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AIM AND SCOPE

Viral Hepatitis Journal (Formerly Viral Hepatit Dergisi) is the regular publishing organ of the Viral Hepatitis Society. This periodical journal covers diagnosis, treatment, epidemiology, prevention and information of hepatitis.
Viral Hepatitis Journal is an open-access journal published 3 times per year (April, August and December). In addition, the special issues are published in some periods. It is a periodic national/international journal, published in English language with abstract and title published also in Turkish language and its editorial policies are based on independent peer-review principles.
The aim of Viral Hepatitis Journal is to continuously publish original research papers of the highest scientific and clinical values specifically on hepatitis, on an international level. Additionally, reviews on basic developments in education, editorial short notes, case reports, original views, letters from a wide range of medical personal containing experiences and comments as well as social subjects are published.
For general practitioners giving first line medical service who are interested in hepatitis, specialists in internal medicine, gastroenterology, microbiology, family physician, public health and hepatology, 'things that must be known' subjects will ensure to involve in Viral Hepatitis Journal.
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INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

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

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

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

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

SCIENTIFIC POLICIES

Scientific and Ethics Responsibility

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

Experimental, clinical and drug studies requiring approval by an ethics committee must be submitted to the Viral Hepatitis Journal with an ethics committee approval report confirming that the study was conducted in accordance with international agreements and the Declaration of Helsinki (revised 2013) (http://www.wma.net/en/30publications/10policies/b3/). The approval of the ethics committee and the fact that informed consent was given by the patients should be indicated in the Materials and Methods section (including approval number). In experimental animal studies, the authors should indicate that the procedures followed were in accordance with animal rights as per the Guide for the Care and Use of Laboratory Animals (http://oacu.od.nih.gov/regs/guide/guide.pdf) and they should obtain animal ethics committee approval.

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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.

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In case of retraction of the text by author(s) for any reason again needs a written and signed application explaining the reasons.

The name of the institution where the authors work and the name of the institution or the department in which the study has been conducted should not be mentioned in the submitted manuscript.

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

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

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

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

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

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

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

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

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Short Announcements

i. Turkish title, english title, author name and institution(s) (Turkish and Engilish)

ii. Turkish and english Abstract (max 300 words)

iii. Turkish and english Key Words

iv. Introduction (max 300 words)

v. Materials and Methods (max 400 words)

vi. Results (max 400 words)

vii. Discussion (max 700 words)

viii. Referances (should not exceed 15), all words 2000 not exceed.

• Author number for review articles should not exceed three.

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

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

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

Manuscripts should be written with Microsoft Word.

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

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

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

ARTICLE SECTIONS

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

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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.

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

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

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

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

Original researches should have the following sections;

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

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

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

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

Conclusion: The conclusion of the study should be highlighted.

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

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Case Reports

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

Review Articles

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

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

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

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

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

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

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

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

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

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

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

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

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

All manuscripts submitted to the Viral Hepatitis Journal are screened for plagiarism using the 'iThenticate' software. Results indicating plagiarism may result in manuscripts being returned or rejected.

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Articles must be complete. They must include the following:

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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.

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EDITORIAL

Dear colleagues,

We are here again with the first issue of 2017 that includes interesting new subjects. This issue starts with review article titled "Current Treatment Approach in Acute Hepatitis C Infection", and continues with the research articles titled "Evaluation of Chronic Hepatitis C Patients from Different Aspects Before the Use of Directly Acting Antivirals", "Distribution of Hepatitis C Virus Genotypes in the Region of 'Istanbul Northern Anatolian Association of Public Hospitals'", "Hepatitis B Virus and Hepatitis C Virus Co-infection: An Evaluation of 82 Patients", "King's Score may be More Effective in the Determination of Severe Fibrosis in Chronic Hepatitis B Infections", "Prevalence of Hepatitis B, Hepatitis C and Human Immunodeficiency Virus Among Cannabis and Opioid Addicts" and "Determination of Resistance Mutation in Chronic Hepatitis B Patients using antiviral drugs at our hospital".

Apart from these, a case report titled "Elbasvir/Grazoprevir Experience-A New Glance at HCV Treatment: Case Report" and a letter to the editor titled "How to Diagnosis the Occult Hepatitis C Virus?" were included in this issue.

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

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

Doi: 10.4274/vhd.91886
Viral Hepatitis Journal 2017;23(1):1-5

Current Treatment Approach in Acute Hepatitis C Infection

Akut Hepatit C Enfeksiyonunda Güncel Tedavi Yaklaşımı

Rahmet GÜNER, Bircan KAYAASLAN

Yıldırım Beyazıt University Faculty of Medicine, Department of Infectious Disease and Clinical Microbiology, Ankara, Turkey

ABSTRACT

Acute hepatitis C virus (HCV) infection has an asymptomatic course in most patients. There is no sufficient data regarding its epidemiological features. Recent advances in the treatment of chronic HCV infection have led also to discuss on acute HCV treatment. The possibility of spontaneous clearance, absence of reduction in response rates with delayed treatment, and being curable easily with new directly acting agents brought interferon-free treatment to the agenda. Current guidelines made some changes in the recommendations for optimal duration of treatment and treatment options for acute HCV infection. We aimed to review the treatment of acute HCV infection in the light of current data.
Keywords: Acute hepatitis C, spontaneous clearance, current treatment approach, interferon-free treatments

ÖZ

Akut Hepatit C virüs (HCV) enfeksiyonu hastaların çoğunda asemptomatik seyreder. Epidemiyolojik özellikleri ile ilgili veriler yetersizdir. Kronik HCV enfeksiyonunun tedavisinde yaşanan yeni gelişmeler, akut HCV tedavisinin de tartışılmasına neden olmuştur. Spontan klirens gelişme ihtimali, gecikmiş tedavi ile yanıt oranlarında azalma olmaması ve yeni direk etkili antiviraller ile enfeksiyonun kolaylıkla tedavi edilebilmesi interferonsuz tedavi rejimlerini gündeme getirmiştir. Güncel kılavuzlar akut HCV enfeksiyonun tedavi süresi ve seçenekleri ile ilgili birtakım güncellemeler yapmıştır. Bu makalede, akut HCV enfeksiyonunun tedavisinin güncel veriler ışığında gözden geçirilmesi amaçlanmıştır.
Anahtar Kelimeler: Akut hepatit C, spontan klirens, güncel tedaviler, interferonsuz tedaviler

Guner R, Kayaaslan B. Current Treatment Approach in Acute Hepatitis C Infection. 2017;23:1-5.

Introduction

Hepatitis C virus (HCV) infection is a global health problem that causes chronic liver disease (1). Acute HCV infection is defined as initial six months of hepatitis C infection after exposure to the virus. It is usually asymptomatic and rarely causes life-threatening disease. It is difficult to diagnose since the infection is usually asymptomatic. The epidemiological data on acute HCV infection are mostly based on chronic HCV infection (2,3). There is no sufficient epidemiological data in our country, as in all over the world. Achieving high rates of sustained virologic response (SVR) with directly acting antivirals (DAAs) in chronic hepatitis C infection necessitated a rethinking of acute hepatitis C treatment. Optimal treatment options and duration of treatment are still not standardized.

Successful treatment of acute HCV infection in high-risk populations, especially in men who have sex with men and intravenous (IV) drug users is very important for the prevention of transmission in population (4,5,6). In the natural course of acute HCV infection, most patients are asymptomatic. Only 10-15% of patients are symptomatic (Figure 1). Jaundice occurs in less than 2% of cases. Fatigue, nausea, and right upper quadrant pain may be seen. The disease continues 2 to 12 weeks in symptomatic patients. Fulminant hepatitis is rarely seen, especially in patients with an underlying chronic liver disease and hepatitis B co-infection (7).

There is no test defined for the diagnosis of acute HCV infection. Acute HCV infection is diagnosed by anti-HCV seroconversion following possible exposure, elevation of liver enzymes and elimination of other acute liver diseases. The development of anti-HCV seroconversion in patients previously known as seronegative is a valid test for the diagnosis of acute infection. Acute HCV infection is usually defined in high-risk populations such as healthcare workers and IV drug users. In clinically high suspicion, definite diagnosis can be made with a positive HCV RNA in anti-HCV-negative patients and anti-HCV seroconversion in the follow-

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up. The algorithms to be followed for diagnostic purposes in post-exposure period are defined in the guidelines (8).

Observation for Spontaneous Clearance

Expected benefit from treatment in acute HCV infection is prevention of chronicity. A substantial part of acute HCV infection is spontaneous clearance. In their review evaluating 675 patients from 31 studies, Micallef et al. (9) reported that the rate of spontaneous viral clearance was 26% in acute hepatitis C. The rate of clearance was reported in the range of 20-42% in these studies. Grebely et al. (10) evaluated the time to spontaneous virologic clearance and factors associated with clearance in the data drawn from international collaboration of nine prospective cohorts. Acute HCV infection in 632 patients from four different European countries was included to the evaluation. Median clearance time was found to be 16.5 weeks. The cumulative clearance rates were reported as 34%, 67% and 83% at months 3, 6 and 12, respectively. Most of the studies showed that spontaneous clearance usually occurs within the first 6 months of the infection. It has been reported that factors influencing spontaneous clearance positively were being under 30 years of age, female gender, symptomatic infection, IL 28BCC genotype and HCV genotype 1, while independent predictive factors have been determined to be female gender, IL 28 B CC genotype and HCV genotype 1 (10,11,12). Spontaneous clearance was reported in 46-67% of symptomatic cases, while no spontaneous clearance observed in asymptomatic patients (13,14). The presence of jaundice may indicate a good immune response resulting in spontaneous clearance. Additionally, spontaneous clearance rates are lower in HIV co-infection (7). HCV infection becomes chronic in most of patients in whom viremia persists at the end of six months.

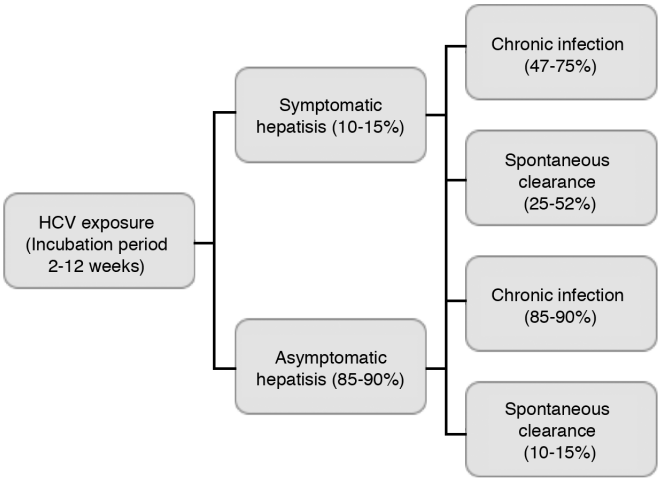

Figure 1. The course of acute hepatitis C virus infection (from Reference 7)

Interferon-Based Therapies

Cure rates are high in acute HCV infection with interferon (IFN)-based therapies. Previous guidelines had recommended follow-up for 12 weeks without therapy for spontaneous clearance in acute HCV infection (5,6). It has been shown that SVR is not adversely affected with IFN-based treatments given after a 12-week observation period for spontaneous clearance. Some of important studies' results on IFN-based treatment are summarized in Table 1. In a German study including 44 cases, the patients were treated with IFN for 24 weeks (5MU of IFN alpha-2b daily for 4

Table 1. Results of some important interferon-based therapy' studies in acut hepatitis C

Ref no.	Number of patients	Treatment regimen and duration	SVR rate (%)
15	44	5MU of IFN alpha-2b daily for 4 weeks and then three times per week for another 20 weeks	98
16	1075	77% peginterferon monotherapy 16% interferon monotherapy 4% peginterferon + RBV 3% interferon + RBV The average duration of treatment: 19.7 weeks	78
17	16	Peg-IFN alpha 2b, 24 weeks	94
18	170	Peg-IFN alpha 2b, 12 weeks	87
19	132	Peg-IFN alpha 2b ± RBV, 24 weeks	90-95
20	102	Peg-IFN alpha 2b, 8 weeks Peg-IFN alpha 2b, 12 weeks Peg-IFN alpha 2b, 24 weeks	67.6 82.4 91.2
21	130	Peg-IFN alpha 2b, 12 weeks Peg-IFN alpha 2b, 24 weeks Peg-IFN alpha 2b + RBV, 12 weeks	81.6 81.6 81.6
22	197*	Peg-IFN alpha 2a/2b ± RBV, 24 weeks	83
23	19** 48	Peg-IFN + RBV + TVR, 12 weeks Peg-IFN + RBV, 24 weeks	84 63
24	57** 73	Peg-IFN + RBV + BOC, 12 weeks Peg-IFN + RBV, 24 weeks	86 84

*Only HCV monoinfected patients' results are summarized, **These studies were conducted on HIV co-infected patients
SVR: Sustained virologic response, Peg-IFN: Pegylated-interferon, RBV: Ribavirin, TVR: Telaprevir, BOC: Boceprevir

weeks and then three times per week for another 20 weeks). Both at the end of treatment and six month after cessation of treatment, HCV RNA were undetectable in 98% of cases (15). This is very important study because it shows that standard IFN treatment can prevent chronic HCV infection. In a meta-analysis including 22 studies on 1075 patients who were treated with pegylated (peg)-IFN alpha or standard IFN monotherapy, SVR rate was reported to be 78%. The authors reported that the highest SVR rates were achieved in patients whose therapies were initiated after a 12-week of observation period for spontaneous clearance. Based on these findings, they recommended 12 weeks of follow-up for spontaneous clearance without treatment and then initiation of treatment if patients could not achieve spontaneous clearance (16). In another study, SVR was reported to be achieved by treatment with peg-IFN alpha 2b for 24 weeks in 94% of 16 patients with acute hepatitis C infection, who were still viremic at the end of the 12-week follow-up period (17). Delaying treatment to 8-12 weeks does not lead to a reduction in SVR rates. Initiation of treatment at week 8 or 12 was shown to be resulted in a high SVR rate in a study by Kamal et al. (18) They initiated peg-IFN alpha-2b treatment at three different time points in 129 patients with aacute HCV infection who had no spontaneous clearance at 8 weeks. SVR rates were reported to be 95.3%, 93.2% and 76.6% with initiating treatment at the weeks 8, 12 and 20, respectively. They reported that the most convenient time to start treatment was 8 to 12 weeks.

In another phase 3 non-inferiority trial conducted as multi-center and randomized study, the efficacy of early and delayed treatment was compared in patients with acute hepatitis C. One group was treated immediately with peg-IFN alpha-2b for 24 weeks and the other group was prospectively followed for 12 weeks and peg-IFN alpha-2b plus ribavirin treatment was initiated if HCV RNA remained positive. one hundred-seven symptomatic and 25 asymptomatic patients were included in the study. SVR rate was reported as 90% in symptomatic patients who were treated immediately and 93% in those given delayed therapy. The authors emphasized that waiting for spontaneous clearance prevents unnecessary treatment, but it would be better to start treatment immediately in patients who cannot be closely followed (19). The study of Kamal et al. (20) is important for comparing the different treatment durations with peg-IFN in acute HCV infection. They reported SVR at the rates of 67.6%, 82.4% and 91.2% with 8, 12 and 24 weeks of peg-IFN treatment, respectively. Age, gender and HCV genotype were found not to be associated with SVR, while rapid virologic response was reported to play an important role in achieving SVR (21).

The other investigated issue is adding ribavirin to the treatment. Data collected from five prospective cohorts of high-risk individuals in Australia, Canada, Germany and the United States provides important data regarding 237 acute HCV patients. Based on this study, the duration of infection, baseline HCV RNA level of <400,000 IU/mL, IL 28B CC genotype and ≥40 years of age were determined as independent predictive factors in terms of achieving SVR in patients with HCV mono-infection. Combination therapy, HCV genotype, asymptomatic infection and gender were not found as an independent risk factor for SVR (22). Therefore, it can be said that the addition of ribavirin to the combination in acute HCV infection is not beneficial. The other choice is the addition of protease inhibitor to peg-IFN + ribavirin combination. Data obtained through the New York acute hepatitis C Surveillance Network showed that a 12-week treatment course with a combination of peg-IFN + ribavirin + telaprevir achieved SVR in 84% of 19 human immunodeficiency virus (HIV)-infected men with acute genotype 1 HCV infection (23). SVR was also detected at the rate of 78% with triple combination treatment with boceprevir (BOC) in the similar patient population and SVR rate was reported to be 95% in the group who achieved rapid virologic response (24). As it has become clear according to the studies, high cure rates were achieved with IFN-based therapies in acute HCV patients who did not develop spontaneous clearance. Today, when DAAs have been successfully used in chronic hepatitis C therapy without IFN, the European Association for the Study of the Liver (EASL) guideline for HCV infection emphasized that the optimal time for starting treatment in acute HCV infection is not clear and ALT elevation may be the starting point of ideal treatment independent of clinical manifestations (25). The American Association for the Study of Liver Diseases (AASLD) guideline for hepatitis C recommends regular HCV RNA monitoring every 4 to 8 weeks until 6-12 months after the diagnosis of acute HCV infection without initiating treatment (26). In addition, it was emphasized that trying to determine the route of the virus exposure during the follow-up period, and the counseling for the behaviors associated with transmission risk should be kept in mind. Especially, it should not be forgotten that IV drug users have a high risk for HCV transmission. Acute hepatitis C patients should also be warned about avoiding hepatotoxic drugs (25,26). The AASLD guideline recommends an observation period of minimum 12 weeks for spontaneous clearance before treatment in patients who cannot wait 6-12 months, and educating the patients who decided to wait 6-12 months regarding rules in chronic HCV infection (26).

Interferon-Free Direct Acting Antiviral Combination Therapies

There are a limited number of studies regarding DAAs treatment in acute hepatitis C and some of them are in the form of report. The HepNet Acute HCV-IV Study aimed to investigate the effectiveness of the 6-week sofosbuvir/ledipasvir combination in genotype 1 acute HCV mono-infected patients. In the cohort including 20 patients, SVR12 was achieved in all patients. It was noted that while the virologic response was found to be slower in patients with high baseline HCV RNA levels, ALT and bilirubin levels rapidly returned to normal in majority of patients (27). In the SLAM-C Study, the effectiveness of the 4-week sofosbuvir/ledipasvir and the 8-week sofosbuvir/simeprevir treatments were evaluated in 24 patients. According to per protocol analysis, SVR12 was achieved in all patients in both groups (28). In a study by Rockstroh et al. (29), sofosbuvir/ledipasvir combination was used for 6 weeks to treat genotype 1 or 4 acute hepatitis C patients co-infected with HIV. The study data has yet to reach SVR4 results. SVR4 was achieved in 85% of patients and recurrence was observed in 15%. The researchers reported a strong association between baseline HCV RNA levels and response to treatment. SVR4 was achieved in all patients with a HCV RNA level of <9 million IU/mL. SVR12 results are not yet available in the study. The SWIFT-C Study investigating sofosbuvir/ribavirin combination therapy for 12 weeks

in HIV-coinfected acute hepatitis C patients reported high relapse rates (30). Upon this, the other 8-week cohort of this study was changed to sofosbuvir/ledipasvir treatment. In the DARE C Study, 19 patients (14 coinfected with HIV) were treated with sofosbuvir and ribavirin combination for 6 weeks. At the end of treatment, HCV RNA became undetectable in 89% of patients (n=17). However, SVR4 and SVR12 rates were only 42% (8) and 32% (6), respectively. No response to treatment (2), posttreatment relapse (9), reinfection (1), and loss to follow-up (1) were the reasons for treatment failure. The authors emphasized that 6 weeks of sofosbuvir/ribavirin combination have a suboptimal efficacy for acute HCV treatment (4). The EASL has listed the recommended treatment options in the current guidelines for use in acute HCV infection as shown in Table 2 (25).

Table 2. The European Association for the Study of the Liver treatment recommendation in acute hepatitis C infection (Transferred exactly from European Association for the Study of the Liver)

Patients with acute hepatitis C should be treated with a combination of sofosbuvir and ledipasvir (genotypes 1, 4, 5 and 6), a combination of sofosbuvir and velpatasvir (all genotypes), or a combination of sofosbuvir and daclatasvir (all genotypes) for 8 weeks without ribavirin (B1).
Patients with acute hepatitis C and HIV coinfection and/or a baseline HCV RNA level >1 million IU/mL (6.0 log IU/mL) may need to be treated 12 weeks with the same combination regimens (B2).
SVR should be assessed at 12 and 24 weeks' post-treatment, because late relapses have been reported (B2).

HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, SVR: Sustained virologic response

Conclusion

Case series with a small number of patients show that direct antivirals, especially sofosbuvir/ledipasvir combination, can be successfully used in acute HCV genotype 1, 4, 5, 6 infections. However, high success rates in the treatment of chronic HCV infection and high spontaneous clearance rates indicate that the treatment can be postponed with determining the eligible patient population and a good counseling before treatment.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

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

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Research Article

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Evaluation of Chronic Hepatitis C Patients from Different Aspects Before the Use of Direct Acting Antivirals

Direkt Etkili Antiviraller Dönemi Öncesi Farklı Yönlerden Kronik Hepatit C Hastalarının Değerlendirilmesi

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ABSTRACT

Objective: Chronic hepatitis C (CHC) virus infection is one of the leading causes of chronic liver disease in all over the world. The prevalence of CHC is almost 0.5-1% in Turkey. Until recently, pegylated-interferon (PEG IFN) alpha in combination with ribavirin was the main treatment of CHC. The aim of the study was to evaluate the real life data of CHC patients.

Materials and Methods: We retrospectively evaluated the demographical data and treatment responses of patients with CHC who were followed and treated in our clinic between January 2008 and December 2015.

Results: A total of 117 patients (67 female and 50 male) with a mean age of 48 (15-65) were included in the study. 105 patients were genotype 1, 3 were genotype 2 and 9 were with genotype 3. The patients were treated with PEG IFN alpha-2a (81/117) or alpha-2b (36/117) combined with ribavirin. We observed sustained virologic response (SVR) in 68% of all genotype 1 patients. While relapse was observed in only 1 patient among those with genotype 2 and 3, SVR was achieved in 11. The rate of SVR was only 42% among patients older than 60 years of age, whereas SVR was achieved in all young patients (range: 15-30). The overall SVR rate was 70%.

Conclusion: As CHC can result in long-term complications (cirrhosis, terminal liver failure and hepatocellular carcinoma), patients without therapy remain at risk of developing progressive liver disease. Since advanced fibrosis is a predictor for poor prognosis and insufficient therapy outcome, early treatment is required to efficiently cope with this health problem, Although the rates of SVR with direct acting antivirals are very high, starting treatment in early stage could reduce the complications of CHC and transmission of the disease.

Keywords: Chronic hepatitis C, pegylated-interferon, ribavirin, sustained virologic response

ÖZ

Amaç: Kronik hepatit C (KHC) virüsü enfeksiyonu, tüm dünyada kronik karaciğer hastalığının önemli bir nedenidir. Türkiye'de hastalığın prevalansı %0,5-1 arasındadır. Yakın zamana kadar, KHC hastalarının standart tedavisinde pegile-interferon (PEG IFN) ve ribavirin kombinasyonu kullanılmaktaydı. Bu çalışmada amacımız KHC hastalarının gerçek yaşam verilerini değerlendirmektir.

Gereç ve Yöntemler: Ocak 2008-Aralık 2015 tarihleri arasında kliniğimizde takip edilen ve tedavisi tamamlanan naif KHC tanılı hastaların dosyaları retrospektif olarak incelendi; hastaların demografik verileri ve tedavi yanıtları değerlendirildi.

Bulgular: Çalışmamızda değerlendirmeye alınan toplam 117 hastanın 67'si kadın olup ortanca yaş 48 (15-65) idi. Hastaların 105'inde genotip 1, 3'ünde genotip 2, 9'unda genotip 3 saptandı. Hastalara PEG IFN alfa-2a (81/117) veya alfa-2b (36/117) ve ribavirin kombine tedavisi başlandı. Genotip 1 hastalarının %68'inde kalıcı virolojik yanıt (KVY) saptandı. Genotip 2 ve 3 hastalarından sadece bir kişide relaps gözlenirken, 11 hastada KVY sağlandı.15-30 yaş grubunda tüm hastalarda KVY sağlanırken, 60 yaş üstünde KVY oranı %42 bulunmuştur. Tüm hastalar değerlendirildiğinde ise, olguların %70'inde KVY saptanmıştır.

Sonuç: KHC uzun dönemde bir çok komplikasyona (siroz, terminal karaciğer yetmezliği ve Hepatoselüler karsinom) neden olabildiğinden, tedavisiz kalan hastalar progresif karaciğer hastalıkları açısından risk altındadır. Bu sağlık sorunuyla etkili bir şekilde başa çıkabilmek için erken tedavi gereklidir, çünkü ileri fibroz kötü prognoz ve başarısız tedavinin önemli bir göstergesidir. Direkt etkili ajanlarla KVY oranları oldukça yüksek olmakla beraber, yine de tedaviye erken başlanması KHC'nin komplikasyonlarından korunmak ve bulaş zincirinin kırılması açısından önemlidir.

Anahtar Kelimeler: Kronik hepatit C, pegile-interferon, ribavirin, kalıcı virolojik yanıt

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Introduction

Chronic hepatitis C (CHC) virus infection is one of the leading causes of chronic liver disease. The estimated global prevalence of hepatitis C virus (HCV) infection is about 3% (1,2,3). According to the guideline prepared by the Turkish Viral Hepatitis Society, the prevalence of CHC is about 0.5-1% in Turkey (1,4,5,6). Patients might develop chronic disease (60-80%), cirrhosis (30%) and hepatocellular carcinoma (1-4%) over the years (3,7). Currently, direct acting antivirals (DAAs), such as sofosbuvir, ledipasvir, paritaprevir, ombitasvir, dasabuvir, simeprevir and daclatasvir have been licensed for the treatment of CHC. In Turkey, these DAAs have been used since July 2016. Before DAAs, pegylated-interferon alpha (PEG-IFNa plus ribavirin (RBV) was standard treatment of CHC (2). Sustained virologic response (SVR) rate is 40-50% for genotype 1 that widely seen in Turkey, and 80% for genotype 2 and 3 (2).

In this study, especially before the new treatments, we evaluated overall demographical, clinical and laboratory characteristics of patients who were followed in our clinic between January 2008 and December 2015 and treatment responses to CHC.

Materials and Methods

A total of 117 patients were included in this study. We retrospectively evaluated the data on demographic characteristics and treatment responses of patients with CHC virus infection who were followed in our clinic between January 2008 and December 2015. These data were collected from the patient files. In the same period, we could not administer treatment in about 90 patients because of various reasons, including older age, underlying disease and intolerance to the drugs.

The treatments were PEG-IFNa-2a (180 µg/week) or PEG-IFNa-2b (1.5 µg/week) combined with RBV (800-1200 mg/day). We monitored HCV RNA levels at 0 (baseline), 4 [rapid virologic response (RVR)], 12 [early virologic response (EVR)] weeks of therapy, end of the therapy (end-of-treatment response) and 24 weeks after the therapy (SVR or relapse).

The planned duration of treatment was 24 weeks for genotype 2 and 3, and 48 weeks for genotype 1. The treatment was stopped at 24 weeks for some genotype 1 patients with RVR.

Statistical Analysis

Data analysis was performed by using the SPSS 20.0 program. The laboratory values of patients were compared with univariate analysis. Afterwards, chi-square test and Mann-Whitney U test were used for categorical variables and continuous variables, respectively. A p value of less than 0.05 ($p \leq 0.05$) was regarded as statistically significant.

Results

A total of 117 patients (67 female, 50 male) were included in the study. The mean age of the patients was 48 (15-65) years. 105 patients were genotype 1, three were genotype 2 and nine were genotype 3 (Table 1).

While all young patients (15-30 years) had SVR, this rate was only 42% among patients older than 60. As the patients got older, hepatic fibrosis stage increased and treatment response decreased (Table 2).

Initial high alanine aminotransferase (ALT) levels (>40 IU/L) and high viral load (>800.000 IU/L) were detected in 81 and 75 patients, respectively. The initial ALT and viral load were statistically unremarkable in terms of SVR. The cut-off value for high viral load is >6.000.000 IU/mL in recent guidelines. According to this data, the SVR rates were found to be 42% and 76% in patients with high and low viral load, respectively. Significant differences were observed compared to 800.000 IU/mL reference value.

Table 1. The baseline characteristics of patients

	Number	Percent (%)
Sex		
Female	67	56
Male	50	44
Genotype		
1	105	90
2	3	2.5
3	9	7.5
HCV RNA		
<800.000 IU/mL	42	36
>800.000 IU/mL	75	64
<6.000.000 IU/mL	98	84
>6.000.000 IU/mL	19	16
ALT		
<40 IU/mL	36	31
>40 IU/mL	81	69
Fibrosis score		
F0-2	25	30
F3-6	58	70
Treatment (RBV+)		
PEG-IFN alpha-2a	81	69
PEG-IFN alpha-2b	36	31

PEG-IFN: Pegylated-interfon, RBV: Ribavirin

Table 2. The rate of treatment response according to age groups

Age	No. of patients N	Genotype 1 N	Genotype 2-3 N	Patients with high viral load n/N (%)	Patients with high ALT level n/N (%)	Patients with advanced hepatic fibrosis n/N (%)	Patients with SVR n/N (%)	Non-responders n/N (%)	Relapse n/N (%)
15-30	15	12	3	4/11 (36)	7/15 (46)	0/11 (0)	15/15 (100)	0/15 (0)	0/15 (0)
31-40	22	19	3	11/22 (50)	17/22 (77)	4/15 (26)	16/22 (72)	3/22 (13)	3/22 (13)
41-50	34	31	3	22/34 (64)	27/34 (79)	5/24 (20)	26/34 (76)	4/34 (11)	4/34 (11)
51-60	33	31	2	23/33 (69)	25/33 (75)	11/25 (44)	20/33 (60)	7/33 (21)	6/33 (18)
61-70	13	12	1	7/13 (53)	8/13 (61)	5/8 (62)	6/13 (46)	4/13 (30)	3/13 (23)

SVR: Sustained virologic response, ALT: Alanine aminotransferase, n: Number of patients of positive for related parameter, N: Number of patients of screening for related parameter

Eighty three patients underwent liver biopsy. Twenty five patients (30%) had moderate-to-advanced fibrosis (F3 and higher according to the Knodell Histological Activity Index) with 44% SVR rate. The rate of SVR in patients with low-to-moderate fibrosis was 86% (p<0.001).

In 29 patients evaluated for RVR, 10 patients (34%) had undetectable HCV-RNA level at the end of 1 month. The treatments of 5 patients with RVR were stopped at the 24th week. At the end of the 12th week, while we detected EVR in 86 (84%) of 102 patients who were evaluated for HCV-RNA level, 1 patient had more than 2-log decline at the 12th week but detectable HCV-RNA at the end of the 24th week. Eighteen patients were considered as non-responders at the 24th week. 80% of genotype 1 patients who were treated for 48 weeks achieved SVR (Table 3). We detected SVR in all 10 patients with RVR.

In our study, while 1 of the 16 relapse cases was genotype 3, the rest of them were genotype 1. Of the 15 genotype 1 patients with relapse, 6 were male (40%). Thirteen patients (86%) had high ALT levels and high HCV-RNA was detected in 9. Four of ten patients who underwent biopsy had moderate-to-advanced hepatic fibrosis. In 12 cases, we had to stop the treatments early due to adverse effects. Only one patient achieved SVR among these patients.

We observed SVR in 68% of all genotype 1 patients. While relapse was observed in only 1 patient among genotype 2 and 3 patients, SVR was achieved in 11. Overall, 70% of the cases achieved SVR.

Discussion

HCV is currently the leading cause of chronic hepatitis (1). Initially, its treatment was IFN-α. The addition of a polyethylene-glycol molecule to standard interferon produces a biologically active molecule with a longer half-life (1,6,8,9). Use of this molecule with RBV has shown to increase SVR rates. Afterwards, combinations with specifically targeted antiviral therapy have been developed (10). Clinical trials have suggested that protease inhibitors (telaprevir or boceprevir) combined with PEG-IFNα+RBV could produce increase of SVR rates but discontinuation of treatment because of adverse events was more frequent (10). Nowadays, DAAs were licensed in the treatment of CHC with or without RBV. The IFN-free regimens are well tolerated than ever before and achieved SVR >90-100%.

PEG-IFNa+RBV treatment is the individualized treatment, a response-guided therapy, which is based on host- and HCV-related factors. Strong predictors of SVR are HCV genotype and the initial virologic response to treatment (10). A number of pre-treatment factors, such as older age, presence of cirrhosis or advanced fibrosis, African-American race, overweight, genotype, viral load, low level of ALT, and low platelet count are known to reduce the SVR rate (10,11).

In Turkey, the most common genotype is 1b (75-97%). 89% of our patients had genotype 1. In genotype 1 group, SVR can be achieved in 40-50% and 91% in genotype 2 and 3 (8). In our study; 68% of genotype 1 and 11 of 12 patients with genotype 2 and 3 achieved SVR.

Male gender and older age have been reported to associate with poor outcome of therapy (8,10,11). We found no difference in SVR rate between genders. In our young patient group (15-30 years of age), all subjects had SVR without relapse.

When comparing the SVR rate between the groups with elevated and normal ALT levels (66% and 80%, respectively), or between subjects with initial high and low viral load, there was no statistically significant difference (70% and 71%, respectively). Unlike our study, it has been reported that high ALT and initial viral load reduced the SVR rate (8,10,12,13,14).

In 83 patients, who underwent biopsy, SVR was achieved in 44% of subjects with moderate to severe stage and in 86% of patients with mild-to-moderate stage (p=0.001). It is well known that low stage is a good prognostic factor for high SVR rate (9,10,15,16).

A higher proportion of patients with advanced age had more severe fibrosis in our study (Table 2). This is probably due to the duration of HCV infection because chronic liver failure and HCV-associated complications may develop many years after infection (1,17).

Patients with RVR have a better likelihood of achieving SVR. We had 10 patients with RVR (m/f: 5/5). Six patients stopped the therapy at the 6th month, 1 patient interrupted the therapy at the 9th month because of severe side effects and, the duration of

Table 3. The characteristics of the patients according to treatment response

	Patients with ETR n/N (%)	Patients with SVR n/N (%)	Non-responders n/N (%)	Relapsers n/N (%)
Male	43/50 (86)	36/50 (72)	7/50 (14)	7/50 (14)
Female	56/67 (83)	47/67 (70)	11/67 (16)	9/67 (13)
Genotype 1	87/105 (82)	72/105 (68)	18/105 (17)	15/105 (14)
Genotype 2-3	12/12 (100)	11/12 (91)	0/12 (0)	1/12 (8)
Patients with HCV RNA <800.000 IU/mL	36/42 (85)	30/42 (71)	6/42 (14)	6/42 (14)
Patients with HCV RNA >800.000 IU/mL	63/75 (84)	53/75 (70)	12/75 (16)	10/75 (13)
Patients with normal ALT level	31/36 (86)	29/36 (80)	5/36 (13)	2/36 (5)
Patients with high ALT level	68/81 (84)	54/81 (66)	13/81 (16)	14/81 (17)
Patients with low hepatic fibrosis	56/58 (96)*	50/58 (86)*	2/58 (3)*	6/58 (10)
Patients with advanced hepatic fibrosis	16/25 (64)*	11/25 (44)*	9/25 (36)*	5/25 (20)

*p<0.05
ETR: End-of-treatment response, SVR: Sustained virologic response, ALT: Alanine aminotransferase, HCV: Hepatitis C virus, n: Number of patients of positive for related parameter, N: Number of patients of screening for related parameter

the therapy was 12 months in 3 patients. In all patients, SVR was achieved. Seven of eight patients with liver biopsy had mild stage.

Only 1 of 16 patients with relapse had genotype 3. This subject had a high viral load (6.450.000 IU/mL) and advanced stage (stage: 3/6). Among the patients with genotype 2 or 3, only this subject had advanced fibrosis and relapse. Fourteen patients (87%) had elevated ALT levels and 11 patients (68%) had high viral load. Five of the 11 patients with liver biopsy had advanced fibrosis. We found statistically significant positive correlation between advanced stage fibrosis and relapse.

All of the 11 patients with RVR and 75 of 87 patients (86%) with EVR had SVR, and the SVR rate in subjects who completed the 48-week therapy was 83%. HCV-RNA decrease, RVR and EVR are supposed to be strong independent on-therapy predictors.

Conclusion

As CHC can result in long-term complications (cirrhosis, terminal liver failure and hepatocellular carcinoma), patients without therapy remain at risk of developing progressive liver disease. To efficiently cope with this health problem, early treatment is required, because advanced fibrosis is a predictor for poor prognosis and insufficient therapy outcome. Although the rates of SVR with DAAs are very high, starting treatment in early stage could reduce the complication of CHC and transmission of the disease.

Etichs

Informed Consent: A retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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

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Research Article

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Distribution of Hepatitis C Virus Genotypes in the Region of İstanbul Northern Anatolian Association of Public Hospitals

İstanbul Anadolu Kuzey Kamu Hastaneler Birliği Hizmet Bölgesinde Hepatit C Virüs Genotiplerinin Dağılımı

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ABSTRACT

Objective: Hepatitis C virus (HCV) is responsible for 20% of acute hepatitis and 70% of chronic hepatitis. Determination of HCV genotype is important in the regulation of treatment and the follow-up of clinical course. In this study, we retrospectively evaluated the results of test performed in the Central Laboratory of İstanbul Northern Anatolian Association of Public Hospitals to determine the genotype distribution of hepatitis C patients in our region.

Materials and Methods: HCV genotypes were determined by real time polymerase chain reaction (Qiagen, Germany) and reverse hybridization line probe assay (Nlm srl, Italy) methods.

Results: Among HCV RNA-positive 554 patients, 312 (56.5%) patients had genotype 1b, 127 (23.1%) - genotype 1a, and 94 (17.3%) patients had genotype 3a. A total of 10 samples were identified to be mixed genotype; 3 (0.5%) - genotype 4c/d, 3 (0.5%) - genotype 2a/c, 3 (0.5%) - genotype 1a/1b, and 1 (0.2%) - genotype 1b/4.

Conclusion: HCV genotype 1b is the most common genotype in our region similar to country-wide results. However, the rates are lower in our country than in the previous years. Probably the difference in this genotype distribution may depend on globalization, developments in technology, changes in human movements and social behaviors. Genotype determination is important for the regulation of treatment and prognosis of HCV infection.

Keywords: Hepatitis C virus genotypes, real time polymerase chain reaction, İstanbul

ÖZ

Amaç: Hepatit C virüsü (HCV) akut hepatitlerin %20'si, kronik hepatitlerin %70'inden sorumludur. HCV'nin genotip tayini tedavide ve klinik sürecin takibinde önemlidir. Bu çalışmada bölgemizdeki HCV genotiplerinin dağılımını belirlemek amacıyla İstanbul Anadolu Kuzey Kamu Hastaneler Birliği Merkezi Laboratuvarı'nda çalışılmış olan test sonuçları retrospektif olarak değerlendirilmiştir.

Gereç ve Yöntemler: HCV genotiplerinin tespiti için real time polimeraz zincir reaksiyonu (Qiagen, Almanya) ve reverse hibridizasyon line probe assay metodu kullanıldı (Nlm srl, İtalya).

Bulgular: HCV RNA pozitif 554 hastada HCV genotiplerinin prevalansı genotip 1b: 312 (%56,5), genotip 1a: 127 (%23,1), genotip 3a: 94 (%17,3) ve genotip 4c/d: 3(%0,5), genotip 2a/c: 3 (%0,5), genotip 1a/1b: 3 (%0,5), genotip 1b/4: 1 (%0,2) olmak üzere 10 hastada (%1) mix tip olarak tespit edildi.

Sonuç: Bölgemizde ülkemizdeki oranlara benzer şekilde HCV genotip 1b en yaygın genotiptir. Ancak oranları ülkemizde önceki yıllarda yapılan çalışmalara göre daha düşüktür. Muhtemelen bu genotip dağılımındaki farklılık, günümüzdeki globalleşme, teknolojideki gelişim, insan hareketlerinin ve sosyal davranışların değişimine bağlı olabilir. Yeni tedavi protokollerinin uygulanabilmesi ve prognozun belirlenmesi açısından HCV hastalarında genotip tayini önemlidir.

Anahtar Kelimeler: Hepatit C virüs genotip, real time polimeraz zincir reaksiyonu, İstanbul

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Introduction

Hepatitis C is a single-stranded positive RNA virus which is found in Flaviviridae family (1). Hepatitis C virus (HCV) is the most important cause of chronic liver disease, cirrhosis and hepatocellular carcinoma around the world (2). Studies using nucleic acid sequence analysis have identified 7 major genotypes and over 100 subtype of HCV (3). Several tests, such as polymerase chain reaction (PCR) amplification and sequence analysis, restriction fragment length polymorphism, reverse hybridization line probe assay (LIPA) and serological genotyping are used for the diagnosis of HCV genotypes (4).

There are differences in the distribution of genotypes of HCV by geographical region. In Europe, United States and Japan, genotype 1 and 2 are the most common genotypes (5). Genotype 3 is the most common genotype in Southeast Asia, genotype 4 in the Middle East, Egypt and Central Africa, genotype 5 in South Africa, and genotype 6 in Asia (6). Genotype 7 is the genotype found in Congo, Africa. It has been reported that the most prevalent genotype in the Mediterranean countries was 1b (7). The risk of developing cirrhosis and hepatocellular carcinoma in patients infected with HCV genotype 1b is higher and this genotype is more resistant to antiviral treatment compared to other genotypes. Genotyping of HCV, collection of epidemiological data, formation of antiviral therapy and the frequency of HCV genotypes in a population varies depending on the age of infection and the route of transmission (8). While preliminary results have shown that genotype 1b was strongly associated with previous blood products transfusion, the incidence of genotypes 1a and 3a due to intravenous drug use has increased (9). It has been found that mean intrahepatic HCV-RNA load was higher in patients infected with genotype 1b (10).

The Northern Anatolian Association of Public Hospitals consists of 11 hospitals, 6 of them are educational and research hospitals. The Central Laboratory services to 11 hospitals. This study was aimed to determine the distribution of HCV genotype in patients who were diagnosed with HCV infection in regions we serve.

Materials and Methods

Patients with the diagnosis of or suspected HCV infection, whose blood samples were retrospectively evaluated for HCV genotypes from January 2016 to January 2017 from 11 hospitals at the Central Laboratory of İstanbul Anatolian North Public Hospitals Association. Sample distribution was as follows, 77.5% chronic HCV infection, 10.5% liver function abnormalities, and 12% other clinic diagnoses.

HCV-RNA positivity was determined by real time PCR (artus HCV QS-RGQ Kit, Qiagen, Germany) method. Real time PCR and reverse hybridization line LIPA (Nlm srl, Italy) methods for genotyping were applied and interpreted according to manufacturers' instructions. In LIPA method, cDNA synthesis was performed first. Hybridization of PCR products obtained from cDNA using biotin-labeled primers to membrane-bound genotype-specific HCV sequences was enzymatically demonstrated. The resulting bands were compared to the guidelines and were genotyped. This study was approved by Haydarpaşa Numune Training and Research Hospital Ethics Committee for Clinical Investigations [Approval number: 08.05.2017 (HNEAH-KAEK 2017/KK/70)].

Results

HCV genotype was identified in a total of 554 HCV RNA-positive samples during a period of one year. Of the patients included in the study; 230 (41.3%) were male and 324 (58.6%) were female, with an average age of 57. HCV genotype 1b was identified as the most dominant genotype followed by genotype 1a and genotype 3a. Of the total of 554 cases, 312 (56.5%) were genotype 1b, 127 (22.9%) were genotype 1a, and 94 (17.3%) were genotype 3a. A total of 10 samples (1.8%) were identified as mixed type; genotype 4c/4d was found in 3 samples (0.5%), genotype 2a/2c in 3 (0.5%), genotype 1a/1b in 3 (0.5%), and genotype 1b/4 in 1 (0.2%).

HCV genotype 1b was found in 56.5% of samples (n=312), in the highest order, followed by genotype 1a with 23.1% (n=127) and genotype 3a with 17.3% (n=94), genotype 4c/4d with 0.5% (n=3) genotype 2a/2c with 0.5% (n=3), genotype 1a/1b with 0.5% (n=3) and genotype 1b/4 with 0.1% (n=1). Eleven (2%) samples were not genotyped (Table 1).

Discussion

Knowing HCV genotypes, collection of epidemiological data, vaccine development studies, treatment design and prognosis are important (3). There are some difficulties in defining genotypes in all methods, including sequence analysis, which is used as the gold

Table 1. Genotype distribution in hepatitis C virus RNA positive cases/gender/age numbers

Genotype Distribution				Age		
Genotype	**Number (%)**	**Female (%)**	**Male (%)**	**18-40**	**41-60**	**60+**
1b	312 (56.3)	181 (32.6)	131 (23.6)	5 (0.9)	177 (31.9)	130 (23.4)
1a	127 (22.9)	72 (12.9)	55 (9.9)	7 (1.2)	71 (12.8)	49 (8.8)
3a	94 (16.9)	58 (10.4)	36 (6.4)	14 (2.5)	53 (9.5)	27 (4.8)
1a/1b	3 (0.5)	2 (0.3)	1 (0.1)	-	2 (0.3)	1 (0.1)
2a/2c	3 (0.5)	1 (0.1)	2 (0.3)	-	-	3 (0.5)
4c/4d	3 (0.5)	2 (0.3)	1 (0.1)	-	3 (0.5)	-
1b/4	1 (0.1)	1 (0.1)	-	-	1 (0.1)	-
Unknown	11 (1.9)	7 (1.2)	4 (0.7)	-	2 (0.3)	9 (1.6)
Total	554 (100)	324 (58.4)	230 (41.5)	26 (4.6)	309 (7)	219 (39.5)

standard method (11). The use of a second method in the laboratory increases sensitivity (12). Studies investigating the effectiveness of interferon and ribavirin combination therapy have shown that the cure rate with antiviral drugs is lower in patients infected with HCV genotype 1b than in those infected with other genotypes (13). In patients with genotype 1b infection, long-term, high-dose therapy results in a higher survival response (14). Furthermore, the rate of permanent response in combination therapy is higher than in interferon therapy alone (15). For this reason, determining HCV genotype may be useful in evaluating the response to treatment and in selecting the most effective treatment regimen (16).

There are geographical differences in the distribution of HCV genotypes. In studies conducted in Turkey, it was seen that genotype 1b was the first most frequently detected type in HCV genotypes with a rate of 66.7%. This is followed by genotype 1a at a rate of 5.8% (1). In our study, the dominant genotype sequence was found to be similar. It is observed that genotypes 2a, 3a, 4, 4c were reported less frequently (3). In their study investigating the distribution of HCV genotypes in 89 Turkish patients, Abacıoglu et al. (15) reported that 75.3% of patients had genotype 1b, 19.1% had genotype 1a, 3.4% had genotype 2 and 2.2% had genotype 4. In a study including.

Seventy-two patients with chronic HCV infection, conducted by Yarkın and Hafta (3) in 2000, using reverse transcriptase (RT) - PCR technique, it was found that 82.2% of patients had type 1b, 14.5% had type 1a, and 3.3% patients had type 2a. Erensoy et al. (17) in 2002, in their study of 45 genotypes in 2002 found genotype 1b in 66.7% of isolites and genotype 1a in 33.3%. In a study by Ural et al. (18) all the 80 HCV RNA-positive cases included in Konya region were found to be genotype 1b (100%). Sönmez et al. (19) analyzed 80 anti-HCV-positive samples using RT-PCR, and detected genotype 1b in 41 samples (69.5%) and mixed type (genotype 1a and 1b) in 3 samples (5.1%). Altuglu et al. (20) investigated serum samples collected from 345 patients with chronic HCV infection and reported that infection with subtype1a and subtype 1b was observed in 9.9% and 87.2% of patients, respectively. Genotypes 2, 3, and 4 were determined in 0.9%, 1.4%, and 0.6% of the patients, respectively. Sağlık et al. (21) in their study including 422 HCV RNA-positive patients performed in 2014, it was determined that 63.3% of subjects (n=267) had genotype 1b, 14.7% (n=62) - genotype 1a, 11.1% (n=47) - genotype 3a, 0.9% (n=4) - genotype 2b, and 0.2% (n=1) of patients had genotype 4; genotype 1 and 4 were observed in 1 patient (0.2%). In genotype 1, 2 and 4 infected patients, subtyping could not be performed in 5.4% (n=23), 2.6% (n=11) and 1.4% (n=6), respectively. In a study performed in Manisa Region by Şanlıdağ et al. (22), a total of 100 HCV-RNA positive patients were included. Genotype 1 was found in 92% of patients (92%) and genotypes 2 and 4 were found in 7% of patients, while HCV genotype could not be identified in one patient (1%). When evaluating the subtypes, genotype 1b was determined in 90 patients (90%), genotype 4a in five patients (5%), genotype 1a in two patients (2%), and genotype 2a in two patients (2%). Kabakçı et al. (23) reported in their study including 500 HCV RNA-positive patients that the most frequent genotype was found to be 1b (93.5%) and the second most frequent genotype was 1a (6.7%). Oztürk et al. (24) found in their study performed in 2014 that the frequency of type 1a (0.31%), 1b (86.73%), 2 (9.26%), 3 (0.93%), and 4 (2.78%) in Antakya was compatible with the nationwide results in Turkey. Altindis et al. (25) who investigated the distribution of HCV genotypes in 7 regions of Turkey reported in their study evaluating 7002 patients with chronic hepatitis C in a six-year period that genotype 1b was the most common genotype (67.7%) followed by untypeable genotype 1 (7.7%), genotype 4 (7.3%) and genotype 3 (6.7%). In 2014, genotype 3 was the second most common one (11.3%) and genotype 4 (9.8%) was the third most common one. A total of 96 (1.3%) patients were found to have mix genotypes. Genotypes 1a/1b were detected in 11 patients, genotypes 2a/2c in - 76 patients, and 9 other genotypes in 9 patients while 2 samples were not genotyped. Uzun et al. (26) reported that genotype 1 was observed in 271 of 308 patients (88%) with chronic HCV infection. Genotype 3 was determined in 15 patients (4.9% of all cases), mix genotype in 9 patients (2.9% of all cases); genotype 2 in 8 patients (2.6% of all cases), and genotype 4 in 5 patients (1.6% of all cases). Of those with mixed genotype, 8 patients had infection with genotype 1/4 and 1 patient with genotype 1/3.

In our study, genotype 1b was dominant genotype and genotype 1a was the second frequently observed genotype. Genotype 3a was the third genotype as reported in studies by Altuglu et al. (20) and Sağlık et al. (21). HCV genotype 1b is the most common genotype in our region similar to that in regions throughout the country. However, the rates are lower in our country than in previous years. Probably the difference in this genotype distribution may depend on globalization, developments in technology, the change of human movements and social behaviors. The incidence of mixed genotypes was 1.8 % and the rates were close to those of Altindis et al. (25) and Uzun et al. (26).

Conclusion

As a result in this context, it is important to determine the molecular epidemiology of HCV infections in our region and to determine the treatment planning and prognosis of HCV infection in terms of follow-up of genotype profile with multi-center country-wide studies.

Ethics

Ethics Committee Approval: This study was approved by Haydarpaşa Numune Training and Research Hospital Ethics Committee for Clinical Investigations [Approval number: 08.05.2017 (HNEAH-KAEK 2017/KK/70)].

Peer-review: Externally and Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.Ö., Concept: S.A., Ü.O.Z., Design: Ü.O.Z., Ş.D.D., Data Collection or Processing: Ü.O.Z., Ş.D.D., Analysis or Interpretation: Ü.O.Z., Literature Search: Ü.O.Z., F.M.Y, Ö.Y., Writing: Ü.O.Z.

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Research Article

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Hepatitis B Virus and Hepatitis C Virus Co-infection: An Evaluation of Eighty-Two Patients

Hepatit B ve Hepatit C Virüs Koenfeksiyonu: Seksen İki Hastanın Değerlendirilmesi

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ABSTRACT

Objective: In this study, we aimed to investigate the characteristics and treatment results of 82 co-infected patients with hepatitis B virus (HBV)/hepatitis C virus (HCV).
Materials and Methods: Four university hospitals evaluated HBV/HCV co-infection cases retrospectively. We analyzed the epidemiological, virological, clinical, and histopathological data and the results of treatment in patients co-infected with HBV and HCV. Pegylated interferon (peg IFN) plus ribavirin treatment was given to patients with HCV dominance. The results of patients receiving different treatment for HBV were evaluated.
Results: The mean age of the patients was 44.3±14.7 years and 52.4% were female. The major risk factors were dental therapy, any surgical procedure, hemodialysis, and blood transfusion. The average HCV RNA level and HBV DNA level were found to be $1.36x10^6$±$3.06x10^6$ IU/mL, and $1.55x10^7$±$4.83x10^7$ IU/mL, respectively. On histopathology, the mean grade of necroinflammation was found to be 4.9±2.6 while the mean stage of fibrosis was 1.7±1.5 in 39 patients. 8.5% of patients were positive for both HCV-RNA and HBV-DNA positive and in 85.7% of cases, HCV infection was found to be dominant. The rate of sustained virologic response was 70.8% in 24 patients receiving peg IFN plus ribavirin therapy. Reactivation of HBV was found in 33.3% of cases. HBV DNA was negative in all patients who received oral antiviral therapy.
Conclusion: In cases where both HCV RNA and HBV DNA were positive, HCV was predominant. This is especially noticeable in hemodialysis patients
Keywords: Hepatitis B virus, hepatitis C virus, co-infection, epidemiology, treatment

ÖZ

Amaç: Bu çalışmada, 82 hepatit B virüs (HBV)/hepatit C virüs (HCV) ko-enfekte hastanın özelliklerini ve tedavi sonuçlarını araştırmayı amaçladık.
Gereç ve Yöntemler: Dört farklı üniversite hastanesinde HBV/HCV ko-enfeksiyonu olan hastalar retrospektif olarak incelendi. HBV/HCV ko-enfeksiyonu olan hastaların epidemiyolojik, virolojik, klinik, histopatolojik verileri ve tedavi sonuçları analiz edildi. HCV enfeksiyonu dominansı olan hastalara pegile interferon (peg IFN) ve ribavirin tedavisi verildi. HBV enfeksiyonu için farklı tedaviler alan hastaların sonuçlarıda değerlendirildi.
Bulgular: Hastaların yaş ortalaması 44,3±14,7 idi ve %52,4'ü kadın hasta idi. HCV enfeksiyonu için major risk aktörleri; diş tedavisi, cerrahi girişim, hemodiyaliz ve kan transfüzyonu idi. Ortalama HCV RNA seviyesi $1,36x10^6$±$3,06x10^6$ IU/mL, ortalama HBV DNA seviyesi $1,55x10^7$±$4,83x10^7$ IU/mL olarak saptandı. Biyopsi yapılan 39 hastanın histopatolojik incelemesinde nekroenflamatuvar aktivitesi ortalama 4,9±2,6 iken, fibrozis evresi otalama 1,7±1,5 olarak saptandı. Hastaların %8,5'inde hem HCV-RNA hem de HBV-DNA pozitifti ve bu hastaların %85,7'sinde HCV enfeksiyonu dominant olarak bulundu. Hastalardan 24 tanesine peg IFN + ribavirin tedavisi başlandı ve bu hastalarda kalıcı viral yanıt oranı %70,8 olarak saptandı. Hastalarının %33,3'ünde HBV reaktivasyon gelişti. Oral antiviral tedavi başlanan hastaların tümünde HBV DNA negatifleşti.
Sonuç: Hem HCV RNA hem de HBV DNA'nın pozitif olduğu durumlarda, HCV baskıntı. Bu durum özellikle hemodiyaliz alan hastalarda belirgindi.
Anahtar Kelimeler: Hepatit B virüs, hepatit C virüs, ko-enfeksion, epidemiyoloji, tedavi

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Introduction

Hepatitis B virus (HBV) and hepatitis C (HCV) virus infections are among the most common causes of advanced chronic liver disease worldwide. Patients co-infected with HBV and HCV have higher rates of progression, faster fibrosis, more severe liver disease, and are at a significantly increased risk of developing hepatocellular carcinoma (HCC) compared to those mono-infected with HBV or HCV (1,2,3). Co-infection with HBV/HCV is rare and epidemiology is not fully defined (4,5). It is estimated that there are about 7-20 million co-infected patients worldwide (6). According to study reports, approximately 5-7% of HBV-infected patients also were positive for anti-HCV and 2-10% of chronic hepatitis C virus (CHC) patients were positive for HBsAg (4,7). In one Turkish Study, 10.165 hepatitis cases were evaluated in 10 hospitals and the co-infection rate was found to be 974/100.000 (8). Combined chronic infection with HBV and HCV is common in areas endemic for either viruses. HCV superinfection in patients with chronic HBV infection is one of the most common clinical conditions in Asian-Pacific countries where co-infection is common (4).

Until this time, there was no standard maintenance recommendation for HBV/HCV co-infection (1,5,9). Pegylated interferon (peg IFN) and ribavirin combination therapy demonstrated similar efficacy in suppressing HCV RNA in co-infected and HCV mono-infection cases. However, re-activation of HBV during therapy is an important question (3,4,5,10).

This retrospective, multicenter study aimed to investigate the epidemiological, virological, clinical, and histopathological characteristics and treatment results and the change in the status of HBV or HCV infection following treatment in 82 co-infected patients with HBV/HCV in Turkey.

Materials and Methods

Study population and data collection

In this study, four university hospitals retrospectively evaluated a total of 82 patients aged 18 years and over with HBV/HCV co-infection. Ethical approval was not required as the study was a retrospective study. This study included patients who were followed-up between 1998 and 2012 and with regular records. The HBV/HCV co-infected patients were diagnosed by serum HBsAg, antibodies to HCV, detectable serum HCV RNA and/or HBV DNA, and compensated liver disease. Data was collected using case records from the doctors in charge in the hospitals involved. We obtained baseline clinical and virological characteristics and results of treatment with the help of retrospective review of medical records, and available histological data before treatment were also recorded. Participant's demographic data, year of diagnosis, the possible transmission routes of viruses, alanine aminotransferase (ALT) levels, markers of hepatitis, results of HCV RNA and HBV DNA tests and the liver biopsy, and treatment results were all evaluated. We analyzed the epidemiological, virological, clinical, and histopathological data and the results of treatment in patients co-infected with HBV and HCV.

Laboratory tests were performed at each hospital. ALT tests were performed with automatic devices. Serological markers (anti-HCV, HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc) were tested with different enzyme immunoassay kits. HBV DNA and HCV RNA were investigated by using real-time polymerase chain reaction (RT-PCR) with different kits [Cobas Ampliprep/Cobas TaqMan HBV/HCV assay (Roche, Molecular System, Pleasanton, CA], Abbott RealTime HBV/HCV assay (Abbott Diagnostics, Chicago, IL), HBV/HCV QS-RGQ (Qiagen, Hilden, Germany). All results were converted to IU/mL. Liver biopsy specimens were scored according to the Ishak's Scoring System (11). The mean grade of necroinflammation and the stage of fibrosis were evaluated. The HCV genotype was determined by different methods [sequence analysis (Pyromark Qiagen-Germany), RT-PCR Fluorion HCV genotyping 1.0 (Iontek AŞ, İstanbul, Turkey)] in patients receiving HCV treatment.

Definition, treatment and evaluation of the treatment responses

Peg IFN-α 2a plus ribavirin or peg IFN-α 2b plus ribavirin treatment was given to HCV dominant patients. Current guidelines were used to determine dose modification and initial dosage of treatment drug (12,13). Patients with genotype 1 or genotype 4 were treated for 48 weeks. All subjects were followed up at least for 24 weeks after cessation of therapy. Responses to therapy were defined according to the American Association for the Study of Liver Diseases guideline (12). The categories and severity of adverse events were registered. HBV DNA was measured at baseline, at week 24 and at week 48 during therapy in all patients. We evaluated the results of patients receiving different treatments for HBV. All patients continued with the antiviral therapy and were followed up at three to six month intervals.

Statistical Analysis

We expressed the clinical and biochemical characteristics of the patients as mean ± standard deviation. Statistical analyses were performed with the Mann-Whitney U test and chi-square test. A p value of less than 0.05 was considered statistically significant. The SPSS (version 16.0) software package was used for statistical analysis.

Results

Demographics and baseline characteristics

The evaluated data were collected from four different academic hospitals in four different regions. 52.4% of patients were female and the mean age of the patients was 44.3±14.7 years. The regional distribution of co-infection was as follows: 41.5% in the Central Anatolian Region, 37.8% in the Southeast Region, 18.3% in the Black Sea Region, and 2.4% in the Marmara Region. Dental therapy, any surgical procedure, hemodialysis and blood transfusion were the major risk factors. Patients' characteristics are summarized in Table 1.

The mean ALT level was 47.8±39.0 IU/L. HBeAg was positive in 14.6% of patients. Of the 82 patients with co-infection, 36 (43.9%) were HCV RNA-positive, while 46 (56.1%) were HBV DNA-positive. HCV RNA levels were found to be between 170 and 1.62×10^7 IU/mL and the average HCV RNA level was found to be $1.36 \times 10^6 \pm 3.06 \times 10^6$ IU/mL. Twenty-eight of the 36 patients (77.8%) had a HCV RNA level of less than 600.000 IU/mL. HBV DNA levels were between 50 and 1.70×10^8 IU/mL and the average HBV DNA level was found to be $1.55 \times 10^7 \pm 4.83 \times 10^7$ IU/mL. Twenty-two of the 46 patients (47.8%) had a HBV DNA level of less than 2.000 IU/mL. Liver biopsy was performed in 39 patients.

The mean stage of fibrosis was 1.7±1.5, and the mean grade of necroinflamation was 4.9±2.6 on histopathology.

Both HCV-RNA and HBV-DNA were positive in 8.5% of patients (n=7) and HCV infection was dominant in 85.7% of cases. In the HCV RNA and HBV DNA-positive group, 5 patients had high ALT levels. HCV RNA levels were in the range of 1139-2.40x10^6 (average: 6.24x10^5±8.38x10^5) IU/mL in 7 patients. HBV DNA levels were 211-5.60x10^6 (average: 8.09x10^5±2.12x10^6) IU/mL in these patients. In 1 patient, HBV DNA level was 5.607.835 IU/mL with low level of HCV RNA (1139 IU/mL). Hemodialysis was the most important risk factor in 2 of s7 cases (28.6%). Serum HCV RNA levels were compared between patients with and without detectable serum HBV DNA. HCV RNA levels were higher in patients with detectable HBV DNA (6.24x10^5±8.38x10^5 IU/mL) than in patients without detectable HBV DNA (1.53x10^3±3.38x10^6 IU/mL). We did not find any statistical significance (p=0.969).

Table 1. Characteristics of eighty two patients co-infected with hepatitis B virus/hepatitis C virus

The mean age, years*	44.3±14.7
Sex, female/male n (%)	43 (52.4)/39 (47.6)
Distribution of patients n (%)	
Central Anatolian Region	41.5
Southeast Region	37.8
Black Sea Region	18.3
Marmara Region	2.4
Risk factors n (%)	
Surgical procedure + dental therapy	14 (17.1)
Dental therapy	12 (14.6)
Surgical procedure	10 (12.2)
Hemodialysis	7 (8.5)
Surgical procedure + dental therapy + blood transfusion	5 (6.1)
Blood transfusion	3 (3.7)
Dental therapy + blood transfusion	3 (3.7)
Dental therapy + suspected sexual transmission	2 (2.4)
Surgical procedure + blood transfusion	1 (1.2)
Surgical procedure + hepatitis in the family	1 (1.2)
Hepatitis in the family	1 (1.2)
Suspected sexual transmission	1 (1.2)
Not found	22 (26.9)
Mean ALT level, IU/L*	47.8±30.0
Virological characteristics	
HCV RNA positivity n (%)	36 (43.9)
Mean HCV RNA level, IU/mL*	1.36x10^6±3.06x10^6
HBV DNA positivity n (%)	46 (56.1)
Mean HBV DNA level, IU/mL*	1.55x10^7±4.83x10^7
HBV DNA and HCV RNA positivity n (%)	7 (8.5)
Histopathological results (n=39)*	
Mean grade of necroinflammation	4.9±2.6
Mean stage of fibrosis	1.7±1.2

*Mean ± standard deviation, ALT: Alanine aminotransferase, HBV: Hepatitis B virus, HCV: Hepatitis C virus

Treatment efficacy

Thirty-four patients had received treatment and in 25 of these, liver biopsy was performed. Peg IFN plus ribavirin therapy was given to 27 patients with dominant HCV infection (peg IFN-α 2a in 12 patients and peg IFN-α 2b in 15 patients). Among patients who received peg IFN plus ribavirin therapy, 25 had HCV genotype 1 and 2, HCV genotype 4. Different treatment regimens were given to 7 patients with dominant HBV infection.

The mean ALT level was 73.4±53.8 IU/L and the mean HCV RNA level was 1.43x10^6±3.16x10^6 IU/mL in group receiving peg IFN plus ribavirin therapy. HCV RNA and HBV DNA were positive in 3 patients. Two of the 3 patients had a baseline HBV DNA level of less than 2000 IU/mL (292 and 403 IU/mL) and the HBV DNA level was 2400 IU/mL in 1 patient. Liver biopsy was performed in 18 patients, and the mean grade of necroinflammation was 5.3±1.8 while the mean stage of fibrosis was 1.8±1.3 in this group. Three patients were excluded since 1 patient could not tolerate medications and 2 were lost-to-follow-up. In 24 patients, the rate of early virologic response (EVR), end-of-treatment response (ETR), and sustained virologic response (SVR) was 83.3%, 79.2%, and, 70.8%, respectively. Relapse was observed in 3 patients (12.5%). 7 patients (29.2%) did not respond to treatment. One of the patients had EVR and two had EVR and ETR (Table 2).

Disappearance of HBV DNA occurred in 1 of 3 (33.3%) patients with positive HBV DNA at baseline. HBV DNA level of above 2000 IU/mL (2400 IU/mL) and SVR was not observed in this patient. After completion of treatment, serum HBV DNA levels remained

Table 2. Characteristics of patients treated with pegylated interferon and ribavirin

The number of patients	27
HCV genotype n (%)	
Genotype 1	25 (92.6)
Genotype 4	2 (7.4)
Mean ALT level, IU/L*	73.4±53.8
Mean HCV RNA level, IU/mL*	1.43x10^6±3.16x10^6
Histopathological results (n=18)*	
Mean grade of necroinflammation	5.3±1.8
Mean stage of fibrosis	1.8±1.3
Results of treatment n (%)**	
EVR	20 (83.3)
ETR	19 (79.2)
SVR	17 (70.8)
Nonresponder	7 (29.2)
Relapse	3 (12.5)
Disappearance of HBV DNA	1 (33.3)
Reactivation of HBV DNA	7 (33.3)

*Mean ± standard deviation, *Three patients were excluded, ALT: Alanine aminotransferase; EVR: Early virological response; ETR: End of treatment response; SVR: Sustained virological response, HCV: Hepatitis C virus, HBV: Hepatitis B virus

positive with the same baseline values in another two patients. Interestingly, 7 of 21 (33.3%) patients with negative HBV DNA at baseline had reactivation of HBV DNA at the 24th week of follow-up; this was not accompanied by significant hepatic flares. The reactivation rate of HBV DNA (50%) with HCV SVR was the same in patients without SVR (50%) (p=1). Serum ALT levels were normal in 4 patients. Serum ALT levels were elevated in 3 patients (mean value: 124±30.0 IU/L; range: 89-161 IU/L), and tenofovir disoproxil fumarate were given to these 3 patients. After three months, the serum HBV DNA of these patients became undetectable and the ALT levels returned to normal.

The mean ALT level was 94.1±46.9 IU/L and the mean HBV DNA level was found to be $4.86x10^7±8.29x10^7$ IU/mL in group receiving treatment for HBV infection. HCV RNA and HBeAg were negative in this group. In 7 patients, the mean stage of fibrosis was 2.9±1.1 and the mean grade of necroinflammation was 6.9±1.2. HBV DNA was found to be negative in all patients who had received oral antiviral therapy. HBV DNA was negative in two patients at the end of the 12th month of lamivudine therapy. HBV DNA was negative in 4 patients treated with entecavir or tenofovir on the 6th month and 12th month of treatment. None of these patients developed HBV reactivation till date. HBV DNA was positive in 1 patient treated with peg IFN alpha-2a at the 6th month, and therapy was stopped. During the treatment, HBsAg clearance and HCV RNA positivity were not observed in the patients.

During treatment with peg IFN and ribavirin, and oral antiviral agents, severe side effects were not observed. Anemia (hemoglobin level <10 g/dL) occurred in 2 patients and the ribavirin dose was reduced to 600 mg/day in these patients.

Discussion

HBV/HCV co-infection is common among persons at high endemic areas due to common transmission routes and high risk for parenteral infections (4,14). The worldwide prevalence of HBV/HCV co-infection is unknown (4,5,6,15). In an Eastern Europe study of 2200 healthy individuals randomly selected, the rate of co-infection was found to be 0.68% (16). The rate of HCV co-infection in chronic HBV patients ranges from 9% to 30%, depending on geographical area (17). The rate of HBV/HCV co-infection was found to be more common in patients over 50 years of age and increased with age in an Italian study (18). These numbers may not reflect the truth, as both were not large-scale studies and concealed infections have not been well-recognized (occult HBV infection) (19). Data related to HCV/HBV co-infection are lacking in Turkey. In one Turkish study, the co-infection rate was reported to be 974/100.000 (8). These results show that the prevalence of HBV or HCV mono-infection is higher than HBV/HCV co-infection.

Organ transplant recipients, patients with human immunodeficiency virus infection, hemodialiysis patients, and intravenous drug users are often at high risk of HBV/HCV co-infection (20). The most common type of HBV/HCV co-infection is HCV superinfection in indivudials with chronic hepatitis B virus (CHB) in areas with high prevalence of HBV infections (4,21). Dental therapy, surgical interventions, hemodialysis and blood transfusion, which are identified as risk factors in this study, reflect the epidemiological differences among the other countries. Zhang et al. (22) reported that the clinical characteristics of HBV/HCV infected patients were significantly different from those of HCV infected patients in different ways.

Epidemiological studies on viral interaction have not revealed consistent results. Some reported no interaction; others reported a sub/supra-additive or multiplicative interaction (10). Additionally, most clinical observations suggest that the interaction between both viruses is often characterized by the inhibition of HCV-mediated HBV replication (4,8). On the other hand, follow-up studies have shown that the virological patterns in co-infection cases had dynamic profiles over time and were widely divergent (4). Coffin et al. (23) has published a case report of profound suppression of CHC after superinfection with HBV and establishment of CHB. It was hypothesized that HBV infection precipitated generalized and/or virus-specific cellular immune responses that profoundly suppressed HCV replication and yet failed to inhibit progression to CHB. Without evidence for direct interference *in vitro*, HBV and HCV can replicate in the same cell (23). In our study, HCV RNA was not higher in patients without detectable serum HBV DNA. However, the difference in the number of patients may have led to this conclusion. HCV-RNA and HBV-DNA were positive in 7 patients and HCV infection was dominant in 6 of the 7 patients. Since HCV is the common cause of infection in patients receiving hemodialysis, the present finding may be due to the high prevalence of HCV hemodialysis and this is especially noticeable in hemodialysis patients (24). It has been reported by Lee et al. (25) that HCV infection suppressed the serum HBV DNA level in hemodialysis patients. In this study, it was found that HBV/HCV co-infection, in comparison with single HBV infection, did not cause more severe liver diseases or reduced patient survival in hemodialysis patients during a 10-year follow-up. For this reason, the viral interference observed in co-infected patients is probably because of indirect mechanisms mediated by innate and/or adaptive host immune responses (26).

Those infected with HBV/HCV tend to have more severe liver injury, a higher likelihood of liver cirrhosis, hepatic decompensation, and a higher incidence of HCC when compared to mono-infected patients (4,5,14). In our study, only 39 patients underwent biopsy and none of these patients had advanced fibrosis. However, the severity of fibrosis in infected patients with HBV/HCV could not be assessed due to the small sample size of the study. The follow-up period was not long enough in our patients. Therefore, evaluation of HCC development or advanced cirrhosis in HBV/HCV co-infected patients was not possible, thus, further studies are needed.

While there are well-established treatment modalities for CHB and CHC patients, currently, there is no standard treatment for patients infected with HBV/HCV. In general, the same treatment criteria should be applied to HBV/HCV patients and mono-infected patients. According to recent studies, there is no significant difference in the rapid virologic response, EVR and SVR rates between HCV monoinfected individuals with peg IFN plus ribavirin treatment and those infected with HBV/HCV. Yu et al. (27) studied combination therapy with peg IFN-α 2a and ribavirin for 24-48 weeks, based on different HCV genotypes in 50 co-infected patients, compared to a control group of HCV-mono-infected patients. The researchers found that patients with the HCV genotype 1 in the co-infection group had a higher rate of partial EVR, ETR and recurrence. However, no significant difference

was seen in the SVR rates. Kim et al. (28) treated 18 patients co-infected with HCV/HBV with combination peg IFN-α 2a and ribavirin. This study reported a SVR rate of 72% in all patients, and 60% and 87.5% in genotypes 1 and 2, respectively. In our study, the overall SVR rate was found to be 70.8% in the HBV/HCV co-infection group with genotype 1 and genotype 4. The relapse rate was 12.5%. The vast majority of Turkish patients with CHC have genotype 1 (29). Successful treatment of CHC infection may correlate with HBV reactivation and flaring (4,6,30,31). In our study, the reactivation rate of HBV infection was 33.3%, however, severe HBV flares were not observed. It was reported by Chuang et al. (32) that co-infected patients who achieved a SVR (compared HCV non-responders) were more likely to have reactivation of HBV (58.8% vs. 12.5%) or HBV flares (44.8% vs. 8.3%), and less likely to achieve HBV DNA clearance (8.3% vs. 100%). Yu et al. (27) found that the reactivation rate of HBV DNA (33.3%) with HCV SVR was significantly higher than that in patients without SVR (8.7%). In our study, the HBV DNA reactivation rate was 33.3% and there was no difference between with or without HCV SVR. Given this risk of HBV reactivation, clinicians must be cautious while treating co-infected patients with the combination of IFN and ribavirin.

There have been not any published studies regarding treatment of co-infected patients with the newer agents adefovir, entecavir and tenofovir. Marrone et al. (33) published a study of lamivudine with IFN for co-infected patients in which eight patients with dually active HBV and HCV were treated with 5 MU IFN and lamivudin (LAM) 100 mg/day for 12 months followed by LAM alone for 6 months. Three patients had clearance of HBV DNA (37.5%) and 3 had clearance of HBeAg. In addition, 4 patients (50%), persistent for 12 months post-treatment, also had clearance of HCV RNA (33). In our study, HBV DNA was found to be negative in all patients who received oral antiviral therapy, however, HBsAg clearance was not observed. HBeAg was negative in all patients receiving therapy.

It has been reported that the majority of Turkish patients with CHB were HBeAg negative (34). On the other hand, particularly in patients with HBV-dominant disease, oral antiviral agents may be useful.

For co-infected patients with double-active HBV/HCV, the addition of peg IFN-α and ribavirin oral nucleotide analogues seems to be a reasonable empirical option, but maintains optimal treatment regime uncertainty (1,4,5).

Conclusion

We found that the majority of the risk factors for HBV and HCV infected patients were; hemodialysis, blood transfusion, a surgical procedure, and dental treatment. In addition, HCV is predominant in cases where both HBV DNA and HCV RNA are positive. This was especially noticeable in hemodialysis patients in our study. At present, peg IFN plus ribavirin should be the treatment of choice in patients with dominant HCV replication. In addition, reactivation of HBV may occur after elimination of HCV, and therefore, close monitoring is recommended for both viruses even for patients with suppressed HBV DNA.

Study Limitations

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

Ethics

Informed Consent: A retrospective study.

Peer-review: Externally and Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B. A., Ö.G., O.Y., M.K.Ç., S.A., Ş.B., C.A., Concept: B.A., O.Y., Design: B.A., O.Y., Data Collection or Processing: B.A., Ö.G., O.Y., M.K.Ç., S.A., Ş.B., C.A., Analysis or Interpretation: B.A., O.Y., Literature Search: M.K.Ç., Ş.B., C.A., Writing: B.A., Ö.G., O.Y.

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Research Article

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King's Score may be More Effective in the Determination of Severe Fibrosis in Chronic Hepatitis B Infections

Kronik Hepatit B Enfeksiyonunda Şiddetli Fibrozisi Belirlemede King Skoru Daha Etkin Olabilir

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ABSTRACT

Objective: The objective of this study was to compare the performances of several noninvasive indirect biochemical markers used to detect advanced fibrosis in patients with chronic hepatitis B (CHB).

Materials and Methods: This study was retrospectively conducted in two centers, and included treatment-naive CHB patients undergoing liver needle biopsies. The following noninvasive biochemical markers were used: the aspartate aminotransferase to platelets ratio index (APRI), Fibrosis 4 (FIB-4) index, Goteborg University Cirrhosis index (GUCI), King's score, FibroQ score, aspartate aminotransferase to alanine aminotransferase ratio (AAR), Cirrhosis Discriminant Score (CDS) Bonacini, and age-platelet (AP) index.

Results: This study included a total of 255 patients (79.6% males), with a median age of 27 years (19-69). The AAR did not show a significant difference in predicting severe fibrosis according to the area under the curve (AUC) and p values (AUC=0.493, p=0.887) of the non-invasive methods. Overall, the APRI, FIB-4, GUCI, and King' score were more effective (all p values <0.001; AUC values: 0.787, 0.768, 0.775, and 0.807; respectively).

Conclusion: In our study group, the highest AUC and positive likelihood ratio (LR+) values were found using King's score. Therefore, King's score seems to be more selective in the classification of patients with severe fibrosis among CHB patients, because of its higher correct predictive value.

Keywords: Chronic hepatitis B, fibrosis, non-invasive, King's score

ÖZ

Amaç: Bu çalışmada kronik hepatit B'li (KHB) hastalarda ileri dönem fibrozisi tespit etmede kullanılan bazı non-invaziv indirekt biyokimyasal belirteçlerin performanslarının karşılaştırılması amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışma retrospektif olarak iki merkezde gerçekleştirilmiştir ve karaciğer iğne biyopsisi yapılan, naiv, KHB hastaları dahil edilmiştir. Non-invaziv biyokimyasal belirteçlerden aspartat aminotransaminaz - Platelet Ratio Index (APRI), Fibrosis 4 (FIB-4) Index, Goteborg University Cirrhosis Index (GUCI), King skoru, FibroQ skoru, aspartat aminotransaminaz - alanin aminotransaminaz ratio (AAR), Cirrhosis Discriminate Score (CDS) Bonacini, age-platelet (AP) index kullanılmıştır.

Bulgular: Çalışmaya %79,6'sı erkek olmak üzere 255 hasta dahil edilmiştir. Yaş ortanca değeri 27 (19-69) yıl olarak hesaplanmıştır. Non-invaziv yöntemlerin eğri altında kalan alan (AUC) ve p değerlerine göre AAR'nin şiddetli fibrosisi belirlemede anlamlı fark oluşturmadığı (AUC=0,493, p=0,887), APRI, FIB-4, GUCI ve King metodlarının diğerlerinden daha etkili yöntemler olduğu saptanmıştır (p değerleri hepsinde <0,001; sırasıyla, AUC değerleri: 0,787, 0,768, 0,775, 0,807).

Sonuç: Çalışma grubumuzda King skoru ile en yüksek AUC ve pozitif likelihood ratio (LR+) elde edilmiştir. Doğru tahmin değerinin daha yüksek olması nedeniyle KHB hastalarında King skorunun şiddetli fibrozis hastalarını sınıflandırmada daha seçici olduğu söylenebilir.

Anahtar Kelimeler: Kronik hepatit B, fibrozis, non-invaziv, King skoru

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Introduction

Development of fibrosis and cirrhosis in the liver plays an important role in the management of patients with chronic hepatitis B (CHB). Evaluation of fibrosis in patients with CHB is required in the determination of both the prognosis and the need for treatment (1). Fibrosis is a nonspecific response to liver damage, with the synthesis of extracellular matrix. The ideal markers of liver fibrosis should have liver-specific, non-invasive, easy to apply, and rapid features, with measurable sensitivity and reproducibility. In addition, they should have features that enable monitoring the progression or regression of fibrosis during the natural course of the disease, or while the patient is under treatment. The serum levels of these markers should not be affected by the changes in the liver, kidney, and reticuloendothelial system functions. In addition, statistically, the area under curve (AUC) value should be as close to 1.0 as possible, and the sensitivity and specificity values should be close to 100%. The AUC values of the vast majority of biochemical markers are between 0.80 and 0.85, and those markers are more helpful in distinguishing low and high fibrosis rather than staging liver damage (2).

The advantages of biochemical markers include reproducibility, easy accessibility, low costs, and applicability in patients who can be treated on an outpatient basis. However, most biochemical markers are not liver specific, and cannot distinguish moderate fibrosis. Moreover, their effectiveness is limited in conditions such as Gilbert's syndrome, hemolysis, and inflammation. Overall, the performance of biochemical markers in the evaluation of cirrhosis is lower than the physical methods used to measure liver stiffness (1). However, most non-invasive methods cannot distinguish between the early stages of fibrosis, so the goal of these methods is to determine the presence of cirrhosis. Currently, there are no biomarkers that can be used instead of a liver biopsy in the determination of advanced fibrosis. Nevertheless, these methods could provide guidance in the identification of those patients requiring biopsies (3).

The objectives of this study were to compare the performances of several noninvasive indirect biochemical markers used in the detection of advanced fibrosis in CHB patients, and to reveal the best method for the determination of biopsy indications.

Materials and Methods

Patients

This study was retrospectively performed in two centers at the second and tertiary levels. It includes treatment-naive CHB patients who presented to the Infectious diseases and clinical microbiology clinics between 01/01/2015 and 01/06/2016 and underwent liver needle biopsies. Patients with hepatitis C or hepatitis D, those with hepatitis B infections, who received antiviral therapy, and those reported to have insufficient biopsy material were excluded from the study. Patients positive for hepatitis B surface antigen, those with a normal or high alanine aminotransferase (ALT) level for six months, and those positive for hepatitis B virus (HBV) DNA were diagnosed with CHB.

Each patient's age and gender, as well as Aspartate aminotransferase (AST), ALT, international normalized ratio (INR), complete blood count, virological, and histopathological outcomes were retrospectively obtained from the patient files. This research was approved by the Etimesgut Military Hospital's Local Ethics Committee (07.07.2015-2015/21).

Liver Histology

Grading and staging of all of the liver biopsy materials were performed using the modified Ishak-modified Histologic Activity Index Grading and Staging System (4). Patients with a fibrosis score from 0 to 2 were considered to have a low level of fibrosis, while those with a score from 3 to 6 had marked fibrosis.

Non-invasive Indirect Biochemical Markers

In this study, among the noninvasive biochemical markers available, the AST-to-platelet ratio index (APRI), Fibrosis-4 (FIB-4) index, Goteborg University Cirrhosis Index (GUCI), King's score, FibroQ score, AST to ALT ratio (AAR), Bonacini Cirrhosis Discriminant Score (CDS), and age-platelet index (AP) were used (5,6,7,8,9,10,11,12). The formulas for these methods are shown in Table 1.

The following cut-off values for the absence of fibrosis have been reported by the researchers who formulated these methods:

Table 1. Formulas of non-invasive methods

Methods	**Formula**							
APRI	(AST, upper limit of normal) / platelet count (10^9/ L) x 100							
FIB-4	Age (year) x AST (U/L) / platelet count (10^9/L) x ALT (U/L)$^{1/2}$							
GUCI	Normalized AST (U/L) x INR x 100 / platelet count (10^9/L)							
King	Age (years) x AST (IU/L) x INR / platelet count (10^9/L)							
FibroQ	[10 x age (years) x AST x INR)/ (platelet count (10^9/L) x ALT]							
AAR	AST (U/L) /ALT (U/L)							
CDS								
	Parametes	**0**	**1**	**2**	**3**	**4**	**5**	**6**
	INR	<1.1	1.1-1.4	>1.4				
	ALT/AST ratio	>1.7	1.7-1.2	1.19-0.6	<0.6			
	Platelet count (x1000/mm^3)	>340	340-280	279-220	219-160	159-100	99-40	<40
AP								
	Age (year)	<30	30-39	40-49	50-59	60-69	>70	
	Platelet count (x1000/mm^3)	>225	200-224	175-199	150-174	125-149	<125	

APRI: Aspartate aminotransferase to platelets ratio, FIB-4: Fibrosis-4 index, GUCI: Goteborg University Cirrhosis Index, AAR: Aspartate aminotransferase- alanine aminotransferase ratio, CDS: Cirrhosis discriminant score, AP-Index: Age-platelet index, INR: International normalised ratio, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

APRI ≤0.5 (5), FIB-4 <1.45 (6), GUCI <0.2 (7), King's score ≥12.3 (8), and FibroQ score <1.6 (9). For marked fibrosis, the following cut-off values were determined by the same researchers: >1.5, >3.25, ≥1.0, ≥16.7, and ≥1.6 for the APRI, FIB-4, GUCI, King's score, and FibroQ score, respectively. The rest of the cut-off values were found in other studies, as follows: AAR ≥1 (10), CDS ≥8 (11), and AP index ≥6 (12).

Statistical Analysis

Statistical analysis was performed using SPSS IBM 22.0 (SPSS Inc., Chicago, IL, USA). The normal distribution of the variables was determined using the Kolmogorov-Smirnov test. Because of their non-normal distribution, the continuous variables were obtained as the median (min-max). The categorical variables were presented as the frequency and percentage.

The AUC was found using a receiver operating characteristics analysis in order to determine the effectiveness of the methods used in showing marked fibrosis. An AUC of ≤0.5 was evaluated as "the test has no diagnostic value". The highest cut-off value giving the sum of the sensitivity and specificity-1 values was found in order to estimate the best compliance between the sensitivity and specificity. The performances of these methods in the determination of fibrosis were evaluated by first using the cut-off values found in this study, and then the cut-off values determined by the researchers who developed the formulas. The sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), diagnostic accuracy (DA), positive likelihood ratio (LR+), and negative likelihood ratio (LR-) were calculated. A p value of less than 0.05 was considered statistically significant. .

Results

This study included a total of 255 patients (79.6% males) with a median age of 27 (19-69) years. And a median HBV DNA value of 72,000 (0-9.9x10^9) IU/mL. Of the 255 patients, 57.3% were negative for the hepatitis B e antigen (HBeAg). The patients' laboratory and histopathological data and noninvasive test outcomes are shown in Table 2.

When the outcomes of the noninvasive biochemical methods were evaluated, the AUC value for determining significant fibrosis was highest with King's score (Figure 1). However, it was seen that the AUC value of the AAR was the lowest (AUC=0.493), and did not produce a significant difference in the determination of severe fibrosis (p=0.887) (Figure 2). In addition, this method was found to be more unsuccessful in the detection of severe fibrosis, according to the cut-off value, than the other methods. According to the other cut-off values of this study, similar values were obtained using the APRI, GUCI, FIB-4, and King's score, which were more efficient in the detection of fibrosis than the other methods (Table 3).

When the performances of the methods used in this study were evaluated with the cut-off values reported in the literature, there were no scores ≥1 with the GUCI method or >8 with the CDS method. In addition, the specificity, PPV, DA, and LR+ values were higher, and the sensitivity and NPV values were lower than our values (Table 3).

Discussion

Certain parameters (such as the AST, ALT, platelet count, and PT/INR) that show the changes in liver function can be detected with routine blood testing (1). There are multiple causes of thrombocytopenia in chronic liver disease. Splenic sequestration of platelets, suppression of their production in the bone marrow, or a decrease in the hematopoietic growth factor (thrombopoietin) activity can reduce the platelet count (13). In advanced liver disease, an increase in AST level is due to a decrease in the clearance or increase in the release of AST as a result of mitochondrial damage. Prothrombin time reflects the synthesis function of the liver, and is one of the earliest markers of cirrhosis (2). In addition to these parameters, the patient's demographic features can also be markers of liver damage. For instance, the severity of fibrosis increases with advanced age, especially in patients negative for HBeAg (14).

In this study, AST, platelet count, age, and INR were found to be more successful in the detection of severe fibrosis. Moreover,

Table 2. Characteristics of patients with chronic hepatitis B (n=225)

Variables	Median	Minimum-Maximum
Age (year)	27	19-69
Sex		
Male (n/%)	179/79.6	
Female (n/%)	46/20.4	
HBeAg negative (n/%)	129/57.3	
HBV DNA (IU/mL)	72000	0-9.9x10^9
Platelet count (10^3/µL)	234	80-431
INR	1.04	0.8-1.46
AST (U/L)	32	11-503
ALT (U/L)	52	10-1053
APRI	0.41	0.11-7.38
FIB-4	0.57	0.10-6.21
GUCI	0.22	0.13-0.67
King	4.4	1.31-156.12
FibroQ	0.84	0.07-6.59
AAR	0.65	0.08-2.66
CDS	3	0-8
AP	1	0-9
Fibrosis scores	1	0-5
F0-2 (n/%)	185/82.2	
F3-6 (n/%)	40/17.8	
HAI scores	5	0-15
G0-7 (n/%)	186/82.7	
G7-18 (n/%)	39/17.3	

HBeAg: Hepatitis B e antigen, INR: International normalised ratio, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, APRI: Aspartate aminotransferase to platelets ratio, FIB-4: Fibrosis-4 index, GUCI: Goteborg University Cirrhosis Index, AAR: Aspartate aminotransferase - alanine aminotransferase ratio, CDS: Cirrhosis discriminant score, AP: Age-platelet index; HAI: Histological activity index

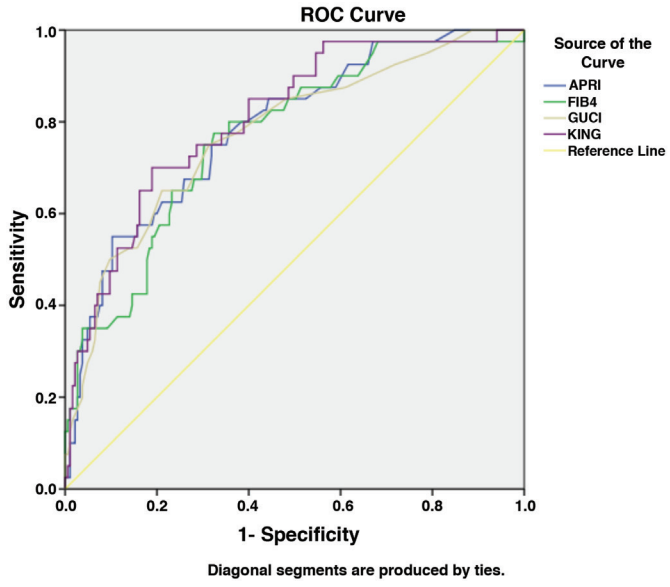

Figure 1. Receiver operating characteristics curves for aspartate aminotransferase to platelets ratio, Fibrosis-4 index, Goteborg University Cirrhosis Index, and King's score for severe fibrosis. King's score had a significantly higher area under the curve than others for the determination of severe fibrosis (F3-6)

ROC: Receiver operating characteristics, APRI: Aspartate aminotransferase to platelets ratio, FIB-4: Fibrosis-4 index, GUCI: Goteborg University Cirrhosis Index

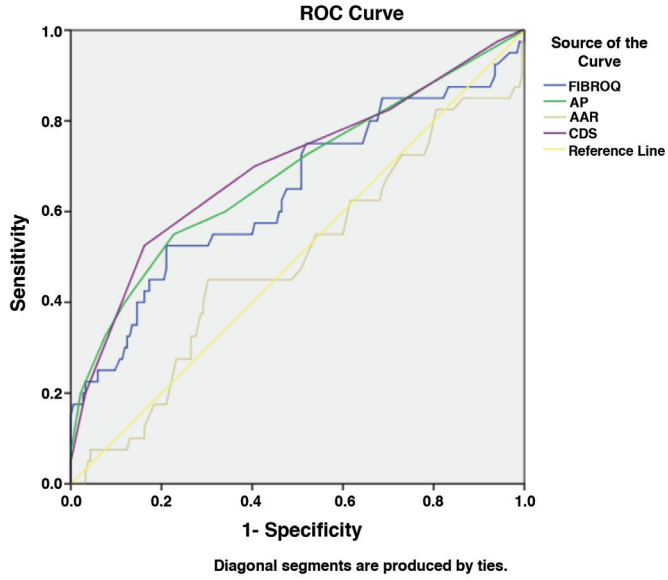

Figure 2. Receiver operating characteristics curves for FIBROQ, age-platelet, aspartate aminotransferase- alanine aminotransferase ratio, and cirrhosis discriminant score for severe fibrosis. The area under the curve value of the aspartate aminotransferase- alanine aminotransferase ratio was the lowest and did not produce a significant difference in the determination of severe fibrosis (F3-6)

ROC: Receiver operating characteristics, AP: Age-platelet index, AAR: Aspartate aminotransferase - alanine aminotransferase ratio, CDS: Cirrhosis discriminant score

Table 3. Performance of the non-invasive methods in detecting significant fibrosis (F3-6)

Methods	Cut-off values	AUC (95% CI)	p value	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	DA (%)	LR+	LR-
APRI	0.47*	0.787 (0.710-0.863)	<0.001	72	68	92	33	69	2.3	0.4
	≤0.5			67.5	73.5	91.3	35.5	72	2.5	0.4
	>1.5			30	96.2	86.1	63.2	84	7.9	0.7
FIB-4	0.73*	0.768 (0.688-0.849)	<0.001	75	70	92.8	34.9	71	2.5	0.4
	<1.45			35	91.9	86.7	48.3	82	4.6	0.7
	>3.25			10	100	84.1	100	84	NA	0.9
GUCI	0.24*	0.775 (0.693-0.857)	<0.001	75	69	92.7	34.1	70	2.4	0.4
	>0.2			92.5	28.1	94.5	21.8	40	1.4	0.3
	≥1			NA	NA	NA	NA	NA	NA	NA
King	5.76*	0.807 (0.734-0.880)	<0.001	73	73	92.4	36.3	72	2.6	0.4
	≥12.3			42.5	91.9	88.1	53.1	83	5.6	0.6
	≥16.7			37.5	93.5	87.4	55.6	84	5.8	0.7
FibroQ	0.915*	0.643 (0.537-0.748)	0.005	57.5	57.3	86.2	22.5	57	1.4	0.7
	≥1.6			42.5	82.7	86.9	34.7	76	2.6	0.7
AAR	0.6*	0.493 (0.389-0.597)	0.887	52.5	47	82.1	17.6	48	1	1
	≥1			10	83.8	81.2	11.8	71	0.7	1.1
CDS	3*	0.700 (0.600-0.799)	<0.001	70	59.5	90.2	27.2	61	1.7	0.5
	≥8			NA	NA	NA	NA	NA	NA	NA
AP	0.5*	0.683 (0.581-0.784)	<0.001	72.5	48.1	89	23.2	52	1.4	0.6
	≥6			20	97.8	85	66.7	84	20	0.9

AUC: Area under the curve, CI: Confidence interval, NPV: Negative predictive value, PPV: Positive predictive value, DA: Diagnostic accuracy, LR+: Positive likelihood ratio, LR-: Negative likelihood ratio, APRI: Aspartate aminotransferase to platelets ratio, FIB-4: Fibrosis-4 index, GUCI: Goteborg University Cirrhosis Index, AAR: Aspartate aminotransferase - alanine aminotransferase ratio, CDS: Cirrhosis discriminant score, AP: Age-platelet index, NA: Not available, * our cut-off value, others are the literateurs' cut-off

NPV, PPV, sensitivity, specificity, and LR+ values were higher in the APRI, FIB-4, GUCI and King's score methods than in the other methods. The NPV value >90% and the very low PPV level found in this study support the fact that these methods can be used effectively in the identification of truly healthy persons. This can be explained by the greater number of patients with mild fibrosis being included in this study. As with other studies, the method selectivity increased as the cut-off value used increased (5,6,7,8).

The APRI, FIB-4, GUCI, and King's score have also been used by other researchers in the determination of fibrosis in patients with chronic hepatitis. In the APRI method, the AUC was in the range of 0.66-0.73 (15,16,17,18). The cut-off value was ≤0.5, sensitivity was 33%, specificity was 91%, PPV was 90%, and NPV was 37% in this method in the determination of severe fibrosis in CHB patients (15). However, as was the case in our study, if the majority of the study group consists of patients with mild fibrosis, with the same cut-off value the first three rates drop to 62.5%, 51.6%, and 25%, respectively, while the NPV raises to 84.2% (17). In the study groups where fibrosis was predominant, the capacity to select the true patients was increased with a cut-off value of 0.535, while the sensitivity, specificity, PPV, and NPV values were 73.2%, 59.4%, 69.8%, and 63.3%, respectively (18).

In particular, the degree of fibrosis in the study group affects the performance of the FIB-4 method. In their study that predominantly included patients with severe fibrosis, Ucar et al. (18) calculated the sensitivity as 70.7% and the specificity as 62.5% using the FIB-4 method, however, our study values were higher. Erdogan et al. (19) found a specificity of 58.8% in their study group, which was similar to our result. Since the AUC was higher in the present research, our sensitivity and specificity values were at a more acceptable level. The closer the AUC value is to 1, the higher the predictive value of the test (20).

Unlike the other methods, it is possible to evaluate the synthesis function of the liver using the GUCI, which includes prothrombin time in the calculations. In our study, the AUC and the other performance characteristics of this test were higher than the results reported by Erdogan et al. (19).

With regard to the King's score, the factor of age is added to the calculations. In a study using this method in CHB patients, the AUC was 0.770, sensitivity was 60%, specificity was 83%, PPV was 66%, and NPV was 76%. In that study, the King's score was reported to show the best performance in detecting fibrosis, when compared with the APRI, CDS, and AP. In the same study, similar to that in the CHB patients, the AUC was 0.783, sensitivity was 61%, specificity was 84%, PPV was 75%, and NPV was 72% in chronic hepatitis C (CHC) patients. However, the performance of King's score in the CHC patients was not found to be superior, as it was in CHB patients (21). In another study conducted with CHC patients, more successful results were obtained with the King's scores than the other methods in terms of the AUC, specificity, PPV, and LR+ values (22).

To the best of our knowledge, evaluation of the King's score method, which is not frequently used in CHB patients, and determination of its performance (more successful) is a distinctive feature of our study. However, since CHC patients were not included, we could not compare the effectiveness of this method with that in CHB patients. Another limitation of our study was that cirrhotic patients were not included in the study group. This was not due to a selective approach, but rather, because no cirrhotic patients were encountered during the study period. Moreover, since the study was performed in two centers, evaluation of fibrosis by two different pathologists may also be a limitation.

Conclusion

The highest AUC and LR+ values were obtained with the King's score in our study group, however, no significant differences were observed in terms of the other performances. When comparing our results with those of other studies King's score was found to perform superior to or similar with the other methods. It can be said that the King's score is more selective in classifying CHB patients with severe fibrosis, because the correct predictive value of this method is higher. Performing a similar study comparing cirrhotic patients may improve the performance of this method, because it would better demonstrate the overall effectiveness of the noninvasive methods.

Ethics

Ethics Committee Approval: This research was approved by the Etimesgut Military Hospital's Local Ethics Committee (07.07.2015-2015/21), **Informed Consent:** The Declaration of Helsinki and Good Clinical Practice Guidelines were respected during the entire process of enrolling the patients in the study and collecting/analyzing/ reporting the data.

Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Concept: Z.K., Design: Z.K., Data Collection or Processing: Z.K., Ö.A., F.Y.K., Analysis or Interpretation: Z.K., Literature Search: Z.K., Writing: Z.K.

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Research Article

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Frequency of Hepatitis B Virus, Hepatitis C Virus and HIV Infections in Cannabis and Opioid Addicts

Esrar ve Opiyat Bağımlılarında Hepatit B Virüsü, Hepatit C Virüsü ve HIV Enfeksiyonları Sıklığı

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ABSTRACT

Objective: There are very few data about the epidemiology of hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV infections in drug addicts in Turkey, whereas several countries have a developed surveillance systems to monitor the spread of HBV, HCV and HIV infections in drug users. In this study, HBV, HCV and HIV prevalence in cannabis and opioid addicts were investigated.

Materials and Methods: Hepatitis B surface antigen (HBsAg), anti-HBs, anti-HCV and anti-HIV tests were analyzed by enzyme-linked immunosorbent assay. The cannabis and opioid metabolites in urine samples of drug addicts were analyzed by cloned enzyme donor immunoassay.

Results: This retrospective study was conducted on 276 individuals with a mean age of 28.89±10.49 years. HBsAg, anti-HBs and anti-HCV prevalence in drug addicts was found to be 4%, 52.3% and 7.9%, respectively. In all the drug addicts, anti-HIV test was negative. Whereas the rate of HBsAg among cannabis users (8.8%) was higher than opioid (4.1%) and both cannabis and opioid users (1.4%), the difference was not statistically significant. Although anti-HCV positivity among cannabis users was not detected, 6.4% of opioid users and 15.9% of both cannabis and opioid users were anti-HCV positive (p=0.009).

Conclusion: This study showed that HCV infection among especially opioid users and both cannabis and opioid users was a problem. Understanding of local status in HBV, HCV and HIV infections is crucial for developing prevention and geographical strategies for these infections.

Keywords: Drug addicts, cannabis, opioid, hepatitis B virus, hepatitis C virus, HIV

ÖZ

Amaç: Bazı ülkelerde uyuşturucu kullanıcıları arasında hepatit B virüs (HBV), hepatit C virüs (HCV) ve HIV enfeksiyonlarının yayılmasını izlemek için gelişmiş bir sürveyans sistemi mevcutken, Türkiye'de uyuşturucu bağımlılarında HBV, HCV ve HIV enfeksiyonlarının epidemiyolojisi hakkında çok az veri mevcuttur. Bu çalışmanın amacı, esrar ve opiyat bağımlıları arasında HBV, HCV ve HIV sıklığını değerlendirmektir.

Gereç ve Yöntemler: Hepatit B yüzey antijeni (HBsAg), anti-HBs, anti-HCV ve anti-HIV testleri, Enzyme-Linked Immunosorbent Assay tekniği ile analiz edildi. Uyuşturucu bağımlılarının idrar örneklerinde esrar ve opiyat metabolitleri, klonlanmış enzim donör immünoassay tekniği ile analiz edildi.

Bulgular: Bu retrospektif çalışma, yaş ortalaması 28,89±10,49 olan 276 olgu üzerinde gerçekleştirildi. Uyuşturucu bağımlılarında HBsAg, anti-HBs ve anti-HCV sıklığı sırasıyla %4, %52,3 ve %7,9 olarak tespit edildi. Tüm uyuşturucu bağımlılarında, anti-HIV testi negatifti. Esrar kullanıcıları arasında HBsAg sıklığı (%8,8), opiyat (%4,1) ve hem esrar hem de opiyat kullanıcılardan (%1,4) daha yüksek iken, bu fark istatistiksel olarak anlamlı değildi. Esrar kullanıcılarında anti-HCV pozitifliği tespit edilmemesine rağmen, opiyat kullanıcılarının %6,4'ü hem esrar hem de opiyat kullanıcılarının %15,9'u anti-HCV pozitifti (p=0,009).

Sonuç: Bu çalışma özellikle opiyat kullanıcıları ile hem esrar hem de opiyat kullanıcıları arasında HCV enfeksiyonunun sorun olduğunu göstermiştir. HBV, HCV ve HIV enfeksiyonlarında lokal durumun anlaşılması, bu enfeksiyonların önlenmesi ve coğrafi olarak stratejilerin geliştirilmesi için önemlidir.

Anahtar Kelimeler: Uyuşturucu bağımlıları, esrar, opiyat, hepatit B virüs, hepatit C virüs, HIV

Karabulut N, Catak Z. Frequency of Hepatitis B Virus, Hepatitis C Virus and Human Immunodeficiency Virus Infections in Cannabis and Opioid Addicts. Viral Hepat J. 2017;23:26-29.

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Introduction

It is estimated that the number of cannabis and opioid users worldwide are 161 million and 16 million, respectively. Cannabis is the most consumed drug in the world (1). Cannabis or marijuana contains the psychoactive chemical delta-9-tetrahydrocannabinol, which is believed to be the main chemical component that produces the psychoactive effect. Cannabis is often used through smoking in hand-rolled cigarettes, pipes, or water pipes (2). Opioids contain hydrocodone, oxycodone, morphine, and codeine, and the illegal opioid, heroin. Heroin is a powerful opiate drug, and used through injecting, smoking, or snorting (3). Hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV prevalence in the world varies depending on geographical location. Turkey is a medium endemic country for both HBV (2%-8%) and HCV (1%-2%). The prevalence of HIV infection in Turkey varies from region to region but still seems low (1,4). On the other hand, since drug addicts show risky behaviors, including unsafe sex and risky injection practices, it is believed that there is a strong link between the spread of infectious diseases and drug abuse. The most important factor increasing their risk of HBV, HCV and HIV transmission is sharing drug preparation or injecting equipment (5). 50%-80% of HCV infection in the developed countries occurs among injection drug users (6). HCV infection is the most common blood-borne infection among drug addicts in the developed countries. HIV, less common than HCV, can also be transmitted during unprotected sex. In several studies, high HBV, HCV and HIV prevalence was described among drug users in the several countries (7,8,9,10). However, there is not enough knowledge about the epidemiology of these infections in drug addicts in Turkey, whereas several countries have a developed surveillance systems to monitor the spread of HBV, HCV and HIV infections in drug users. Therefore, in this study, it was aimed to investigate the frequency of HBV, HCV and HIV infections in cannabis and opioid addicts admitted to the drug addiction treatment center at Elazığ Mental Health Hospital.

Materials and Methods

Subjects

This retrospective study included 276 drug addicts in Drug Addiction Treatment Centre at Elazığ Mental Health Hospital in 2015. There is a drug addiction treatment center providing medical care and social support for drug addicts in the hospital. This study was approved by Fırat University Ethics Committee (08/11/26.04.2016).

Serological Analysis

Blood samples collected from the drug users were analyzed in the center clinical laboratory of the hospital. Hepatitis B surface antigen (HBsAg), anti-HBs, anti-HCV (GBC, Taiwan, ROC) and anti-HIV (DIA.PRO, Milano, Italy) tests were performed by enzyme-linked immunosorbent assay using the Triturus analyzer (Grifols, Parets del Valles, Spain). Each study included both positive and negative controls. For HBsAg, anti-HCV and anti-HIV parameters, samples with a cut-off value of <1 were considered negative and samples with a cut-off value of ≥1 were considered positive. For anti-HBs, samples with <10 mIU/mL were considered negative and samples with ≥10 mIU/mL were considered positive. The samples detected to be positive in the first run were tested again. For samples which were repeatedly positive for HIV, the results were confirmed by the Western blot test.

Drug Analysis

The cannabis and opioid metabolites in urine samples of drug addicts were analyzed in the center clinical laboratory of the hospital. All tests for drug detection (CEDIA, Fremont, USA) were performed by cloned enzyme donor immunoassay, using the Roche Hitachi Modular P800 analyzer (Diamond Diagnostics, Holliston, USA).

Statistical Analysis

Statistical analyses were performed by SPSS 20 (SPSS Inc, Chicago, USA). The differences in results between two groups were assessed by the chi-square test. The Kruskal-Wallis test was performed to compare hepatitis parameters between cannabis, opioid and mixed drug users. A p value of less than 0.05 was considered statistically significant.

Results

All the 276 drug addicts in the study were male and the mean age of the subjects was 28.89±10.49 years (range: 18-68 years). As shown in Table 1, 34 and 173 drug addicts who were admitted drug addiction treatment center have used only cannabis and only opioid, respectively. The number of mixed drugs users in whom both cannabis and opioid metabolites were detected in the urine samples was 69. 55.9% of cannabis users, 53.8% of opioid users and 47.8% of both cannabis and opioid users were in the 20-29 age group (Figure 1).

In drug addicts, HBsAg, anti-HBs and anti-HCV rates were 4%, 52.3% and 7.9%, respectively. Anti-HIV test was negative in all the drug addicts. HBV and HCV rates among cannabis, opioid and mixed drugs users are shown in Table 2. Whereas the rate of HBsAg among cannabis users (8.8%) was higher than opioid (4.1%) and mixed drugs users (1.4%), this difference was not

Table 1. Drug use distribution

Drug use	The number of subject	Percentage (%)
Cannabis only	34	12.3
Opioid only	173	62.7
Mixed drugs	69	25.0

Mixed drugs: Both cannabis and opioid users

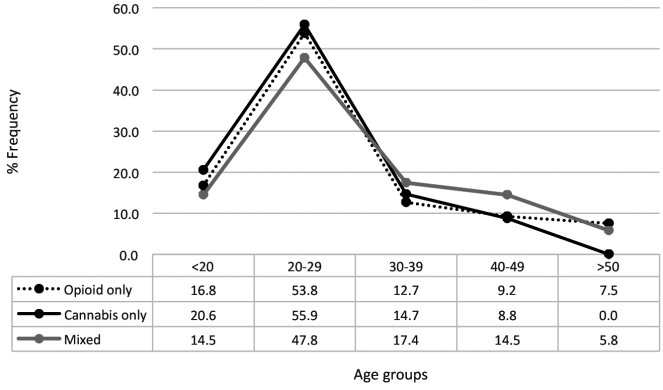
Table 2. The frequency of hepatitis B surface antigen, anti-HBs and anti-hepatitis C virus among drug users

	Overall n (%)	Cannabis only n (%)	Opioid only n (%)	Mixed drugs n (%)	p
HBsAg positive	11 (4)	3 (8.8)	7 (4.1)	1 (1.4)	0.200
Anti-HBs positive	145 (52.3)	18 (52.9)	91 (52.6)	36 (52.2)	0.997
Anti-HCV positive	22 (7.9)	0	11 (6.4)	11 (15.9)	0.009

Mixed drugs: both cannabis and opioid users, HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C virus

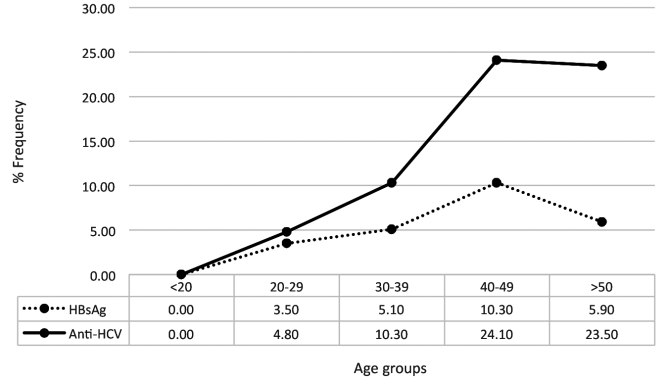
statistically significant. The rate of anti-HBs among cannabis, opioid and mixed drugs users was similar (52.9%, 52.6% and 52.2%, respectively). Whereas cannabis users were anti-HCV-negative, 6.4% of opioid users and 15.9% of mixed drugs users were anti-HCV positive (p=0.009).

The highest HBsAg seropositivity was detected in the 40-49 age group while the highest anti-HCV seropositivity was determined in the 40-49 group and in those older than 50 years of age. Seropositivity of HBsAg and anti-HCV was not detected in the <20 age group (Figure 2). The frequency of anti-HBs was found to be 80.4% in the <20 age group (Figure 3).



	<20	20-29	30-39	40-49	>50
Opioid only	16.8	53.8	12.7	9.2	7.5
Cannabis only	20.6	55.9	14.7	8.8	0.0
Mixed	14.5	47.8	17.4	14.5	5.8

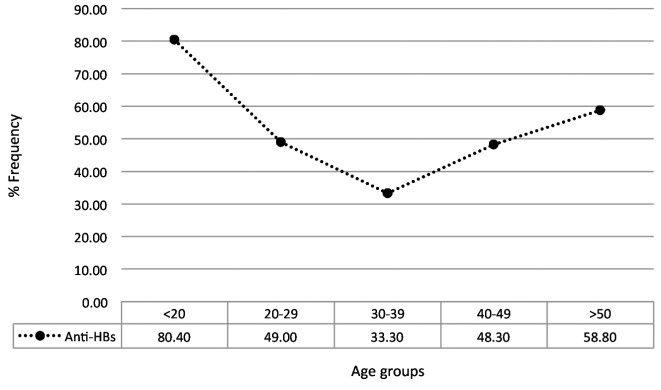
Figure 1. The distribution of according to age groups of drug users



	<20	20-29	30-39	40-49	>50
HBsAg	0.00	3.50	5.10	10.30	5.90
Anti-HCV	0.00	4.80	10.30	24.10	23.50

Figure 2. The frequency of hepatitis B surface antigen and anti-HCV in age groups

HBsAg: Hepatitis B surface antigen



	<20	20-29	30-39	40-49	>50
Anti-HBs	80.40	49.00	33.30	48.30	58.80

Figure 3. The frequency of anti-HBs in age groups

Discussion

HBV, HCV and HIV infections are a serious problem among drug addicts and remain an important public health issue. The high HBV, HCV and HIV prevalence among drug users in various parts of the world is still at the alarming rate. Unfortunately, there is limited data about HBV, HCV and HIV prevalence in drug addicts in Turkey. In our previous study performed between September 2011 and October 2012, HBsAg, anti-HBs and anti-HCV frequency in drug addicts were found to be 2.6%, 38.3% and 9.4%, respectively. Additionally, anti-HIV positivity was not detected (1). The present study showed that the frequency rate of HBsAg, anti-HBs and anti-HCV in drug addicts was 4%, 52.3% and 7.9%, respectively. All the drug addicts in this study were HIV-negative, and this result might be related to the size of the HIV reservoir in Turkey. HBV and HCV are more stable in environmental conditions and transmitted more easily than HIV. The incidence rates for HBV and HCV infections are also typically much higher than HIV infection (11).

HBV and HCV prevalence in injecting drug users varies between regions in Europe over 40% and 40-90%, respectively. In a study carried out in Luxembourg, it was detected that the prevalence of HCV was 81.3% in injecting drug users and 19.1% in non-injecting drug users. The authors claimed that these results put Luxembourg in the upper range of the prevalence of HCV among injecting drug users in Europe (7). In the European Economic Area and the European Union, the estimated HIV prevalence among injecting drug users ranges from less than 1% to more than 60%. A significant increase in HIV infection among injecting drug users in Greece and Romania in 2011 has been reported (8). A serious increase in the number of newly diagnosed HIV infection among injecting drug users, more than 10-fold, was reported in Greece during the first seven months of 2011 (9). In the United States, HCV seroprevalence has been reported in 18%-38% of shorter duration injection drug users and 75%-90% of long-term injection drug users (10).

The high HBV, HCV and HIV prevalence in drug addicts depends on multiple factors, including the number of needle-sharing partners, the frequency of needle sharing and the social network structures among drug addicts. Additionally, the types of drugs, risky sexual behaviors, the size of the drug addicts population, and awareness of risks and prevention measures are other important factors. In a study conducted among drug users in St. Petersburg, Russia, it was shown that the type of the injected drug was associated with the prevalence and incidence of HIV infection (12). In the present study, whereas anti-HCV was not detected among cannabis users, 6.4% of opioid users and 15.9% of mixed drugs users were anti-HCV positive. This study showed that the type of drug has an effect on the rate of anti-HCV.

This study showed that the highest rate of drug use was in the 20-29 age group. Therefore, effective programs preventing HBV and HCV infection among drug addicts should target young persons. Additionally, our results were consistent with previous studies (10,13) which demonstrated that frequency of anti-HCV increases with age. This status may be related to the duration of drug use. Longer duration of drug use may lead to more sharing of needles and other equipment, resulting in a greater likelihood of transmission. On the other hand, this study showed that the rate

of anti-HBs positivity was 80.4% in those younger than 20 years of age. In Turkey, hepatitis B vaccine was included in the national vaccination program in 1998. While the vaccine is given to any adult in the risk groups who desires protection, all infants are vaccinated with three doses (14,15).

Study Limitations

This study had several limitations. The tests showing acute or chronic infection could not be performed. Tests distinguishing HBV vaccine from past HBV infection could not be performed in anti-HBs positive patients. Lastly, the risk factors for transmission of HBV and HCV transmission could not be determined among the drug addicts due to the retrospective design of the study.

Conclusion

This study demonstrated that HBV and HIV prevalence among drug addicts was not higher than in the general population in Turkey. However, it may be said that HCV infection among especially opioid users and mixed drugs users was a problem. Reducing HBV, HCV and HIV transmission among drug users should be an important public health objective. Understanding of local status in HBV, HCV and HIV infections is important for developing prevention strategies.

Ethics

Ethics Committee Approval: This study was appoved by the Fırat University Ethical Committee, 08/11/26.04.2016, **Informed Consent:** This study was a retrospective and no informed consent was obtained.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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

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Research Article

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Determination of Resistance Mutation in Chronic Hepatitis B Patients Using Antiviral Drugs at Our Hospital

Hastanemizde Antiviral İlaç Kullanan Kronik Hepatit B Hastalarında Direnç Mutasyonlarının Saptanması

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ABSTRACT

Objective: In this study, it is aimed to determine the mutations responsible for drug resistance in patients with chronic hepatitis B virus (HBV) infection received/receiving antiviral treatment at our hospital and to examine the patients in terms of the treatment applied and their HBV-DNA levels.

Materials and Methods: One hundred and thirty-one samples taken from patients diagnosed with chronic hepatitis B infection between January 2010 and January 2015 at Necmettin Erbakan University Meram Faculty of Medicine Hospital were studied with reverse hybridization principle-based INNO-LiPA HBV DR v2 method and the results were evaluated retrospectively.

Results: Mutation was determined in 12 samples (9.1%). While tyrosine, methionine, aspartate, aspartate (YMDD) pattern change causing lamuvidine resistance was determined in 10 samples, 7 of them were observed to be M204I tyrosine, isoleucine, aspartate, aspartate (YIDD) and 4 were M204V tyrosine, valine, aspartate, aspartate (YVDD). Multiple mutations were determined in six samples (M204V+M204I+L180I, YVDD+L180M+V/G173L, YIDD+L180M, YIDD+L80V in one each and YIDD+L80I, YVDD+L180M in two each) and single mutation was determined in 3 samples (YIDD in two samples and N236T and L80V in one each). Control HBV-DNA levels were evaluated in patients with resistance gene after 6-12 months and a decrease in DNA level was observed in 11 of 12 patients.

Conclusion: Since a limited number of mutations can be examined via LiPA method, it is concluded that different mutation patterns causing drug resistance cannot be determined and it will be beneficial to use an additional method such as sequencing that enables to determine these genes. Additionally, as a result of treatment failure due to drug resistance, if the treatment will be continued with a novel drug that is not used before, it is considered that the possibility of the presence of mutations causing a resistance against this antiviral should not be neglected.

Keywords: Hepatitis B, drug resistance, mutation

ÖZ

Amaç: Bu çalışmada, hastanemizde antiviral terapi almış/alan kronik hepatit B virüs (HBV) enfeksiyonu olan hastalarda ilaç direncinden sorumlu mutasyonların belirlenmesi, hastaların verilen tedavi ve HBV-DNA düzeyleri yönünden incelenmesi amaçlandı.

Gereç ve Yöntemler: Necmettin Erbakan Üniversitesi Meram Tıp Fakültesi Hastanesi'nde Ocak 2010 - Ocak 2015 tarihleri arasında kronik hepatit B enfeksiyonu tanısı ile takip edilen hastalardan alınan ve hastanemiz mikrobiyoloji laboratuvarına gönderilen 131 örnek ters hibridizasyon temeline dayalı INNO-LiPA HBV DR v2 yöntemi ile çalışıldı ve sonuçlar retrospektif olarak incelendi.

Bulgular: Yüz otuz bir örneğin 12'sinde (%9,1) mutasyon saptanmıştır. On örnekte lamuvidin direncine neden olan tirozin, metiyonin, aspartat, aspartat (YMDD) motif değişikliği belirlenirken bunların 7'si M204I tirozin, izolösin, aspartat, aspartat (YIDD), 4'ü M204V tirozin, valin, aspartat, aspartat (YVDD) şeklinde izlenmiştir. Altı örnekte çoklu mutasyon (birer örnekte M204V+M204I+L180I, YVDD+L180M+V/G173L, YIDD+L180M, YIDD+L80V; ikişer örnekte YIDD+L80I, YVDD+L180M), 3 örnekte tekli mutasyon (iki örnekte YIDD, birer örnekte N236T ve L80V) saptanmıştır. Direnç geni tespit edilen hastaların 6-12 ay sonra yapılan kontrol HBV-DNA düzeyleri incelenmiş ve 12 hastanın 11'inde DNA düzeyinde düşüş izlenmiştir.

Sonuç: LiPA yöntemi ile sınırlı sayıda mutasyon incelenebildiği için, alınan ilaca karşı dirence neden olan farklı mutasyon paternlerinin saptanamayacağı, bu genleri saptamaya olanak veren sekanslama gibi ek bir yönteme başvurmanın faydalı olacağı kanaatine varılmıştır. Ayrıca ilaç direnci nedeniyle meydana gelen tedavi başarısızlığı sonucunda, daha önce kullanılmamış yeni bir ilaç ile tedaviye devam edilecekse, bu antivirale karşı direnç oluşturan mutasyonların da bulunma ihtimalinin göz ardı edilmemesi gerektiği düşünülmüştür.

Anahtar Kelimeler: Hepatit B, ilaç direnci, mutasyon

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Introduction

Although viral hepatitis B can be prevented via vaccination, it continues to be a threat for public health. 5-10% of the adult population is chronically infected in sub-Saharan Africa and East Asia and an estimated 240 million people in the world are having chronic hepatitis B (CHB). According to the data of the World Health Organization, cirrhosis or liver cancer develops in 20-30% of chronically infected patients and more than 686.000 people die each year due hepatitis B complications such as liver insufficiency, cirrhosis and especially hepatocellular carcinoma. In our country, it is estimated that approximately 3-4 million people carries this virus. None of the treatment methods used can completely eradicate hepatitis B virus (HBV) infection. Basis of all the treatments is to provide lifelong continuation of HBV suppression. The drugs used in HBV antiviral treatment consist of immuno-modulator agents (interferon and peginterferon) and oral antiviral agents (nucleotide and nucleoside analogues) (1,2,3).

The use nucleotide/nucleoside analogues resulted in important problems such as mutation and drug resistance. In patients receiving drug treatment, it is known that mutations determined to be related to various drugs in viral polymerase genes are observed and they have caused *in vitro* reduction in drug sensitivity. Determination of these mutations in early period and prevention of unnecessary drug usage have a vital importance in terms of treatment success and it will also avoid toxicity caused by unnecessary drug usage and prevent unnecessary economic burden on the healthcare system.

In this study, it is aimed to determine the mutations causing antiviral resistance in patients with chronic HBV infection received/receiving antiviral treatment and to evaluate the patients in terms of the treatment received and their HBV DNA levels.

Materials and Methods

We retrospectively evaluated the results obtained from 131 serum samples that were taken from patients diagnosed with CHB infection and were sent to the microbiology laboratory at Necmettin Erbakan University Meram Faculty of Medicine Hospital between January 2010 and January 2015 in order to determine drug resistance. Samples were studied with reverse hybridization principled INNO-LiPA HBV DR v2 (INNO-LiPA HBV DR; INNOGENETICS N.V., Ghent, Belgium) method. According to the manufacturer's recommendations, purified DNA samples were amplified by using primers. In the biotinylated PCR product, the presence of mutation was investigated via reverse hybridization method by using oligonucleotide probes specific to the mutation points on the nitrocellulose strips. Thirty two HBV probe lines showing wild type, mutant and mixed sequences for 80th, 173rd, 180th/181st, 204th and 236th polymerase codons were monitored colorimetrically and the bands formed were assessed by means of guide strips. HBV-DNA levels were examined simultaneously with the determination of mutation and during follow-up evaluation performed after 6-12 months by real-time PCR (COBAS TaqMan, Roche Diagnostics, France) method.

Results

Mutation was determined in 12 of 131 patients (9.1%). While YMDD (Y: tyrosine, M: methionine, D: aspartate, D: aspartate) pattern change was determined on 204th codon in 10 patients, 7 of them were in the form of M204I tyrosine, isoleucine, aspartate, aspartate (YIDD) and 4 in the form of M204V tyrosine, valine, aspartate, aspartate (YVDD) and in one patient, it was observed to be multiple mutations as M204V+M204I+L180I. M204I mutation was seen singly in two samples, together with L80I mutation in two samples, with L180M mutation in one and with L80V mutation in one. L180M was determined in two and L180M+V/G173L mutation was determined in one of the samples with M204V mutation. Sole N236T and L80V mutation was determined in each patient sample. It was determined that the patient with YIDD resistance gene was receiving adefovir treatment and the patient with YVDD+L180M resistance gene was receiving entecavir treatment. It was specified that the patients with other resistance genes had received lamivudine treatment. No information was obtained regarding the treatment of the patients with N236T and YIDD+L80I/V resistance profile. Observing HBV-DNA levels of the patients simultaneously with the determination of mutation and during follow-up evaluations performed after 6-12 months showed that DNA levels in 11 of 12 patients was decreased. An increase was observed in the DNA level in only one patient during the follow-up. Resistance profiles, drugs used and DNA levels of the patients are given in Table 1.

Discussion

HBV is a rapidly replicating virus. Mutation rate is high due to the absence of proofreading function of the reverse transcriptase enzyme in replication and it causes heterogeneous virus population in infected people. It is known that viral genome also generates adaptive mutations resulting in drug resistance formation especially under the suppression effect of the antiviral treatment. Antiviral drug resistance is also affected by the factors of host characteristics such as virus-infected hepatocyte, immune response, and genetic factors (4,5).

Studies performed on various patient groups in our country revealed that there were various mutations reducing the drug

Table 1. Tyrosine, methionine, aspartate, aspartate patterns, received treatments and DNA levels of patients

YMDD pattern changes	Treatment received	DNA level follow-up (copy/mL) (Initial measurement - control measurement)
YVDD+YIDD+L80I	Lamivudine	62060-0
YIDD+L80I	-	515620-0
YVDD+L180M	Lamivudine	6032-0
YIDD	Lamivudine	1210-0
YVDD+L180M+V/G173L	Lamivudine	>986x10^6-28188
YVDD+L180M	Entecavir	638x10^6 -15x10^6
YIDD+L80I	Lamivudine	887400-148
YIDD	Adefovir	>986x10^6-28188
YIDD+L180M	Lamivudine	6612-2243
YIDD+L80V	-	174x10^6-328x10^6
N236T	-	-
L80V	Lamivudine	1815400-<116

YMDD: Tyrosine, methionine, aspartate, aspartate, YVDD: Tyrosine, valine, aspartate, aspartate, YIDD: Tyrosine, isoleucine, aspartate, aspartate

sensitivity at varying frequencies in untreated individuals or in inactive carriers and investigation of nucleotide and nucleoside analogues resistance mutations before the treatment was contentious in terms of price and efficiency (6,7,8,9,10).

It has been shown that hybridization tests had high sensitivity and they could determine the mutations in some HBV populations which cannot be determined via sequences; their superiority over sequencing method in terms of interpretation, experience of personnel, conformance with the program used, time spent, and economic conditions have been revealed (11,12,13).

It is known that the mutations occurred in the amino acid sequence in YMDD pattern in the gene area coding the HBV polymerase enzyme and expressed as rtM204V/I/S cause resistance development against lamivudine. In our study, among 10 patients with YMDD mutation, M204I (YIDD) was determined in 7 patients and M204V (YVDD) was determined in 4 patients. It has been shown that L180M/C mutation was almost present with YMDD mutations at all times and this has increased both replication and lamivudine resistance. It is noted that YIDD type-mutations can be seen singly (14,15,16,17). In a study by Akarsu et al. (8), it was noted that L180M accompanied by YMDD pattern change in 8 of 13 patients and YVDD pattern change was present in all cases having L180M mutation. In our study, it was observed that in 3 samples, YVDD and in one, YIDD mutations were accompanied by L180M mutation and no sole L180M mutation was found. In two patients, sole YIDD mutation was observed. In some studies, it has been shown that sole YVDD mutation could be seen and L180M mutation might accompany this mutation in the future and, in our study, no isolated YVDD mutation was observed (8,14,18).

It has been shown that the lamuvidin resistance mutation rtM204I/V together with rtT184G+rtS202I/C or rtM250V+rtI169T mutation is responsible for entekavir resistance (16,19,20). In addition, it has been shown that adefovir resistance is related with rtN236T and/or rtA181T/V mutation (13,16,21,22). In our research, it was seen that a patient with YVDD+L180M mutation was receiving entecavir treatment and another patient with isolated YIDD mutation was receiving adefovir treatment. The possibility that these patients had previously received lamivudine treatment was also considered but no relevant data was obtained. Also it is suggested that there are other mutations causing entecavir and adefovir resistance, but since a limited number of mutations can be examined via LiPA method, these mutations could not be determined. Sequencing can be the method of choice to detect other mutations. Altındiş et al. (23) revealed compensatory mutations in treatment-naive CHB patients who have received both nucleoside/nucleotide analogues and lamivudine and/or adefovir treatments by sequencing method. rtQ149K, Q215S, Q215H, Q249K and V214A mutations were found to be associated with lamivudine and adefovir treatment; rtL91I mutation was found to be associated only with telbivudine and N238D with only adefovir.

Thus, in case of non-response to the treatment, using alternative methods for determining different potential mutations, such as sequencing, and revealing complex mutation patterns will be more useful for conducting the treatment. In addition, if there is a failure of treatment due to drug resistance and the treatment will be continued with a novel drug that has not been used before, the possibility of the presence of mutations causing a resistance against this antiviral should not be neglected.

It has been shown that rtL80V/I mutation was related with lamivudine resistance, 85% of the lamivudine-resistant isolates coded rtL80I and this mutation increased the replication skills of rtM204V/I mutants (24). In our study, rtL80V/I mutation was observed in a total of 5 patients; together with YMDD mutations in 4 patients and as isolated in one patient and, the rate of co-existence of YMDD mutations was found to be 50%.

HBV DNA level follow-up is used as a significant parameter in monitoring the treatment efficiency. The presence of antiviral drug resistance genes mostly shows itself via viral load increase in patients receiving treatment. However, viral load is affected by the adherence of the patient to treatment and pharmacogenomic factors; it should be kept in mind that this parameter is not suitable to be used as a direct drug resistance indicator (4). In this study, DNA levels in patients with resistance gene were examined during the follow-up evaluation performed after 6-12 months. As a result of the resistance gene determination and the treatment plan that was rearranged afterwards, a decrease in HBV DNA levels was observed in 11 of 12 patients. An increase was observed in the DNA level in one patient during the follow-up. In this study, treatment follow-up was not monitored; it is considered that for patients with no decrease in DNA level, parameters such as qualification of the treatment regime change, presence of other possible mutations and the period of adherence to treatment should be investigated.

It has been shown that rtV173L mutation was seen in 9% of lamivudine-resistant cases and it has increased the replication capacity of lamivudine-resistant HBV and, anti-HBs binding capacity has decreased due to the change in the HBsAg structure of the virus with rtV173L+rtL180M+rtM204V triple mutation (19,25,26). In our study, this triple mutation was determined in one patient (8.3%) and the HBV DNA level in this patient was at the levels exceeding measurable upper limit of mutation (>986 copy/mL) and a decrease was observed during the follow-up evaluation performed after 6 months (28.188 copy/mL).

Conclusion

The samples sent to our laboratory for determination of resistance were retrospectively examined and mutation profiles, HBV DNA levels and the treatment received by the patients with resistance were evaluated. It was revealed that mutations have developed under antiviral treatment and this could be in the form of various drug resistance mutations. Further studies in which antiviral treatment periods are followed up for a longer term can demonstrate new and different information regarding mutation development. Early determination of the mutations causing drug resistance is required to provide opportunity to alternative treatment options and to provide a more efficient treatment.

Ethics

Ethics Committee Approval: A retrospective study, **Informed Consent:** A retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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

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Case Report

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Elbasvir/Grazoprevir Experience - A New Glance at HCV Treatment: Case Report

HCV Tedavisinde Yeni Bir Bakış Elbasvir/Grazoprevir Deneyimi: Olgu Sunumu

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ABSTRACT

Hepatitis C is a viral disease having a worldwide importance and posing a risk for liver complications. With the new treatment options, it is easy to manage with higher rates of success. Among them, one of the most recent one is elbasvir/grazoprevir option. This study presents the results of two treatment-naive patients treated with elbasvir/grazoprevir. The first case was a male non-cirrhotic patient and the second one was a female who suffered from compensated hepatic cirrhosis. Both patients received elbasvir/grazoprevir 50/100 mg in a single tablet for 12 weeks. Persistent viral response was achieved in both patients and no side effect was observed during the treatment. Elbasvir/grazoprevir combination, one of the recent treatments, was considered effective and tolerable.

Keywords: Hepatitis C virus, oral antiviral treatment, elbasvir/grazoprevir

ÖZ

Hepatit C dünya genelinde önemini sürdüren ve karaciğer komplikasyonları açısından da hala risk oluşturan bir viral hastalıktır. Yeni tedavi seçenekleri ile yöntemi daha kolay ve başarısı daha yüksektir. Bunlar içersinden en yenilerden biri de elbasvir/grazoprevir seçeneğidir. Bu çalışmada elbasvir/grazoprevir tedavisi alan iki hastamıza ait sonuçlar paylaşıldı. İki olgunun değerlendirildiği bu çalışmada olgularımızdan ikisi de tedavi deneyimsiz olup; birinci olgu non-sirotik erkek hasta diğeri ise kompanse karaciğer sirozu olan kadın hastadır. Her iki hasta 12 haftalık elbasvir/grazoprevir 50/100 mg tek doz tablet ile tedavi edildi. Her iki hastada da kalıcı viral yanıt elde edilirken ilaç kullanımı esnasında da herhangi bir yan etki izlenmedi. Yeni tedavi seçeneklerinden biri olan elbasvir/grazoprevir kombinasyonu hem etkin hem de tolere edilebilir olarak değerlendirildi.

Anahtar Kelimeler: Hepatit C virüs, oral antiviral tedavi, elbasvir/grazoprevir

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Introduction

Hepatitis C virus (HCV) is a viral disease which still has a worldwide importance and poses a risk of liver complications. It is one of the major reasons for hepatic failure, cirrhosis and liver cancer. Conventional treatment using interferon-based drug regimens, which has been available for several years, was an option difficult to tolerate due to its low rate of treatment success and serious side effects. The first generation protease inhibitors, which have been involved in the treatment in the recent years, were among the options difficult to be used in terms of side effects. However, the new generation oral antivirals were introduced as a satisfactory treatment with better results and less side effects thanks to the option of single tablet with short-term treatments and no interferon content. Among them, one of the most recent one is elbasvir/grazoprevir combination (1,2,3). This study presents the results of two patients treated with elbasvir/grazoprevir. The recommended dose of elbasvir/grazoprevir combination is one tablet of 50 mg/100 mg taken orally once daily (4,5).

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Case

Grazoprevir, a NS3/4A protease inhibitor, and elbasvir, an NS5A inhibitor, have become in place in HCV treatment in the dose of 100 mg/day and 50 mg/day, respectively, combined in a single tablet with a recommended treatment duration of 12 weeks. Two patients were treated in our hospital with this treatment option. Elbasvir/grazoprevir is indicated for the treatment of patients with genotype 1, 4 and 6, cirrhotic and non-cirrhotic, both treatment-naive and treatment-experienced ones.

Case 1

The first case was a 50-year-old male HCV-infected patient who has been followed up since 2003. The patient was diagnosed with diabetes mellitus (DM) in 2006. With a HCV-RNA level of 342.000 IU/mL at baseline, he was reported to have genotype 1B. No decompensation was observed and liver ultrasound was normal. The patient had no co-infection and did not drink alcohol. The patient was administrated elbasvir/grazoprevir combination starting with a single dose daily. The laboratory values at baseline and during the follow-up period are given in Table 1. The patient had negative HCV-RNA test in the first week. Persistent viral response was achieved at the end of the treatment which was tolerated well in terms of side effects although nausea, toothache and itching were observed. Persistent viral response was achieved with negative HCV-RNA test in the sixth month, at the end of the treatment. Tolerating the treatment with no significant adverse effect, the patient's compliance to treatment was found to be high.

Case 2

The second case was a treatment-naive 63-year-old female patient with compensated hepatic cirrhosis. She had refused interferon treatment for years, since she witnessed that a relative of her suffered from the side effects developed during the use of pegylated interferon. Diagnosed with DM in 2009, the patient has been followed up since 2005. With genotype 1B and a HCV-RNA load of 2.300.000 IU/mL, the patient had never received treatment. The patient suffered from hypertension and no co-infection. The patient was considered as Child-pugh A. She was administered elbasvir/grazoprevir combination in fixed dose. The details of the treatment process are given in Table 2. Persistent viral response was achieved with a negative HCV-RNA test in the sixth month, at the end of the treatment. She experienced no other significant side effects except for frequent urination, dizziness and nausea.

Table 1. Outset of treatment for the case 1 and values observed during the treatment

	HCV-RNA	ALT/AST	HGB	PLT	INR	AE	SAE
Screen	342.000	41/35	12.1	189.000	1.1	-	-
Visit-2	314.000	36/12	11.7	200.000	1.1	-	-
Week 1	0	29/21	11.8	213.000	1.05	-	-
Week 2	0	28/20	12.0	256.000	1.1	Nausea	-
Week 4	0	27/21	11.8	198.000	1.01	-	-
Week 6	0	28/23	12.3	242.000	0.9	-	-
Week 8	0	34/29	11.9	201.000	1.1	Toothache	-
Week 10	0	26/23	12.8	230.000	1.1	-	-
Week 12	0	28/22	11.8	221.000	1.05	-	-
Week 16	0	27/22	11.7	187.000	1.1	Itching	-
Week 24	0	32/36	12.1	209.000	1.03	-	-

HCV: Hepatitis C virus, ALT/AST: Alanine aminotransferase/aspartate aminotransferase HGB: Hemoglobin, PLT: Trombosit, AE: Adverse events, SAE: Serious adverse events

Table 2. Blood values for the case 2

	HCV-RNA	ALT/AST	HGB	PLT	INR	AE	SAE
Screen	2.300.000	23/35	13.1	109.000	1.3	-	-
Visit-2	2.415.000	28/41	12.3	110.000	1.34	-	-
Week 1	0	49/51	12.8	103.000	1.35	Dizziness	-
Week 2	0	38/50	13.0	96.000	1.23	Nausea	-
Week 4	0	24/41	12.8	98.000	1.09	-	-
Week 6	0	22/33	13.3	102.000	1.19	-	-
Week 8	0	34/39	13.9	101.000	1.19	-	-
Week 10	0	36/43	12.8	130.000	1.27	Pollakiuria	-
Week 12	0	38/52	12.8	121.000	1.10	-	-
Week 16	0	37/42	13.7	107.000	1.23	Pollakiuria	-
Week 24	0	32/36	13.1	109.000	1.20	-	-

HCV: Hepatitis C virus, ALT/AST: Alanine aminotransferase/aspartate aminotransferase HGB: Hemoglobin, PLT: Trombosit, AE: Adverse events, SAE: Serious adverse events

Discussion

Grazoprevir, NS3/4A protease inhibitor, and elbasvir, NS5A inhibitor, have taken place in HCV treatment with the recommended dose of elbasvir/grazoprevir combination of 50 mg/100 mg taken orally once daily for 12 weeks. It is effective in patients with genotype 1, 4 and 6, regardless of being treatment-naive or treatment-experienced, with a persistent viral response of up to 100%.

In a clinical study of phase 3 in which both treatment-naive and treatment-experienced HCV patients were involved, elbasvir/grazoprevir combination was compared with sofosbuvir plus pegylated interferon alpha. In this study including a total 257 patients, the treatment was administered for 12 weeks. The rate of sustained viral response was found to be 99.2% for elbasvir/grazoprevir, while it was 90.5% for sofosbuvir with pegylated interferon alpha. In patients in the group of sofosbuvir and pegylated interferon alpha combination, the frequency of adverse effects and non-compliance with treatment were higher than in elbasvir/grazoprevir combination (4). Compliance with the treatment was high in both of our cases and no significant side effects were reported.

Conclusion

HCV is a viral disease which still has a worldwide important posing a risk for liver complications. It is one of the major reasons for hepatic failure, cirrhosis and liver cancer. Elbasvir/grazoprevir single dose regimen is a new drug with a high sustained virological response at chronic HCV patients.

Ethics

Informed Consent: A consent form was completed by all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: C.A., Design: İ.A., Analysis or Interpretation: R.T., Literature Search: K.B., Writing: M.K.Ç.

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

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How to Diagnosis the Occult Hepatitis C Virus?

Okült (Gizli) Hepatit C Virüs Tanısı Nasıl Olmalı?

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Keywords: Occult, HCV, diagnosis
Anahtar Kelimeler: Okült, HCV, tanı

Aslan FG, Altındiş M. How to Diagnosis the Occult Hepatitis C Virus? Viral Hepat J. 2017;23:37-38.

Dear Editor;

Occult hepatitis C (OHC) virus infection, defined as the presence of hepatitis C virus (HCV) RNA in the liver and in peripheral blood mononuclear cells (PBMCs) in the absence of detectable viral RNA in serum, can be found in anti-HCV positive patients with normal serum levels of liver enzymes and in anti-HCV negative patients with persistently elevated liver enzymes of unknown etiology. OHC has been described using highly sensitive nucleic acid amplification assays with a sensitivity of <3 IU/mL. Different studies have revealed that HCV RNA can persist and replicate in immune cells but the relevance of its presence and persistence over time is still unknown. As the contribution of this extrahepatic reservoir could have several clinical implications in viral transmission, treatment response and disease pathogenesis, future studies are required to improve our knowledge of the extrahepatic manifestations of HCV infection and its possible consequences (1).

Individuals with OHC usually have normal serum liver enzymes and most of them are reactive for anti-HCV antibodies. These individuals could have a history of resolved chronic hepatitis C due to antiviral therapy, spontaneous recovery from hepatitis C or asymptomatic exposure to HCV. Low levels of HCV RNA have also been detected in a significant portion of patients with persistently elevated liver enzymes of unknown etiology that were anti-HCV antibody nonreactive (2). The data gathered using highly sensitive assays showed serum HCV-RNA positivity in the majority of persons with a sustained virologic response (SVR) or after spontaneous recovery from hepatitis C in those who were repeatedly negative for HCV RNA by standard clinical assays (3). Some studies using a highly sensitive real-time polymerase chain reaction (RT PCR) assay have shown the presence of residual HCV RNA in a small number of individuals up to 5 years after apparent spontaneous or treatment induced viral clearance. In addition to sera, HCV RNA was detected in PBMCs. In a large study among 400 patients with SVR, 98% had undetectable hepatic HCV RNA, while only 2% (7 patients) had detectable hepatic HCV RNA. In this study, it could be noticed that the detection of OHC virus was related to the presence of high pretreatment viral load. This factor is well known as prognostic indicator of viral response to antiviral therapy (4).

The principle of HCV RT PCR in PBMC: For PCR performed in PBMCs, five mL of blood was aspirated in sterile tubes containing EDTA, mixed well and lymphocytes were separated by centrifugation on a density gradient (i.e., Ficoll-Hypaque, Pharmacia Biotech) (3). The separation of PBMCs consists of a series of steps. Initially, 3 mL peripheral blood sample is collected into an EDTA-containing tube from each individual. Ficoll hypaque solution is added into a gel-free tube. Peripheral blood samples are transferred to this tube slowly and centrifuged at 1600 rpm for 15 minutes. In the middle of the tube, a cloudy appearance occurred is PBMCs, 1 mL of these cells is transferred to falcon tube by micropipette. Four mL phosphate buffered saline is added and centrifuged at 1300 rpm for 10 minutes. This washing process is repeated four times to purify the cells. Inactivated fetal calf serum is added into the 50 mL of RPMI 1640 medium, 1 mL of this broth is transferred to Eppendorf tubes, 100 µL of 10% dimethyl sulfoxide (DMSO)

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is added and this mixture is transferred into the cells as a last step. PBMCs are kept at -80°C until use. Another peripheral blood sample collected into EDTA-containing tube from each individual is centrifuged then plasma is stored at -80°C and used for PCR. RNA isolation procedure is applied to the plasma samples and PBMCs, HCV RNA is extracted from the plasma samples and PBMCs by using RT PCR. Measurement range of the PCR test must be 25-3.91x108 IU/mL and the sensitivity of the test is 25 IU/mL (5).

The principle of HCV real-time PCR: It is a highly sensitive assay that combines simultaneous amplification and fluorescence detection of target nucleic acid. The fluorescence signal generated during PCR is directly proportional to the target amount in the sample. A synthetic internal control is stabilized within the nucleic acid extraction tubes to be co-purified with the HCV target nucleic acid. HCV genotypes are amplified with similar efficiency applying probes and primers specific for a subsequence of the HCV 5′ untranslated region. Primers used are designed to detect the positive sense RNA. Amplification of HCV RNA in samples and internal control RNA is measured independently at different wavelengths due to probe labeling with different fluorescence reporter dyes (HCV RNA; FAM, Internal control RNA; Yakima Yellow). The detection limit of this assay was found to be 3 synthetic HCV RNAs per PCR run (1).

HCV RNA in PBMCs is recommended to detect residual infection in patients with SVR, especially in those with high serum HCV RNA levels before treatment (6). Future works should deal with the possible incidence of OHC or B infections and their pathologic and infective relevance specially in drug abusers, healthcare workers, patients who had received multiple blood transfusions, and patients on hemodialysis, etc. to know the prevalence and spread of this infection in these populations (1,7).

Ethics

Peer-review: Externally and Internally peer-reviewed.

Authorship Contributions

Concept: M.A., Design: M.A., Data Collection or Processing: M.A., F.G.A., Literature Search: M.A., F.G.A., Writing: M.A., F.G.A.

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