALT. Recent studies (19,20) and guidelines (7,12,13) showed that the normal upper limit of ALT should be lowered to 19 U/L for women and to 30 U/L for men, but in most of studies including HBeAg-negative patients with PNALT, conventional values (basal normal range values) were frequently used. In a study using a conventional ALT value of 40 U/L (20), if the ALT level was determined as 19 U/L for women and 30 U/L for men, there would be no significant differences between groups of LNALT and HNALT according to histological indication for treatment (18% vs. 28%, p=0.2). In another study that separated the two groups according to upper or lower ALT level of 23 U/L, no considerable differences were found between groups of HNALT and LNALT for fibrosis and HAI score (p=0.86 and p=0.091, respectively) (28). In our study, HBV DNA level, fibrosis and HAI scores were significantly higher in HNALT group than in LNALT group (p<0.001, p=0.031, p=0.005, respectively). We assume this is important because a slightly increased ALT but still within the normal ranges, is associated with increased risk of death from liver disease, as was shown in a study (29). However, this relationship is not as strong as with HBV DNA.

In this study, it was found that there was no difference in HAI score and fibrosis level between genders. In a study from Iran including 132 HBeAg-negative CHB patients with PNALT genotype D, hepatitis, fibrosis and histological activity scores were higher in men than women (28). Fattovich et al. (11) have reported that advanced age, male gender and cirrhosis at entry and absence of sustained remission predicted liver-related death. However, we assume that there is no considerable association between age over 40 years and HAI fibrosis score.

Study Limitation

Our study has several limitations. Monitoring of patients could not be continued after biopsy due to the study design. Additionally, number of patients could have been higher. Also patients with a HBV DNA level of lower than 2000 IU/mL were excluded because of restriction of treatment according to the regulations of the social security institution.

Conclusion

In patients with HBeAg-negative chronic infection phase HBV DNA is the most significant value for determining chronic hepatitis. Age, gender, BMI, alkaline phosphatase, and other parameters, such as platelets, alpha fetoprotein were found not to be useful in chronic hepatitis distinction. We recommend starting treatment regardless of any other criteria if HBV DNA level is higher than 20.000 IU/mL and close monitoring or biopsy in patients with HBV DNA values within the limits of 2.000 and 20.000 IU/mL.

Ethics

Ethics Committee Approval: This study was approved by the Local Ethics Committee (Şişli Hamidiye Etfal Training and Research Hospital, approval number:12.01.2010, 1119).

Informed Consent: Written informed consent received.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practise: O.Ö., A.R.K., E.A., M.B., C.A., B.Y.Ö., Concept: O.Ö., S.Y., E.A., C.A., Desing: O.Ö., S.Y., A.R.K, E.A., C.A., B.Y.Ö., Data Collection or Processing: O.Ö., A.R.K,

E.A.,M.B., C.A., Analysis: O.Ö., S.Y., B.Y.Ö., Literature Search: O.Ö., S.Y., A.R.K., E.A., M.B., Writing: O.Ö., S.Y., E.A.

Conflict of Interest: All authors declare to have no conflict of interest.

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Research Article

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Circulating Glucose-Regulated Protein 78 Levels in Patients with Chronic Hepatitis B Infection

Kronik Hepatit B Enfeksiyonlu Hastaların Dolaşımında GRP78 Seviyesi

Selma CIRRIK[1], Yeliz ÇETİNKOL[2], Arzu ALTUNÇEKİÇ YILDIRIM[3], Mustafa Kerem ÇALGIN[2], Tevfik NOYAN[4]

[1]*Ordu University Faculty of Medicine, Department of Physiology, Ordu,Turkey*
[2]*Ordu University Faculty of Medicine, Department of Medical Microbiology, Ordu,Turkey*
[3]*Ordu University Faculty of Medicine, Department of Infectious Diseases, Ordu, Turkey*
[4]*Ordu University Faculty of Medicine, Department of Medical Biochemistry, Ordu,Turkey*

ABSTRACT

Objectives: The role of endoplasmic reticulum (ER) stress in the pathogenesis of hepatitis B virus (HBV) has been reported. However, serum levels of glucose-regulated protein (GRP) 78 which is an ER stress marker both in tissue and circulation have not been reported yet. This study aimed to evaluate serum GRP78 levels in patients with chronic HBV infection.

Materials and Methods: A total of 60 patients with HBV infection and 50 control subjects were included in this study. According to HBV-DNA levels, patients with HBV infection were subdivided into low, mild and high HBV-DNA subgroups (n=20, in each). Serum GRP78 level was measured by ELISA and then correlation analysis was performed between GRP78 and alanine aminotransferase (ALT), aspartate aminotransferase (AST), HBV-DNA or hepatitis B surface antigen (HBsAg).

Results: HBsAg levels were significantly higher in each HBV subgroup compared to controls. ALT and AST levels were significantly increased in the high HBV-DNA subgroup. There was no significant difference in serum GRP78 level between controls and HBV subgroups and no correlation between serum GRP78 levels andother variables.

Conclusion: As a result of our study, there was no relationship between the serum level of GRP78 and the HBV infection parameters. Since ER stress is an important mechanism in HBV-related liver injury, other ER stress markers, such as GRP94, might be examined in future studies.

Keywords: Hepatitis B, glucose-regulated protein 78, endoplasmic reticulum stress

ÖZ

Amaç: Hepatit B virüsünün (HBV) patogenezinde, endoplazmik retikulum (ER) stresinin rolü daha önceden gösterilmiştir. Ancak, hem dokuda hem de dolaşımda bir ER stres belirteci olan glukozla düzenlenen protein (GRP) 78'in serumdaki seviyesi bugüne kadar çalışılmamıştır. Bu çalışmada, kronik HBV enfeksiyonlu hastaların serum GRP78 seviyelerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışma kontrol (n=50) ve HBV (n=60) olmak üzere iki grup ile yapılmıştır. HBV hastaları, HBV-DNA miktarına göre düşük, orta ve yüksek HBV-DNA alt gruplarına (her birinde n=20) bölünmüştür. Serum GRP78 seviyesi ELISA ile ölçülmüş ve arkasından GRP78 ile alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), HBV-DNA veya hepatit B yüzey antijeni (HBsAg) arasında korelasyon analizi yapılmıştır.

Bulgular: Kontrol ile kıyaslandığında, her bir HBV alt grubunda HBsAg seviyesi önemli yüksek bulunmuştur. ALT ve AST seviyesindeki artış, yüksek HBV-DNA alt grubunda önemli bulunmuştur. Serum GRP78, hem kontrol hem de HBV alt gruplarında benzer düzeylerde olup, serumdaki değişkenlerle bir korelasyon göstermemiştir.

Sonuç: Çalışmamızın sonuçlarına göre, HBV hastalarının parametreleri ile serum GRP78 seviyesi arasında bir ilişki yoktur. ER stresi, HBV ile ilişkili karaciğer hasarında önemli bir mekanizma olduğundan, daha sonraki çalışmalarda GRP94 gibi diğer ER stres belirteçleri incelenebilir.

Anahtar Kelimeler: Hepatit B, glukozla düzenlenen protein78, endoplazmik retikulum stresi

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Address for Correspondence: *Selma Cırrık, PhD, Ordu University Faculty of Medicine, Department of Physiology, Ordu,Turkey*
Phone: +90 452 226 52 14 E-mail: selmacrrk@yahoo.com ORCID ID: orcid.org/0000-0001-8474-0185 **Received:** *31.05.2018* **Accepted:** *31.10.2018*

Introduction

The endoplasmic reticulum (ER) is a membranous organelle required for folding and processing of nascent proteins. Physiological, pathological or pharmacological insults that disrupt ER function induce accumulation of unfolded or misfolded proteins in the ER lumen. This condition is defined as ER stress and triggers a conserved cellular response called unfolded protein response (UPR) (1). UPR aims to relieve ER stress via different mechanisms, including enhancement of protein folding and degradation processes as well as specific inhibition of protein synthesis in the cell. UPR-related mechanisms are mediated by three different signaling pathways: protein kinase R-like ER kinase (PERK), activated transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1) (1,2). Although UPR provides a defense mechanism for cells, ER stress can trigger apoptosis depending on the severity and duration of stress (2,3,4). It has been reported that a number of pathophysiological conditions, such as diabetes mellitus, cardiovascular diseases, obesity, cancer, neurodegenerative diseases and hepatic steatosis are associated with excessive or persistent ER stress (5,6,7,8,9,10).

Hepatocytes possess quite well-developed ER and high capacity for protein synthesis similar to other secretory cells. Therefore, ER stress and UPR play an important role in various liver diseases such as non-alcoholic steatohepatitis, alcoholic liver disease, and ischemic reperfusion injury as well as toxic liver damage (11,12,13,14,15). Moreover, different studies conducted on mammalian cells, including hepatocytes, have reported that viruses were also able to induce ER stress. Increased viral protein synthesis in the infected cells induces ER stress by disturbing protein folding mechanisms and enhancing protein aggregates in the ER lumen (16). It is known that hepatitis B virus (HBV) induces ER stress and UPR activation, which contributes to liver pathogenesis during HBV infection (16,17,18,19).

Glucose-regulated protein 78 (GRP78) is an ER resident chaperone protein that monitors ER stress and regulates UPR-induced survival or apoptosis. During ER stress, expression of GRP78 significantly increases; hence, increased expression of GRP78 indicates UPR induction (1,2,3,4). Khadir et al. (20) reported that obesity, which induces hepatic ER stress, caused an elevation in GRP78 levels both in plasma and liver. These results suggest that GRP78 can pass into blood from tissue during hepatic ER stress (20,21). In HBV-infected patients, activation of UPR and its contribution to HBV pathogenesis have been shown previously, however, no circulating marker has been reported. Therefore, alterationsin circulating GRP78 level in patients with chronic HBV infection is evaluated in the present study.

Materials and Methods

In the current study, samples collected from 60 patients with chronic HBV infection whose serum specimens were processed for HBV-DNA quantification in the molecular microbiology laboratory were evaluated for GRP78 analysis. Control serums were obtained from 50 hepatitis B surface antigen (HBsAg)-, anti-hepatitis C virus- and anti-human immunodeficiency virus-negative subjects who had no chronic disease including chronic liver disease. According to HBV-DNA levels, HBV-infected patients were subdivided into low (20-1x10^2 IU/mL, n=20), mild (1x10^3-1x10^5 IU/mL, n=20), and high HBV-DNA (1x10^6- 1.7x10^8 IU/mL, n=20). All the samples were stored at -40 °C until assayed throughan ELISA. This study was approved by the Clinical Research Ethics Committee in Ordu University (2017-157).

Real-time polymerase chain reaction: Quantification of HBV-DNA was performed via a real-time polymerase chain reactionmethod using the COBAS AmpliPrep/COBAS Taqman 48 system (Roche, Branchburg, NJ, USA). Viral nucleic acids were extracted from serum (500 µl) using Cobas AmpliPrep automatic extractor system according to the manufacturer's instructions. The assay range for HBV-DNA was 20-1.7x10^8 IU/mL.

Detection of GRP78: Levels of circulating GRP78 were determined in serum using the human GRP78 ELISA kit (Elabscience; E-EL-H5586) with a detection range of 0.63-40 ng/mL. Samples and standards were added to the appropriate micro ELISA plate wells pre-coated with an antibody specific to human GRP78 and then the manufacturer's instructions were followed. The optical density was measured spectrophotometrically at the wavelength of 450 nm (BioTek, ELx800 brand REF ELX508 SN1310149). The level of GRP78 in the samples was calculated by comparing the optical density of the samples with the standard curve.

Statistical Analysis

All data are given as mean ± standard deviation. Statistical analysis was performed with One-Way ANOVA and Tukey's test.A p value of less than 0.05 was considered statistically significant.

Results

Sixty patients with chronic HBV infection (42.1±16.0 years; 28 female, 32 male) and 50 control subjects (53.9±19.1; 21 female, 29 male) were included in this study. HBV-DNA content in the

Table 1. Hepatitis B surface antigen, alanine aminotransferaseand aspartate aminotransferaselevels in control (n=50) and hepatitis B virus subgroups (n=20, in each subgroup)

	Control	Chronic HBV infection		
		HBV-DNA low	HBV-DNA mild	HBV-DNA high
HBsAg (IU/L)	0.54±0.07	3668±3262*	3869±2397*#	2397±1576*
ALT (U/L)	11.26±5.01	18.8±8.2###	25.0±13.1###	82.6±100.1*
AST (U/L)	13.50±4.23	19.1±5.3###	21.4±5.9##	59.7±74.5*

HBV patients divided into 3 subgroups as HBV-DNA low; 20 - 1x10^2 IU/mL (n=20), HBV-DNA mild; 1x10^3- 1x10^5 IU/mL (n=20) and HBV-DNA high; 1x10^6- 1.7x10^8 IU/mL (n=20). *p<0.001 vs. control; #p<0.05, ##p<0.01, ###p<0.001 vs. HBV-DNA high
HBV: Hepatitis B virus, HBsAg: Hepatitis B surface antigen

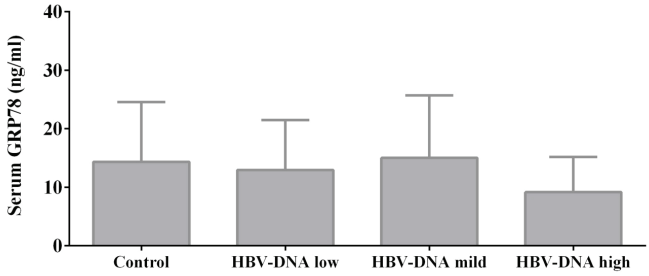

Figure 1. Serum glucose-regulated protein 78 levels in the control and hepatitis B virus subgroups; i.e. hepatitis B virus-DNA low, hepatitis B virus-DNA mild and hepatitis B virus-DNA high
HBV: Hepatitis B virus, GRP78: Glucose-regulated protein 78

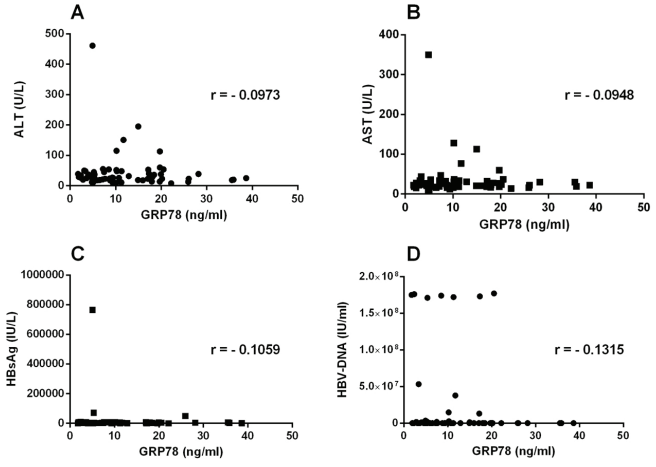

Figure 2. Correlation between serum glucose-regulated protein 78 concentration and serum levels of alanine aminotransferase (A), aspartate aminotransferase (B), hepatitis B surface antigen(C) and hepatitis B virus-DNA (D) in patients with chronic hepatitis B virusinfection. All the patients were included in the correlation analysis (n=60)
HBsAg: Hepatitis B surface antigen, GRP78: Glucose-regulated protein 78, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HBV: Hepatitis B virus

serum samples of the patients with chronic HBV infection was in three different ranges of low (20 - $1x10^2$ IU/mL, n=20), mild ($1x10^3$ - $1x10^5$ IU/mL, n=20), and high ($1x10^6$-$1.7x10^8$ IU/mL, n=20). Serum HBsAg, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in all patients are presented in Table1.

The serum GRP78 concentration was found to be 14.36±10.22 ng/mL in controls. As seen in Figure1, there was no significant difference in GRP78 concentration between the HBV subgroups (12.97±8.5, 15.01±10.7 and 9.18±6.02 ng/mL, respectively). In the control group, no correlation was found between GRP78 and serum ALT or AST levels. In each HBV subgroup, changes in serum GRP78 did not show a correlation with the serum variables such as ALT, AST and HBsAg or HBV-DNA content. When all the HBV-infected patients were included in the analysis, we did not determine a correlation between GRP78 and the other variables (Figure2).

Discussion

Accumulation of unfolded or misfolded proteins in the ER lumen causes a condition termed ER stress (1,2,3,4). During ER stress, UPR is activated to restore cellular homeostasis, however, delayed or insufficient responses to ER stress are implicated with pathological consequences, including fat accumulation, insulin resistance, inflammation, and apoptosis, all of which play important roles in liver pathologies (11,12,13,14,15).

HBV infection is a serious health problem affecting approximately 260 million people worldwide and causing acute and chronic hepatitis, liver cirrhosis, and even hepatocellular carcinoma (22). To date, many studies have reported on the molecular mechanisms for the relationship between HBV infection and pathogenesis of hepatic diseases, but the mechanisms are still not fully understood (23-25). Recent studies indicate that ER stress may play a role in the pathogenesis of HBV infection (16,17,18,19). It has been reported that some products of HBV genome might be involved in the activation of UPR in hepatocytes. For instance, Li et al. (26) reported that regulatory X protein (HBx), which is a product of HBV genome, mediated UPR activation in Hep3B and HepG2.2.15 cells via ATF6 and IRE1-XBP1 pathways. Further studies confirmed HBx-induced ER stress in different cell lines (27,28). Additionally, Wang et al. (29,30) reported that the pre-S mutant large HBsAgs were retained in the ER lumen and induced UPR signals, leading to the increased expression of ER chaperones such as GRP78 and GRP94.

In the present study, serum GRP78 levels were evaluated as an ER stress marker in patients with chronic HBV infection whose HBV-DNA levelranged between 80 and $1.7x10^8$ IU/mL. As expected, these patients displayed higher levels of serum HBsAg compared to controls. Since the measurement of serum ALT and AST levels is a common laboratory assay to monitor liver functions, we evaluated these parameters in the subjects. In the control group, the ALT and AST levels were within the normal range. In the HBV subgroups, as the HBV-DNA content raised, serum ALT and AST levels gradually increased. However,a statistically significant elevation was observed only in the HBV subgroup with the highest HBV-DNA content. These results are consistent with the previous reports and suggest that hepatic functions declined during high viral replication (31,32,33).

GRP78 is an ER-resident molecular chaperone which also regulates ER stress and is upregulated during UPR (1,2,3,4). Since HBV induces hepatic ER stress (16,17,19,26,27,28,29,30), we hypothesized that circulating GRP78 increased in HBV-infected patients and then, measured serum GRP78 levels in controls and in patients with chronic HBV infection.Contrary to our expectation, present results showed that during chronic HBV infection, circulating GRP78 levels remained unchanged without any relationship with serum variables. These results might be attributed to a mechanism that prevents GRP78 release from hepatocytes. Li et al. (26) showed that HBx protein and GRP78 were co-localized in ER lumen and displayed a direct interaction which may results in increased GRP78 retention in ER lumen. The aforementioned HBx-GRP78 interaction or another unknown mechanism may prevent GRP78 release to the blood and be responsible for the unchanged serum GRP78 levels in HBV-infected patients. Nevertheless, GRP78 measurements are needed both in liver biopsy and serum samples to confirm this suggestion.

Study Limitations

The most important limitations of the current study are small sample size, which included only Turkish subjects, and absence of liver biopsies. It would be valuable data if we had shown the presence of ER stress in liver biopsies obtained from a larger and heterogenous subject population, despite unchanged serum GRP78 concentration.

Conclusion

Although the involvement of ER stress in HBV-induced liver damage is well-documented, the present results show that serum GRP78 remained unchanged during chronic HBV infection and there was no relationship between serum level of GRP78 and the parameters of HBV infection. Since ER stress is an important mechanism in HBV-related liver injury, other ER stress markers, such as GRP94, might be examined with liver biopsies in future studies conducted with larger patient group.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee in Ordu University (2017-157).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: S.C., Design: S.C., Y.Ç., Data Collection or Processing: S.C., Y.Ç., M.K.Ç., Analysis or Interpretation: S.C., A.A.Y., T.N., Literature Search: S.C., A.A.Y., Writing: S.C., Y.Ç.

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

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Does Pegylated-Interferon Still Have High Efficacy Treatment Properties Against Chronic Hepatitis B?

Pegile-İnterferon Kronik Hepatit B'de Hala Yüksek Etkili Tedavi Özelliklerine Sahip mi?

Figen SARIGÜL YILDIRIM[1], Ülkü USER[1], Murat SAYAN[2,3], Nefise ÖZTOPRAK[1]

[1]*University of Health Sciences, Antalya Training and Research Hospital, Clinic of Infectious Disease and Clinical Microbiology, Antalya, Turkey*
[2]*Kocaeli University Faculty of Medicine, Clinical Laboratory, PCR Unit, Kocaeli, Turkey*
[3]*Near East University, Research Center of Experiment Health Sciences, Nicosia, Northern Cyprus*

ABSTRACT

Objectives: Pegylated-interferon (Peg-IFN) alpha 2a/2b and nucleoside and/or nucleotide analogues (NAs) are currently the only two treatment approaches approved for the chronic hepatitis B (CHB). To date, few studies have compared Peg-IFN with NAs in the treatment of CHB. We aimed in this study to evaluate the effectiveness of Peg-IFN and potent NAs (entecavir and tenofovir disoproxil) and to compare cumulative virological and serological responses in Turkish CHB patients treated between 2006 and 2016.

Materials and Methods: In this observational retrospective study, we divided a total of 331 patients, who were diagnosed with CHB, into 3 groups: Peg-IFN treatment group (n=62), entecavir treatment group (n=131) and tenofovir disoproxil treatment group (n=138).

Results: Virologic response rate in the Peg-IFN treatment group (90%) at 12 months was higher than in the NAs treatment groups (80% for entecavir and 76% for tenofovir disoproxil) ($p<0.05$). Sustained virologic response (SVR) rate at 24 months was 61% in the Peg-IFN group ($p<0.001$). The rate of hepatitis B e antigen seroconversion was significantly higher in the Peg-IFN group (25%) than in the NAs groups (16% for entecavir, 13% for tenofovir disoproxil). Hepatitis B surface antigen (HBsAg) seroconversion rate was also higher in the Peg-IFN than in the NAs treatment groups (7.9% vs. 0.9% and 0%, respectively) ($p<0.001$). After HBsAg seroconversion, the titers of anti-HBs were retained for over six months.

Conclusion: Peg-IFN treatment was found to be effective with high SVR and hepatitis B e antigen and HBsAg seroconversion rates than NAs treatment in long-term follow-up of patients with CHB. Peg-IFN appears to be the first-choice treatment approach in patients with CHB until a new era in which hepatitis B is cured.

Keywords: Chronic hepatitis B, pegylated-interferon, nucleoside/nucleotide analogues, hepatitis B surface antigen, seroconversion

ÖZ

Amaç: Pegile-interferon (Peg-IFN) alfa 2a/2b ve nükleozid ve nükleotid analogları (NA), şu anda kronik hepatit B (KHB) tedavisi için onaylanmış iki tedavi yaklaşımıdır. Bugüne kadar birkaç çalışma Peg-IFN ve NA'lar arasındaki tedavinin etkinliğini karşılaştırmıştır. Bu çalışmada, 2006-2016 yılları arasında Türk KHB hastalarında Peg-IFN ve potent NA'ların (entekavir ve tenofovir disoproksil) kümülatif virolojik ve serolojik yanıtlarını karşılaştırarak etkinliklerini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Retrospektif gözlemsel olan bu çalışmada KHB tanılı total 331 hasta (Peg-IFN tedavi grubu, n=62, entekavir tedavi grubu, n=131 ve tenofovir disoproksil tedavi grubu, n=138) değerlendirildi.

Bulgular: Virolojik yanıt oranı Peg-IFN tedavi grubunda 12. ayda (%90), NA tedavi grubundan (entekavir için %80, tenofovir disoproksil için %76) daha yüksekti ($p<0,05$). Peg-IFN'nin 24. ayda kalıcı viral yanıt (KVY) oranı %61 idi ($p<0,001$). Hepatit B e antijen serokonversiyonu Peg-IFN grubunda (%25) NA grubuna (entekavir için %16, tenofovir disoproksil için %13) göre belirgin yüksekti. Hepatitis B yüzey antijeni (HBsAg) serokonversion oranı Peg-IFN grubunda NA tedavi grubuna göre daha yüksekti (%7,9 vs %0,9 ve %0) ($p<0,001$). HBsAg serokonversiyonundan sonra anti-HBs titreleri altı aydan fazla korundu.

Sonuç: KHB hastalarının uzun sureli takibinde Peg-IFNtedavisi, NA'larına göre daha yüksek KVY ile daha yüksek HBsAg ve HBeAg serokonversiyon ile ilişkili bulundu. Peg-IFN hepatit B virüsün tedavi edildiği yeni bir döneme kadar KHB hastalarında ilk seçenek tedavi yaklaşımı olarak görünmektedir.

Anahtar Kelimeler: Kronik hepatit B, pegile-interferon, nükleozid/nükleotid analog, hepatit B yüzey antijen, serokonversiyon

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Address for Correspondence: *Figen Sarıgül Yıldırım MD, University of Health Sciences, Antalya Training and Research Hospital, Clinic of Infectious Disease and Clinical Microbiology, Antalya, Turkey*
E-mail: drfigensarigul@yahoo.com.tr ORCID ID: orcid.org/0000-0001-8646-2203 **Received:** *19.04.2018* **Accepted:** *10.07.2018*

Introduction

Antiviral therapy is administered to prevent the progression of liver damage to cirrhosis, hepatocelluler carcinoma (HCC) and death in patients with chronic hepatitis B (CHB). Currently, the available treatments for patients with CHB include pegylated-interferon (Peg-IFN) alpha 2a/2b and nucleoside/nucleotide analogues (NAs) (1,2). Peg-IFN is an immunomodulator with antiviral activity against hepatitis B virus (HBV) (3). NAs (i.e., lamivudine, telbivudine, entecavir, adefovir, tenofovir disoproxil and tenofovir alafenamide) inhibit reverse transcriptase activity of HBV polymerase (***pol***) (4). A therapy leading to hepatitis surface antigen (HBsAg) seroconversion would avoid requirement of additional treatments. The difficulty in eliminating HBV is due to the persistence of covalently closed circular DNA (cccDNA) that integrates into the host DNA (5).

Most guidelines recommend entecavir or tenofovir (disoproxil/alafenamide) and Peg-IFN, as monotherapy for the management of CHB (1,2,3,6). Deciding on the type of treatment depends on the patient's clinical status, HBV DNA load and the level of alanine aminotransferase (ALT) enzyme, hepatitis B e antigen (HBeAg) status, and liver histology (1,2,3). Entecavir is preferred in older patients due to the low risk for nephrotoxicity and acceleration of bone loss. Tenofovir disoproxil is very effective against NAs-resistant and wild type HBV strains (7,8). Entecavir and tenofovir disoproxil, having similar high potency and genetic barriers, are used much more than Peg-IFN even in CHB patients with no contraindications to IFN (9). However, Peg-IFN is preferable in some circumstances, particularly in young patients eligible for shortened treatment duration. Peg-IFN has the advantages of absence of drug resistance, providing immune-mediated control of the HBV infection, the possibility of achieving a sustained virologic response (SVR), and the possibility of HBsAg loss in patients with an HBV DNA load of <2000 IU/mL. An additional advantage of Peg-IFN treatment is clearly established treatment duration of 48 weeks. On the contrary, the duration of NA therapy is yet to be clearly outlined (1,2,3).

A systematic review of 339 studies on the prevalence of CHB in Turkey revealed that the prevalence of HBsAg was 4.5% in the Turkish population. This rate may increase to 9.8% in the eastern part of Turkey (10,11). In our country, it is estimated that one out of every three people over 18 years of age has encountered HBV, in addition, more than 2 million adults have been reported to be HBsAg-positive (12). The rate of drug spending for CHB patients is 0.7% of the total and unfortunately, the rate for on treatment CHB patients is only 13.5-15% in Turkey (13). According to the data of the Intercontinental Market Services (IMS Health) in 2016, 61544 CHB patients in Turkey were receiving entecavir and tenofovir disoproxil treatment. The number of Peg-IFN-treated CHB patients has been reported to be 947 in 2016 in Turkey (14).

In this study, we aimed to evaluate the effectiveness of Peg-IFN and potent NAs. However, we compared cumulative virologic and serological responses in CHB patients over a given period of time.

Materials and Methods

Patients

In this retrospective observational study, we included 334 CHB patients treated with Peg-IFN (n=63), entecavir (n=131) and tenofovir disoproxil (n=140) between 2006 and 2016 in the department of infectious disease at University of Health Sciences Antalya Training and Research Hospital.

According to the European Association for the Study of the Liver guideline, the diagnosis of CHB was made based on histological, virologic, serological and biochemical data (2). Grade and stage of the CHB were assessed using the Ishak (14) modified histological activity index (HAI) (15).

Patients with co-infection with HIV and other viral hepatitis viruses, immunodeficiency disorders, decompensated cirrhosis, HCC or any other malignancies, injecting and other drug users, solid organ recipients, those non-compliant with treatment and patients who received treatment less than 12 months, or less than 3 months due to adverse effect or intolerance, pregnant women, and any patient under the age of 18 years were excluded.

All the patients were followed up by hepatitis B serology, biochemistry, and virology at 1, 3 and 6 months. HBV DNA and serology parameters were recorded at 3-month intervals after 12 months of therapy with Peg-IFN and NAs.

During NAs treatment, their HBV DNA, evaluated using polymerase chain reaction (PCR), was negative, showing virologic response. The duration of Peg-IFN treatment was 48 weeks for all patients. Virologic response has been defined as a HBV DNA value of <2000 IU/mL (2). Both treatment groups were were evaluated for virologic response at 6 months and at the end of the treatment. HBeAg seroconversion was defined as loss of HBeAg and development of anti-HBe on at least two consecutive follow-ups. HBsAg seroconversion was defined as absence of serum HBsAg and presence of anti-HBs after a 6-month period.

Compliance to therapy was accepted if patients took their pills once daily at the same time (0.5 or 1 mg entecavir and 245 mg tenofovir disoproxil per day) and were injected Peg-IFN (180 mcg for 2a, 1.5 mcg/kg for 2b) weekly and regularly, without interruption (except for their physician advices). After the treatment started, any symptom or abnormal laboratory and clinical findings were accepted as drug-related adverse effect.

Laboratory Analysis

Serological markers of HBV were quantified by using chemiluminescence assay (Cobas platform, Roche Diagnostics, Mannheim, Germany), which has been the preferred test in the hospital since 1998. HBV DNA levels were quantified by sensitive real-time PCR (Abbott TagMan 2000, Illinois-Des Plaines USA) (lower limit as quantification, 10 IU/mL) and has been in use in the hospital since 2008.

Antiviral resistance analysis was made in Kocaeli University by the Sanger dideoxy sequencing method as follows; oligonucleotides (forward primer: 5' - TCG TGG TGG ACT TCT CTC AAT T - 3' / reverse primer: 5'- CGT TGA CAG ACT TTC CAA TCA AT - 3') were used for the HBV ***pol*** gene amplification. There are the PCR reactions: 95 °C - 10 min. for 35 cycles and then 95 °C - 45 s, 60 °C - 45 s, finally 72 °C - 45 s. The primers concentration was 0.3 mM. The amplicon size was 740 bp. A drug resistance tool that was Genafor/arevir (http://coreceptor.bioinf.mpi-inf.mpg.de/) used on the interpretation of HBV resistance mutations.

Statistical Analysis

One-Way ANOVA was conducted to compare the continuous variables such as baseline demographic and laboratory characteristics (age, baseline HBV DNA load, HAI, patient follow-up

time, baseline ALT, fibrosis) between the subjects of Peg-IFN group and those of entecavir and tenofovir disoproxil group. Categorical variables (gender, HBeAg positivity, treatment status, and original type of therapeutic molecule) were compared using a chi-square test. The two-tailed t-test was conducted to compare the means of the HBV DNA monitoring between HBeAg-positive entecavir and tenofovir disoproxil treatment groups, Peg-IFN and HBeAg-negative entecavir patients as well as Peg-IFN and HBeAg-negative tenofovir disoproxil patients. Seroconversion rates were analysed using a chi-square test. A p value of ≤0.05 was considered statistically significant. SPSS v13 programme was used for statistical analyses.

Results

There was no statistically significant difference in baseline age (p=0.5), gender (p=0.5), pretreatment mean ALT (p=0.5), pretreatment HBV DNA (p=0.05), and duration of follow-up (p=0.6) between the groups. NAs were also similar in terms of being original and generic drugs (p=0.5). Baseline demographic and clinical and laboratory characteristics of patients are shown in Table 1. Basal liver biopsy was done in all patients in Peg-IFN and in NAs treatment group. The fibrosis score was significantly lower in the Peg-IFN treatment group (p=0.001).

Peg-IFN was injected in 63 patients (one patient was excluded due to signs of depression), entecavir was given to 131 patients, and tenofovir disoproxil was given to 140 patients, two of whom were receiving combination treatment with tenofovir disoproxil and entecavir as high HBV DNA levels were keeping on with entecavir monotherapy without any resistance and incompliance. These two patients were excluded from evaluation. Only two patients had entecavir resistance, in these patients the mutations were identified at the gene loci rtL180M, rtM204V and rtS202G. The treatment was therefore changed to tenofovir disoproxil. Subsequently, in these two patients, renal toxicity developed because of tenofovir disoproxil treatment, in turn the patients were instead treated with entecavir.

Amongst the Peg-IFN group, there were only four HBeAg-positive patients; evaluation of virologic response was not conducted for this group. Amongst HBeAg-negative patients, the the difference in the rate of HBV DNA suppression between the Peg-IFN and NAs groups were statistically significant for each month (p<0.001 at 3, 6, 24, 36 and 48 months, p<0.05 at 1 month and 12 month). Until 12 months of treatment, HBV DNA suppression was higher in the Peg-IFN group. By the second year of treatment (24 month), in the NAs group, the rates of HBV DNA suppression became higher (p<0.001). In our study, the rate of virologic response was 90% at the end of the therapy and the SVR rate was 61% at 24 months amongst the Peg-IFN-treated patients. No statistically significant differences was found in HBV DNA suppression in NAs group between HBeAg-positive and negative patients (p>0.1). In NAs, the suppression of HBV DNA was above 90% after 48 months of treatment. HBV DNA suppression rates are presented in Table 2.

The rates of HBeAg seroconversion was 25%, 16% and 13% in the Peg-IFN, entecavir and tenofovir disoproxil treatment groups, respectively. The difference between the treatment groups was statistically significant (p<0.001). No statistically significant difference was found in HBeAg seroconversion rate between the two NAs (p>0.1). HBsAg seroconversion rates were 7.9% and 0.9% in the Peg-IFN and entecavir treatment groups (p<0.001). However, HBsAg seroconversion was detected in the tenofovir disoproxil treatment group. HBeAg and HBsAg seroconversion rates are presented in Table 3.

Some patients had anti-HBs titers after HBsAg seroconversion. HBsAg seroconversion was accepted based on the disappearance

Table 1. Baseline demographic and laboratory characteristics of the study patients

Characteristic	Pegylated-interferon	Nucleos(t)ide analogue	
		Entecavir	Tenofovir disoproxil
Patient, n	62	131	138
Gender (F), n (%)	27 (44)	48 (37)	59 (43)
Age, median years (range)	40 (21-55)	36 (20-68)	39 (22-73)
Baseline ALT, median U/L (range)	90 (12-330)	48 (15-300)	40 (10-300)
HBeAg positivity, n (%)[a]	4 (6)	34 (26)	43 (31)
Baseline HBV DNA load, median IU/mL (range)	2.4+E6 (2.5+E3-1.3+E9)	1.0+E8 (2.0+E4-1.7+E10)	1.2+E9 (1.0+E1-1.7+E10)
Biopsy status, median (range)			
HAI	6 (3-13)	7 (2-16)	7 (2-18)
Fibrosis[b]	1 (0-3)	2 (0-5)	2 (0-5)
Treatment status, n (%)[a]			
Naive	62 (100)	107 (82)	76 (56)
Experienced	-	24 (18)	62 (44)
Type of therapeutic molecule, n (%)[a]			
Original	62 (100)	70 (53)	67 (49)
Generic	-	61 (47)	71 (51)
Patient follow up time, median month (range)	30 (12-156)	36 (12-120)	36 (12-108)

F: Female, ALT: Alanine aminotranferase, HAI: Hepatic activity index, HBeAg: Hepatitis B e antigen, HBV: Hepatitis B virus
[a]For the mean comparisons across groups; p<0.001, [b]p<0.01

of serum HBsAg and the presence of anti-HBs for more than 6 months. Anti-HBs titers occurred in five patients in the Peg-IFN treatment group and in one patient in the NAs treatment group. Anti-HBs occurred only in the entecavir treatment arm. However, in the second tests, there was an increase in anti-HBs antibodies and continued for a further six months. The period of anti-HBs occurrence and anti-HBs titers detected are presented in Table 4.

Discussion

In the present study, the purpose was to compare the effectiveness of Peg-IFN and potent NAs treatments in CHB patients over an extended period (10 years). We used the standard parameters in both the serological and virologic responses. The rate of virologic response in the Peg-IFN group (90%) at 12 months was higher than in the NAs treatment group (80% for entecavir and 76% for tenofovir disoproxil). In addition, in our study, the SVR at 24 months was higher (61%) in the Peg-IFN group than in other studies. Information available regarding the treatment efficacy of Peg-IFN and NAs groups is limited. There is only one study from Turkey which compared the difference between Peg-IFN and adefovir treatment amongst a small CHB patient group (16). The rates for the virologic response at 48 weeks were similar to our study (90% for Peg-IFN, 80% for entecavir and 76% for tenofovir disoproxil). In a multicenter cohort study from Korea, after the end of Peg-IFN treatment, virologic response rate in HBeAg-negative patients was 30% in a period of one year (17). However, Yamazhan et al. (18) reported that the response rate of Peg-IFN treatment was low at the end of treatment whereas one-year SVR in HBeAg-negative cases was 33%. In the current study, patients in all treatment groups displayed similar outcome in terms of baseline parameters; the fibrosis score however was significantly lower in patients treated with Peg-IFN. The lower SVR responses in other studies may depend on patients selected for Peg-IFN treatment.

High virologic and SVR rates may lead to long-term viral suppression that is known to reduce the degree of liver damage and the risk of end-stage liver disease (19,20). In the present study, HBV DNA suppression as a result of NAs treatment occurred in over 90% of cases after 48 months. It was long duration but long-lasting if the compliance to treatment provided perfect results could have been in the CHB patients treated with potent NAs. In a retrospective multicenter study conducted in Turkey, virologic response in patients treated with entecavir or tenofovir disoproxil therapy over a period of 4 years was similar to that in our NAs treatment group (21). On the other hand, there is a relationship between the concentration of HBV DNA and cirrhosis and HCC in patients with CHB (19). In our patients, who were on long-term follow-up, there were no HCC and cirrhosis in the two groups because Peg-IFN produced a high sustained off-treatment response, NAs evoked a high virologic response during uninterrupted therapy. Long-term NAs therapy has several disadvantages such as side effects, non compliance to therapy, reactivation and the risk of emergence of drug resistance by mutations (22). Although, the updated guidelines recommend discontinuation of NAs therapy (evidence level II-2, grade of recommendation 2) (23), patients

Table 2. Comparison of hepatitis B virus DNA supression in the pegylated-interferon and nucleos(t)ide analogue treatments in the study patient groups

HBV DNA monitorization, (month)	HBV DNA supression				
	Pegylated-interferona, n (%)	Nucleos(t)ide analogueb, n (%)			
		Entecavir		Tenofovir disoproxil	
		HBeAg (+)	HBeAg (-)	HBeAg (+)	HBeAg (-)
1	22 (35)	2 (6)	19 (20)	2 (5)	18 (19)
3	52 (84)	4 (12)	35 (36)	5 (12)	38 (40)
6	58 (94)	6 (18)	52 (54)	11 (26)	53 (56)
12	56 (90)	10 (29)	77 (78)	15 (35)	72 (76)
18	50 (80)	NA	-	NA	-
24[&]	38 (61)	17 (50)	82 (85)	24 (56)	85 (90)
36	41 (66)	22 (65)	88 (92)	37 (86)	90 (96)
48	27 (44)	33 (97)	96 (100)	42 (98)	92 (98)

The rule of HBV supression; [a]In the Peg-IFN treatment; HBV DNA concentration should be less than 2000 IU/mL, [b]In the oral antiviral treatment; HBV DNA should be undetectable or less than 10 IU/mL by a sensitive PCR
NA: Not available, [&]The 12th month virological response of Peg-IFN at the end of the treatment, HBeAg: Hepatitis B e antigen, HBV: Hepatitis B virus, Peg-IFN: Pegylated-interferon

Table 3. Hepatitis B e antigen and hepatitis B surface antigen seroconversion rate of the study patients

Seroconversion status	Chronic hepatitis B treatment		
	Pegylated-interferon	Entecavir	Tenofovir disoproxil
Before tratment HBeAg positive, n (%)[a]	4 (6)	34 (26)	43 (31)
HBeAg seroconversion, n (%)[a,b]	1 (25)	6 (16)	5 (13)
HBsAg seroconversion, n (%)[a,b]	5 (7.9)	1 (0.9)	ND

[a]For the mean comparisons across groups (pegylated-interferon and NAs) p<0.001, [b]For the mean comparisons across groups (entecavir and tenofovir disoproxil) p>0.1, HBeAg: Hepatitis B e antigen, HBsAg: Hepatitis B surface antigen

Table 4. Anti-hepatitis B surface level in pegylated-interferon and nucleos(t)ide analoque treated patients

Therapy	Patient	Time of seroconversion, years	Anti-HBs titer, IU/mL		
			First testing[a]	Second testing (6th months)	Duration of titer (>6th months)
Pegylated-interferon	1.	5th	30	50	200
	2.	4th	20	400	400
	3.	4th	50	60	500
	4.	6th	50	500	400
	5.	5th	20	40	350
Entecavir	1.	5th	80	90	100
Tenofovir	ND	ND	ND	ND	ND

Hepatitis B surface antigen serocon version was the disappearence of serum hepatitis B surface antigen and presence of anti- -hepatitis B surface for >6 months.
[a]The time was that the anti-hepatitis B surface titres were detected for the first time
ND: Not determined, Anti-HBs: Anti-hepatitis B surface

in whom the therapy is discontinued, must be closely followed for reactivation (2,3,24). In low-income countries, the high cost of therapy limits the number of patients who receive treatment, which can ultimately influence the emergence of viral resistance on/off-treatment NAs in CHB patients (24).

As serological responses; HBeAg seroconversion rate was found to be significantly higher in the Peg-IFN group (25%) than in the NAs group (16% for entecavir and 13% for tenofovir disoproxil). Despite the long-term follow-up, HBeAg seroconversion rates were lower in the NAs group. This finding was also demonstrated by Xing et al. (24) that in long-term treatment with potent NAs, HBeAg seroconversion with the therapy was lower compared with spontaneous HBeAg seroconversion rate (25). The latest study conducted by Marcellin et al. (26) in 2017 showed remarkable results of Peg-IFN treatment in long-term follow-up of CHB patients (26). In HBeAg-positive patients, HBeAg seroconversion increased from 23% at the end of treatment to 38% after 3 years of therapy. ENUMERATE which is the largest "real-world" entecavir treatment study showed that five-year HBeAg seroconversion was 33.7% (27). A study with tenofovir disoproxil treatment also demonstrated a HBeAg seroconversion rate of 40% (28). In our real-world data, Peg-IFN showed durable e seroconversion but in the NAs treatment group, there was some seroreversion, and an additional 16% and 13% of the patients would achieve HBeAg seroconversion in HBeAg-positive CHB patients in the long-time period. Because of the immunomodulating effect of Peg-IFN that was sustained for a long time even after the end of therapy, no anti-HBe seroconversion in the Peg-IFN group was detected in the current study over the 10 years period.

In studies by Marcellin et al. (26), Ahn et al. (27), and Petersen et al.(28), HBsAg seroconversion rate was also clearly higher in Peg-IFN groups than in the NAs treatment groups (7.9%, 0.9% and 0%, respectively). The rate of HBsAg clearance 3 years after treatment was 2% in HBeAg-positive patients and 5% in HBeAg-negative patients treated with Peg-IFN; the rate of HBsAg loss was 4.6% for entecavir and the rate of HBsAg seroconversion was 0.8% for tenofovir disoproxil during long-term therapy (26,27,28). The production of HBsAg is associated with HBV replication and the amount of intrahepatic cccDNA. Higher HBsAg seroconversion rate in the long-term Peg-IFN treatment may be due to their good immunomodulating, weak antiviral activity and potential of immune-mediated control of HBV infection characteristics. The possibility of treatment discontinuation in CHB patients was more likely in the Peg-IFN therapy group in our study.

We found that in the Peg-IFN group, anti-HBs titers of five patients were lasted longer than six months and one patient from the entecavir treatment group had the anti-HBs titer durability. Following the discontinued Peg-IFN therapy, SVR off-treatment in long-term treatment had a chance of HBsAg seroconversion and durable immunity when compared with NAs which also requires long-term administration (2). Actually, there is limited information regarding the level of anti-HBs titer and their duration related with the endpoint of CHB treatments and their long-term efficacy.

There are some limitations in our long-term based retrospective study: firstly, genotype D is known with the lowest rate of virologic response in Peg-IFN treatment (29). In Turkey, genotype D is predominant in CHB patients (30,31). It is worthwhile to note that the CHB patients distributed into the various treatment groups which displayed virologic and serological responses were infected with HBV genotypes other than genotype D. Secondly, quantitative HBsAg is not available in our hospital, therefore, the comparison, between the Peg-IFN and the NAs treatment groups could not be established. There may be more efficient parameters for the long-term surveillance of serological responses to the treatment.

Conclusion

In conclusion, Peg-IFN treatment was found to be highly efficient based on SVR, HBeAg and HBsAg seroconversion rates when compared with NAs treatment during long-term follow-up in Turkish CHB patients. Therefore, Peg-IFN appears to be the first-choice treatment approach in particular patients with CHB until a new era in which HBV is cured.

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Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Antalya Hospital of Health Sciences University (decision no: 7/13-04.13.2017).

Informed Consent: All patients were given informed consent.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: F.S., Design: F.S., Data Collection or Processing: F.S., Ü.U., Analysis or Interpretation: F.S., M.S., Literature Search: F.S., M.S., Writing: F.S., M.S.

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Case Report

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Failure of Direct-Acting Antiviral Agents Due to Incomplete Hepatitis C Virus Genotyping

Eksik Hepatit C Virüs Genotip Tayini Nedeniyle Direkt Etkili Antiviral Tedavi Başarısızlığı

Esragül AKINCI[1], Esra YURDCU[2], Bahadır ORKUN ÖZBAY[1], Hürrem BODUR[1]

[1]*University of Health Sciences, Ankara Numune Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Ankara, Turkey*
[2]*Ankara University Faculty of Medicine, Department of Hepatology, Ankara, Turkey*

ABSTRACT

Current direct-acting antivirals (DAAs) have high success rates in the treatment of chronic hepatitis C virus (HCV) infection. However, 1-15% of patients fail to achieve viral eradication. Some factors may play a role in the treatment failure and relapse. In this paper, we present two cases of hepatitis C in which DAAs failed due to incomplete HCV genotyping.

Keywords: Chronic hepatitis C, direct-acting antivirals, hepatitis C virus

ÖZ

Günümüzde direkt-etkili antiviral (DEA) ilaçlar ile kronik hepatit C virüs tedavisinde yüksek başarı oranlarına ulaşılmıştır. Bununla birlikte %1-15 hastada viral eradikasyon sağlanamamaktadır. Bazı faktörler tedavi başarısızlığında ve relapslarda rol oynayabilir. Bu olgu sunumunda, eksik genotip tayini nedeniyle tedavide başarısız olunan iki olgu sunulmuştur.

Anahtar Kelimeler: Kronik hepatit C, direkt etkili antiviraller, hepatit C virüs

Akıncı E, Yurdcu E, Orkun Özbay B, Bodur H. Failure of Direct-Acting Antiviral Agents Due to Incomplete Hepatitis C Virus Genotyping. Viral Hepat J. 2018;24:96-98.

Introduction

Current direct-acting antivirals (DAAs) have high success rates in the treatment of chronic hepatitis C virus (HCV) infection. Despite important advances in HCV clearance by DAA therapies, about 1-15% of patients fail to achieve virological eradication (1). Some factors may play a role in treatment failure and relapse. These factors are difficult-to-treat genotypes, advanced fibrosis, sub-optimal treatment regiments, poor compliance of patients, drug-drug interactions, drug resistance, and inaccurate or incomplete genotyping (1,2). Genotyping errors, such as indeterminate results, incomplete genotyping in mixed genotype infections, wrong genotyping or subtyping may cause treatment failure in up to 10% of cases (1). In this paper, two cases of chronic hepatitis C in which DAAs failed due to incomplete HCV genotyping are presented.

Patients permitted their data for publication.

Cases

Case 1

A 39-year-old male patient was admitted to the hospital with the diagnosis of genotype 1b chronic hepatitis C infection in November 2017. He had no risk factor for transmission of HCV such as blood transfusion or intravenous (IV) drug abuse. His HCV RNA level was 3310000 IU/mL, alanine aminotransferase (ALT) was 95 U/L and aspartate aminotransferase (AST) was 50 U/L. The liver biopsy revealed fibrosis 3/6 and histology activity index (HAI) 10/18. He was treatment-naive and ombitasvir/paritaprevir/ritonavir + dasabuvir was started for 12 weeks. At follow up, HCV RNA was negative in the second month of therapy. However, one month after completion of the therapy, HCV RNA [real time-polymerase chain reaction RT-PCR)] was found to be positive (22600000 IU/mL) and ALT and AST levels were elevated (281 U/L and 92 U/L,

Address for Correspondence: *University of Health Sciences, Ankara Numune Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Ankara, Turkey*
Phone: +90 312 508 48 41 E-mail: esragulakinci@gmail.com ORCID ID: orcid.org/0000-0002-2627-219X **Received:** *03.10.2018* **Accepted:** *22.10.2018*

respectively). At follow up, increased liver enzymes on liver function test continued. One month later, ALT and AST levels reached to 467 U/L and 197 U/L, respectively. Prothrombin time (PT), international normalized ratio (INR), thrombocyte, albumin and biluribin levels were within the normal ranges. Other hepatitis serology (hepatitis A and B) and autoimmune markers were negative. His physical examination was normal. He had no complaints and no risk of exposure to HCV in the last 6 months. Relapse was thought and genotype testing was repeated by sequencing analysis. NS5B and 5'UTR region of HCV genome were analyzed (ABI, BigDye Terminator v3.1 Cycle Sequencing Kit). At this time, genotype 3a was detected. Because, there was no risk of exposure to HCV, re-infection was excluded. Mixed genotype infection (1b + 3a) was thought with higher probability. The patient was followed weekly. After 3 months, his ALT and AST levels decreased to the normal levels but HCV RNA persisted positive (54300 IU/mL).

Case 2

A 23-year-old male patient was admitted to the hospital with the diagnosis of genotype 1b chronic hepatitis C infection in December 2017. He had a history of IV drug abuse, but he gave up two years ago. His HCV RNA level was 841000 IU/mL (RT-PCR), ALT: 86 U/L and AST: 35 U/L. Liver biopsy revealed fibrosis stage of 2/6 and HAI score of 7/18. He was treatment-naive and ombitasvir/paritaprevir/ritonavir + dasabuvir was started for 12 weeks. At follow up, HCV RNA levels were negative at the first and second months of therapy. However, at the end of therapy, HCV RNA was found to be positive (7240 IU/mL) and the level of ALT was elevated (60 U/L). Increased liver enzymes on liver function test and rise of HCV-RNA continued. A month later, the level of ALT was 305 U/L, AST was 97 U/L and HCV-RNA was 392000 IU/mL. PT, INR, thrombocyte, albumin and bilirubin levels were within the normal ranges. Other hepatitis serology (hepatitis B and A) and autoimmune markers were negative. He had no clinical symptoms and his physical examination was completely normal. His genotyping test was repeated by sequencing analysis. NS5B and 5'UTR region of HCV genome were analyzed (ABI, BigDye Terminator v3.1 Cycle Sequencing Kit) and genotype 2b was detected. He had no risk of exposure to HCV and IV drug abuse in the last 2 years, thus, re-infection was excluded. Mixed genotype infection (1b + 2b) was thought and he was followed weekly. After 3 months, his ALT and AST levels were within the normal ranges, but HCV RNA was still positive (45100 IU/mL).

Discussion

We have a small amount of real-life data on DAA failure in patients with chronic hepatitis C. In a recent study, characteristics of 87 patients with failure to interferon-free regimens were reported (3). Of these 87 patients, misclassified HCV genotype was detected in 13 (14.9%), 16 patients (18.4%) were treated with sub-optimal DAA regimen and 19 (21.8%) received simeprevir-based regimen. Nearly half of the patients (39, 44.8%) were treated with an optimal DAA regimen. In the 10 of 13 misclassified genotypes, genotype 3 was the wrongly detected genotype and these patients were treated with an ineffective DAA regiments. The authors emphasized that especially misidentification of genotype 3 by commercial assays may cause a trouble in clinical practice and underlined the need for accurate detection of the HCV genotype in order to prevent ineffective treatment. HCV genotyping by more recent methods or by sequencing may warrant the identification of correct genotypes or subtypes.

The efficacy of DAAs varies according to the HCV genotype. Thus, treatment regimen is tailored to the genotype of the virus. So that, accurate diagnosis of mixed genotype infections is needed for the success of treatment. The prevalence of mixed genotype HCV infections varies between studies depending on the study design, patient populations and genotype detection methods. In a study performed in the United Kingdom (UK), it was observed that infection by more than one HCV found in 9% of 44 injecting drug users and in 19% of 37 patients with bleeding disorders (4). In another recently published study from the UK, sera samples of 506 individuals diagnosed with either genotypes of 1a or 3 infection were re-screened for mixed infections by genotype-specific PCR and deep sequencing (5). The total rate of mixed genotype infection was found to be 3.8%. As 6.7% of samples diagnosed with genotype 3 were harboring genotype 1a, 0.8% of samples diagnosed with genotype 1a were harboring genotype 3 ($p<0.05$). Mixed genotype infection samples included major and minor genotypes. Minor genotype constituted less than 21% of the total viral load and less than 1% of the viral load in 67% of cases. A study form Turkey also suggested being careful with mixed genotype HCV infections. In this study, 21 of 495 (4.2%) patients with chronic hepatitis C had mixed genotype infections and of them, 15 (71%) were IV drug users (6). Genotype 1b-4 (7 patients) and genotype 2-3 (6 patients) were the most frequent mixed genotypes.

In this case report, coincidentally, these two patients were admitted to the hospital consecutively and HCV RNA reversed to positive at the end of therapy. There was no risk of exposure in the last 6 months. In both of them, repeated HCV RNA tests revealed different genotypes from the first ones. In the first genotyping tests, the RT-PCR (Rotor Gene Real Time PCR, Qiagen) method was used. In the second genotyping tests, a more sensitive and more specific test, sequencing analysis, was preferred. The NS5B and 5'UTR regions of the HVC genome were amplified by PCR. Amplified PCR products were sequenced directly and different genotypes were detected. In these patients, the first genotype (1b) was accepted as the major genotype with higher viral loads and the second genotypes (3a and 2b) were as minor genotypes with lower viral loads. After treatment with ombitasvir/paritaprevir/ritonavir + dasabuvir, which is effective in major genotype (1b), HCV RNA became negative. However, as a result of activation of minor genotypes (3a, 2b), which are out of the spectrum of the antiviral therapy, HCV RNA reversed to positive and liver enzymes were elevated again.

Resistance-associated substitutions (RASs) may play a role in treatment failure of DAA therapy. In patients with virologic breakthrough, RASs are mostly observed. In addition, RASs are detected between 53%-91% in patients with virologic relapse (1). However, clinical impact of RASs is much more limited (1,2,7). Currently, clinically, the most important RASs are in the NS5A position for genotypes 1a and 3 (8). In our two cases, resistance associated mutation was not detected.

In conclusion, these cases indicated that mixed genotype HCV infections should be kept in mind especially in IV drug users and haemophiliacs. The HCV population structure involved a major and a minor genotype in mixed genotype infections. Thus, low viral load of minor genotype may not be detected by less sensitive and less specific PCR techniques. So that, especially in patients with risk of mixed genotype HCV infections, more effective genotyping methods should be preferred. If it is not possible, pangenotypic DAAs may be chosen.

Ethics

Informed Consent: Patients permitted their data for publication.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Medical Practices: E.A., B.O.Ö., Design: E.A., Data Collection or Processing: E.A., B.O.Ö., Analysis or Interpretation: E.Y., E.A., Literature Search: E.A., Writing: E.A., B.O.Ö., H.B.

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