VIRAL HEPATITIS SOCIETY

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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

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

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

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

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

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

The authors should acknowledge and provide information on grants, contracts or other financial support of the study provided by any foundations and institutions or firms.

The articles sent to be published in the journal shouldn't have been published anywhere else previously or submitted and accepted to be published. However, a complete report that follows publication of a preliminary report, such as an abstract can be submitted. If authors intend to discard any part of the manuscript, a written application should be sent to the Editor.

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

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

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

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i. Turkish title, English title, author(s)' name(s) and institution(s) (Turkish and English)
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vi. Results (max 400 words)
vii. Discussion (max 700 words)
viii. Referances (should not exceed 15), all words 2000 not exceed.

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

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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.

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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.

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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.

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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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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.

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

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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.

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

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

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• Keywords (minimum 3; maximum 6)

• Article divided into appropriate sections

• Complete and accurate references and citations

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Research Article

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Relationship between Viral Load and Hepatic Histopathology in Patients with Chronic Hepatitis B

Kronik Hepatit B Tanılı Hastalarda Viral Yük ile Karaciğer Histopatolojisi İlişkisi

Damla Akdağ[1], Tansu Yamazhan[1], Hüsnü Pullukçu[1], Meltem Işıkgöz Taşbakan[1], Raika Durusoy[2]

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ABSTRACT

Objectives: It is not always possible to determine a clear relationship between the DNA level of hepatitis B virus (HBV) and histology. In this study, we aimed to determine the relationship between HBV-DNA level and liver histopathology in patients with chronic hepatitis.

Materials and Methods: Between 2008 and 2016, 361 patients diagnosed with chronic HBV infection were retrospectively examined with age, sex, hepatitis B e antigen status, alanine aminotransferase (ALT) and HBV-DNA levels and liver biopsy scores according to modified Ishak criteria. Patients were divided into five groups (10^5, 10^5-10^6, 10^6-10^7, 10^7-10^8, ≥10^8) based on their HBV-DNA level (IU/mL) and upon histopathological evaluation, hepatic injury was divided into two groups - mild and moderate/severe- according to Ishak score (grade 1-6: mild, 7-18: moderate/severe and stage 0-2: mild, 3-6: moderate/severe) to investigate the statistical relationship between HBV-DNA levels and grade/stage scores. Cross-table and Pearson's chi-square test were used in the analyses.

Results: Of the three hundred and sixty-one patients, 62.3% (225/361) were male and the average age was 40.32±12.79. Anti-HBe (296/361) was positive in 82% of the patients, ALT, HBV-DNA averages were 83.17 U/L (±125.1), 57298951.01 IU/mL during biopsy, and grade and stage averages were 5.34 and 1.76 respectively. HBV-DNA groups with the grade's 2-binary groups when compared to moderate/high group, respectively, HBV-DNA <10^5 and 17.2%, 10^5-10^6 37%, 10^6-10^7% in 46.9, 107-10^8% and 48.6 and 35.1 in ≥108 were found. The difference between the groups was found to be statistically significant (p<0.000). Similarly, HBV-DNA groups when compared to stage-binary groups, in the middle/high group, respectively, HBV-DNA 17.2% in <105, 32.6% in 10^5-10^6, 51% in 10^6-10^7, 48.6% in 10^7-10^8 and 35.1% in ≥10^8 group were found and all of them were statistically significant (p<0.000).

Conclusion: A HBV-DNA level was not found to be a threshold determining moderate/severe histopathological level. However, in group analysis, the histopathological relationship with the DNA level is directly proportional. Liver histology is an important marker determining the progression of the disease.

Keywords: Viral hepatitis, hepatitis B, histopathology, viral

ÖZ

Amaç: Hepatit B virüs (HBV)-DNA düzeyi ile histoloji arasında her zaman net bir ilişki saptamak mümkün olmamaktadır. Bu çalışmada kronik hepatit tanılı hastalarda HBV-DNA düzeyi ile karaciğer histopatolojisi arasındaki ilişkinin ortaya konması amaçlanmıştır.

Gereç ve Yöntemler: 2008-2016 yılları arasında kronik hepatit B tanılı 361 hastanın; yaş, cinsiyet, hepatit B e antijeni durumu, alanin aminotransferaz (ALT) ve HBV-DNA düzeyleri ile modifiye Ishak kriterlerine göre karaciğer biyopsi skorları retrospektif olarak incelenmiştir. HBV-DNA düzeyi ile grade/stage skorları arasındaki istatistiksel ilişkinin araştırılması açısından hastalar, HBV-DNA düzeyine göre 5 gruba (<10^5, 10^5-10^6, 10^6-10^7, 10^7-10^8, ≥10^8), histopatolojik değerlendirmede ise grade: 1-6 hafif, 7-18 orta/yüksek; stage 1-2 hafif, 3-6 orta/yüksek olmak üzere olmak üzere 2'şerli gruplara ayrılmıştır. Analizlerde çapraz tablo ve Pearson'un ki-kare testi kullanılmıştır.

Bulgular: Üç yüz altmış bir hastanın %62,3'si (225/361) erkek olup yaş ortalaması 40,32±12,79idi. Hastaların %82'sinin anti-HBe'si (296/361) pozitif olup, biyopsi esnasındaki ALT, HBV-DNA ortalamaları sırasıyla; 83,17 U/L (±125,1); 57298951,01 IU/mL saptanmış olup, grade ve stage ortalamaları sırasıyla; 5,34 ve 1,76 olarak bulunmuştur. HBV-DNA ve grade'nin ikili grupları karşılaştırıldığında orta yüksek grupta sırasıyla HBV-DNA <105 iken %17,2, 10^5-10^6'de %37, 10^6-10^7'de %46,9, 10^7-10^8'de %48,6 ve ≥10^8 olan grupta %35,1 olarak tespit edilmiştir ve gruplar arasındaki fark istatistiksel olarak anlamlı bulunmuştur (p<0,000). Aynı şekilde HBV-DNA grupları ile stage ikili grupları karşılaştırıldığında orta yüksek grupta sırasıyla HBV-DNA <10^5 iken %17,2, 10^5-10^6'de %32,6, 10^6-10^7'de %51, 10^7-10^8'de %48,6 ve ≥10^8 olan grupta %35,1'dir ve gruplar arasındaki fark istatistiksel olarak anlamlı bulunmuştur (p<0,000).

Sonuç: Orta/ileri histopatolojik düzeyi belirleyen eşik bir HBV-DNA düzeyi bulunamamıştır. Ancak grupsal analizde DNA düzeyi ile histopatolojik ilişki doğru orantılıdır. Kronik HBV tanılı hastalarda karaciğer histolojisi, hastalığın progresyonu belirleyen önemli bir belirteçtir.

Anahtar Kelimeler: Viral hepatit, hepatit B, histopatoloji, viral yük

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Introduction

Chronic hepatitis B virus (HBV) infections that are still commonly seen in our country are responsible for approximately one million deaths each year due to their fatal complications such as cirrhosis, hepatocellular carcinoma, and liver failure (1). A substantial proportion of these complications can be prevented with anti-viral therapy. Serum HBV-DNA levels and the level of hepatic necroinflammation and fibrosis are the two current criteria employed in the decision-making process to start antiviral treatment. In general, as the viral load increases, an evident deterioration in hepatic histology is expected, however at which HBV-DNA levels this histological degradation is predominant is not clear. This study aimed to statistically establish the relationship between HBV-DNA level and hepatic histopathology in patients who were diagnosed with chronic hepatitis and planned to start treatment and to find a HBV-DNA level which could be a reference point to determine the extent of moderate or advanced histopathological damage (2,3).

Materials and Methods

This study retrospectively examined the biopsy results of 361 patients diagnosed with chronic HBV infection between 2008 and 2016 and who underwent liver biopsy to initiate treatment. The age, gender, hepatitis B e antigen (HBeAg) status, alanine aminotransferase (ALT) and HBV-DNA (RealArt HBV-polymerase chain reaction (PCR), Abbott, USA) levels of patients were evaluated compared with Ishak liver biopsy scores (4). Patients were divided into five groups ($<10^5$, 10^5-10^6, 10^6-10^7, 10^7-10^8, $\geq 10^8$) based on their HBV-DNA level (IU/mL). Upon histopathological evaluation, hepatic injury was divided into two groups - mild and moderate/severe- according to Ishak score. Based on this, the first three-quarters (1-6) of the necroinflammatory activity, which is graded from 0 to 18 points, were classified as mild, and 7 and above (7-18) were classified as moderate/severe. Likewise, the scoring used to grade fibrosis from 0 to 6 points was divided into two groups where 0-2 points were classified as mild, 3-6 points were classified as moderate/severe fibrosis.

Statistical Analysis

A Spearman correlation analysis was conducted to explore a possible association between HBV-DNA and grade and stage as unclassified, continuous variables. A chi-square test and trend analysis was performed to compare the distribution of patients into the two grade and stage categories according to their classified HBV-DNA levels. Received operating characteristic curve analyses were performed to see if HBV-DNA as a continuous variable could be a good predictor of grade and stage classified into two categories. The HBV-DNA values of the naive and treatment-experienced groups were compared using Mann-Whitney U test. P values below 0.05 were considered statistically significant.

Results

Among the 361 participants, 62.3% (225/361) were male with mean age being 40.32 (±12.79). Among the patients, 82% were anti-HBe-positive (296/361), 345 (95.6%) were naive and 16 (4.4%) were treatment-experienced. ALT, HBV-DNA means and grade and stage means of patients during biopsy are presented in Table 1.

Patients' distributions in binary groups (mild and moderate/severe) by their necroenflammatory activity and fibrosis status when they are divided into 5 groups ($<10^5$, 10^5-10^6, 10^6-10^7, 10^7-10^8, $>=10^8$) based on their HBV-DNA (IU/mL) levels are presented in Table 2.

There was a moderate, positive correlation between the HBV-DNA values and grades of the patients (r_S=0.344, p<0.0005) and a weak, positive correlation between their HBV-DNA values and their stages (r_S=0.257, p<0.0005).

Table 1.	
Sex	
Male	225 (62.3%)
Female	136 (37.7%)
Age	40.3±12.8
HBeAg	
Positive	65 (18.0%)
Negative	296 (82.0%)
ALT(U/L)	83.17±125.1 (median 52)
HBV-DNA (IU/mL)	57.298.951 (median 110.901)
Treatment	
Naive	345 (95.6%)
Experienced	16 (4.4%)
Ishak	
Grade	5.3±2.6 (minimum: 0, maximum: 17, median 5)
Stage	1.8±1.5 (minimum: 0, maximum: 6, median 2)

HBeAg: Hepatitis B e antigen, ALT: Alanine aminotransferase HBV: Hepatitis B virus

Table 2. Grade and Stage Distributions by HBV-DNA levels (n, percent to total)

	Grade (p<0.0005)		**Stage (p<0.0005)**	
HBV-DNA	**Mild**	**Moderate/Severe**	**Mild**	**Moderate/Severe**
$<10^5$	144 (82.8)	30 (17.2)	144 (82.8)	30 (17.2)
10^5-$<10^6$	29 (63.0)	17 (37.0)	31 (67.4)	15 (32.6)
10^6-$<10^7$	26 (53.1)	23 (46.9)	24 (49.0)	25 (51.0)
10^7-$<10^8$	18 (51.4)	17 (48.6)	18 (51.4)	17 (48.6)
10^8 and higher	37 (64.9)	20 (35.1)	37 (64.9)	20 (35.1)
Total	254 (70.4)	107 (29.6)	254 (70.4)	107 (29.6)

HBV: Hepatitis B virus

There was a statistically significant difference among grade groups compared to DNA level groups. As HBV-DNA levels increased, the proportion of patients in the moderate/severe group increased, which was statistically significant (p<0.0005, Table 2).

Similarly, there was a statistically significant difference between distributions by HBV-DNA level and by Stage with an increasing trend (p<0.0005, Table 2).

There was no significant difference between the mean HBV-DNA values of the naive and treatment-experienced groups (U=2108.5, p=0.110).

The area under the curve of HBV-DNA to predict grade 2-3 was 0.65, while it was 0.63 for predicting stage (Figure 1a and 1b). For example if HBV-DNA is cut from 10^5, its sensitivity to predict grade becomes 72.0% and specificity 66.7% while if it is cut from 10^6, its sensitivity to predict grade becomes 56.1% and specificity 68.1%. Similarly, if HBV-DNA is cut from 10^5, its sensitivity to predict stage becomes 72.0% and specificity 66.7% while if it is cut from 10^6, its sensitivity to predict stage becomes 57.9% and specificity 68.9%.

Discussion

In addition to ALT elevation, HBV-DNA level and the extent of liver damage are two criteria that are employed in the decision-making process to initiate antiviral treatment for chronic HBV infection.

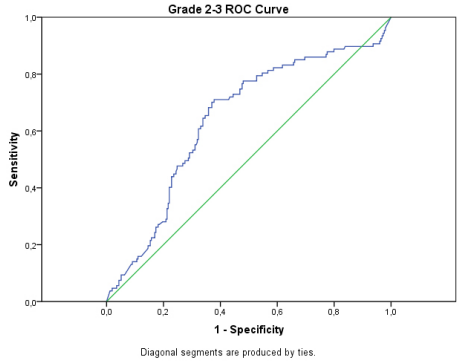

Figure 1a. Receiver operating characteristic curve of hepatitis B virus-DNA in predicting Grade 2-3

ROC: Receiver operating characteristic

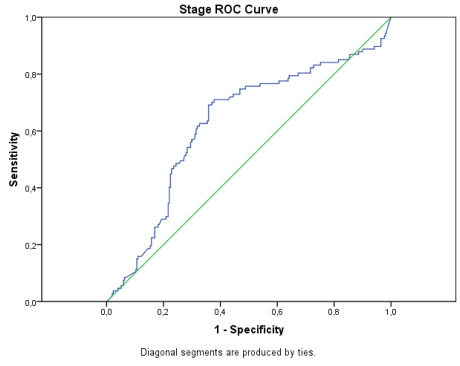

Figure 1b. Receiver operating characteristic curve of hepatitis B virus DNA in predicting stage

ROC: Receiver operating characteristic

National and international guidelines emphasized the importance of HBV-DNA level to determine the threshold to initiate treatment, and standardization was attempted to establish in this regard with no consensus being reached at the end. In the guideline updated by Asian Pacific Association for the Study of the Liver in 2016, treatment initiation threshold were defined as 2000 IU/mL and above for HBV-DNA for cirrhotic patient groups who have ALT level within normal ranges and non-cirrhotic patients groups who are HBeAg-negative (5). In the guideline published by the European Association for the Study of the Liver in 2017, the treatment limit was set at 2000 IU/mL and treatment was recommended to be initiated in patients with HBV-DNA above 20.000 IU/mL and who had elevated ALT, regardless of fibrosis level[2]. World Health Organization stated in its guideline that treatment should be initiated regardless of HBeAg status if HBV-DNA level is >20.000 IU/mL in non-cirrhotic patients with elevated ALT levels over 30 years of age (6).

This study attempted to find a threshold level range for HBV-DNA at which hepatic damage began in patients that were divided into 5 groups based on a $\log^{10}$ increase of serum HBV-DNA levels between 10.000 (10^5) and 100.000.000 (10^8). The aim of finding a highly sensitive and specific threshold HBV-DNA level to predict moderate/advanced liver damage which would be an indication for treatment could not be achieved in the statistical analysis. Low HBV-DNA levels in patients with mild liver damage, although statistically significant, were already expected (7). Unexpectedly, only in the last group, ie., in the group with HBV-DNA level higher than 10^8, the number of patients with low grade and stage was found to be higher than in the previous subgroup (10^7-10^8). This can be explained by the presence of, although in small numbers, immunotolerant patients in this group.

Similar studies have shown that serum HBV-DNA correlates with necroinflammation and fibrosis, and that as the levels of HBV-DNA increase, the risk of cirrhosis significantly increases. In a study published by Iloeje et al. (8) in 2006, 365 out of 3582 patients with chronic hepatitis B were diagnosed with cirrhosis in 11 years of follow-up and the incidence of cirrhosis was found to be 4.5% in patients with hepatitis B viral load less than 300 copies/mL and 36.2% in patients with hepatitis B viral load more than 106 copies/mL (p<0.001). In a study by Nabuco et al. (9) conducted for similar purposes in 78 blood donors who were HBsAg-positive, HBV-DNA levels were significantly higher in patients with chronic hepatitis or cirrhosis compared with patients without histologic hepatic disease, although histologic lesions were mild in the majority of patients (25.260.000 vs 9480 copies/mL; p<0.001). There was also a significant correlation between HBV-DNA levels and necroinflammatory score (r=0.59) and fibrosis (r=0.50). However, 25% of the subgroup (of HBeAg-negative patients) with HBV-DNA levels less than 30.000 copies/mL was reported to have HBV-related histologic disease.

Similarly, there are many studies showing a significant relationship between HBV levels and risk for hepatocellular carcinoma, and these studies emphasize the relationship between the level of HBV-DNA and the severity of the histological lesion in the course of HBV infection (10,11). In the study by Chen et al. (10), the incidence rate of hepatocellular carcinoma was 1.3% when HBV-DNA level was 300 copies/mL and lower vs.

14.9% when HBV-DNA level was one million copies/mL and above. However, there are also studies showing that there is no correlation with the extent of hepatic necro-inflammatory activity or fibrosis in patients with chronic HBV infection, and that some patients have progressive liver disease although HBV-DNA levels are undetectable and ALT levels are consistently within normal ranges (12).

Determination of hepatic histology by liver biopsy in patients with chronic hepatitis B is an important predictor of disease progression. However, in recent years, the avoidance of invasive methods such as liver biopsy, alternatively employing non-invasive techniques such as serum fibrotic markers or fibroscans have been proposed in the guidelines as well (2). The most important non-invasive method leading the diagnostic and therapeutic methods used in chronic hepatitis B infection is the measurement of HBV-DNA level. With the introduction of sensitive molecular diagnostic tests, particularly PCR based on amplification of the target working principle, HBV-DNA was measured at detectable levels in the majority of individuals with chronic HBV infection, including those who were inactive carriers (13). These results give rise to important questions regarding the relationship of HBV-DNA levels measured by non-invasive methods with hepatic histopathology of patients diagnosed with clinically significant and chronic HBV infection.

Study Limitations

Although a correlation was found between viral load level and histopathology in our study, a sensitive and specific cut-off value could not be determined. One of the reasons for this is that there is no history of viral load, necroinflammation and fibrosis grouping in the literature, therefore they were performed subjectively. In addition, subgroup analysis was not performed based on patients' HBeAg status, ALT levels, age and history of alcohol use. Therefore, we think that statistically significant results can be obtained from advanced studies with subgroup analyzes performed and a different grouping method employed.

Conclusion

No threshold HBV-DNA level was found to determine the moderate/severe histopathological level. However, in group analysis, the histopathological relationship with DNA level were proportional. Liver histology in patients with chronic hepatitis B is an important predictor of disease progression.

Ethics

Ethics Committee Approval: This study was approved by Ege University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology (approval number: 60770832, date:15.01.2016).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.A., T.Y., H.P., M.T., Concept: D.A., T.Y., H.P., M.T., Design: D.A., T.Y., H.P., M.T., Data Collection or Processing: D.A., Analysis or Interpretation: D.A., T.Y., R.D., Literature Search: D.A., T.Y., Writing: D.A., T.Y.

Conflict of Interest: Authors declare no conflict of interest.

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Research Article

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Hepatitis B Surface Antigen Seroprevalence of Turkish and Foreign Patients of Reproductive Age in 2014-2017

Üreme Çağındaki Türk ve Yabancı Uyruklu Kadın Hastalarda 2014-2017 Yıllarındaki HBs Antijen Seroprevalansı

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ABSTRACT

Objectives: Cross-country migration may affect the prevalence of hepatitis B virus (HBV). Due to the recent war in Syria, there has been a serious exodus towards Turkey. In this study, it was aimed to investigate the hepatitis B surface antigen (HBsAg) seroprevalence among women of reproductive age of foreign origin, both Turkish and mostly Syrian immigrants.

Materials and Methods: In this study, we retrospectively evaluated the HBsAg results of 55.057 patients, mostly pregnant and aged between 15 and 49 years who presented to a Maternity and Children Hospital between January 1st, 2014, and December 31st, 2017.

Results: In both Turkish and foreign origin patients, the seropositivity of HBsAg was found to be 1.1%. However, in women over 40, those of foreign origin were higher than Turkish women.

Conclusion: Our region is low endemicity in terms of HBsAg seroprevalence in women of reproductive age. In addition, women of foreign origin are not different than women of Turkish origin in this respect. However, in future years, the migrant population may be disadvantaged if they do not receive adequate health care.

Keywords: HBsAg seroprevalence, Syrian refugees, reproductive age, women

ÖZ

Amaç: Ülkeler arası göç hepatit B virüs (HBV) yaygınlığını etkileyebilmektedir. Suriye'de son yıllarda yaşanan savaş nedeniyle, Türkiye'ye doğru ciddi bir göç yaşanmaktadır. Bu çalışmada, hem Türk, hem de çoğunluğu Suriyeli göçmenlerden oluşan yabancı kökenli üreme çağındaki kadınlarda hepatit B yüzey antijeni (HBsAg) seroprevalansının araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışmada, 1 Ocak 2014 ve 31 Aralık 2017 tarihleri arasında bir kadın doğum ve çocuk hastanesi'ne başvuran, çoğunluğu hamile olan, ve yaşları 15 ile 49 yaş aralığında bulunan 55,057 hastanın HBsAg sonuçları retrospektif olarak değerlendirildi.

Bulgular: Hem Türk hem de yabancı kökenli hastalarda HBsAg seropozitivitesi %1,1 olarak tespit edildi.Ancak 40 yaş üstü kadınlarda, yabancı kökenli olanların Türk kadınlarına göre daha yüksek olduğu görüldü.

Sonuç: Bölgemiz, üreme çağındaki kadınlarda HBsAg seroprevalansı açısından düşük endemisitededir. Ayrıca, yabancı kökenli kadınlar bu açıdan Türk kökenli kadınlardan farklı değildir. Bununla birlikte, gelecek yıllarda göçmen nüfusu yeterli sağlık hizmeti almazlarsa dezavantajlı durumda olabilir.

Anahtar Kelimeler: HBsAg seroprevalansı, Suriyeli göçmenler, üreme çağı, kadınlar

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Introduction

Hepatitis B virus (HBV) infection is still an important public health problem in the world. It is estimated that approximately two billion people across the world face HBV and 257 million people are chronic HBV carriers (1,2). HBV is transmitted through contact with skin and mucous membranes of infected blood and body fluids. Vertical transmission of HBV infection from the mother to the newborn is commonplace in areas with high endemicity, whereas in areas with middle endemicity, sexual transition is predominant (2). In the technical report of the European Centre for Disease Prevention and Control (ECDC) in 2010, Turkey was classed as a middle endemic region in terms of the prevalence of hepatitis B, and Hepatitis B Surface Antigen (HBsAg) seropositivity was reported to be approximately 5.2% (3). Although the risk of acute HBV infection is independent of age, the risk of chronicity of HBV infection is inversely proportional to the age at which the infection is transferred. The 90% rate of chronicity of HBV infection in the newborn decreases to 5% in adulthood (2,4).

Hepatitis B vaccination is the most effective way to protect against HBV infection and complications (5). Turkey added HBV vaccination to the routine childhood immunization program in 1998 (6,7). In addition, it was recommended that HBsAg screening should be performed for pregnant women during the first follow-up in the Prenatal Care Management Guidelines, and HBV vaccination is recommended for pregnant women with negative HBsAg and anti HBs during or after pregnancy (8).

In order to reduce the global prevalence, governments should be determined in screening and preventive measures, the awareness of individuals should be raised, and access of carriers to health services should be enabled. Assuming that approximately one-quarter of the world's population is women of reproductive age, it is possible for 65 million women to infect their babies with HBV (1). Although horizontal transmission is more frequent in Turkey, vertical transmission from mother to baby is also important (9,10).

HBsAg seropositivity among pregnant women was reported to be between 1.2% and 9.3% in Turkey over the last two decades (11). However, migration between countries may also affect the prevalence of HBV (1). Due to the war in Syria in recent years, significant immigration has occurred toward Turkey from this region (12).

In this study, we aimed to investigate the HBsAg seroprevalence among women of reproductive age of Turkish and foreign origin, the latterlargely comprising Syrian refugees.

Materials and Methods

Study setting

Kahramanmaraş is a city located in the Eastern Mediterranean Region of Turkey, with a population of 1.127.623. The population of women aged 15-49 years in the province of Kahramanmaraş is 283.949 (13). According to the Migration Report of the Republic of Turkey Ministry of Interior Directorate General of Migration Management, 86.964 Syrians were registered under temporary protection in Kahramanmaraş in 2016. There are also temporary shelter centers for Syrian refugees to Turkey in Kahramanmaraş and 17.968 Syrians reside in these shelters, receiving health services under the management of the Republic of Turkey Ministry of Health (12).

Ethical considerations

The study protocol was approved by Kahramanmaraş Sütçü İmam University Clinical Research Ethics committe (approval number: 02, date: 06.02.2019). Informed consent wasn't obtained.

Study type and participants

This study is planned in a descriptive design. In this study, the HBsAg results of 55.057 patients, the majority of whom were pregnant, aged between 15 and 49 years (reproductive period) who presented to a Maternity and Children Hospital between January 1st, 2014, and December 31st, 2017, were assessed. In the same year, repeated data of participants with multiple serum HBsAg concentrations were excluded. Following the removal of duplicate cases, the HBsAg results of 54.201 women were evaluated, retrospectively.

Measurement of seropositivity

HBsAg and Hepatitis B surface antigen antibody (anti-HBs) seropositivity rates were determined using ELISA. The values of the anti-HBs of 10 IU/mL and the HBsAg concentration of 1 IU/mL were considered to be positive.

Statistical Analysis

The independent variables of the study were the age and nationality of the patients. Descriptive statistics are expressed as number, mean, standard deviation, and percentage. The chi-square test and Spearman's Rho test were used for statistical analyses and $p<0.05$ was accepted as the level of statistical significance. Statistical analyses were performed using the SPSS 15.0 package program.

Results

Of the 54.201 patients, 42.679 (78.7%) were women of Turkish origin and 11.522 (21.3%) were of foreign origin. Of the foreign women, 11.361 (98.6%) were Syrian and the rest were women from other countries. The mean age of all women was 26.64±6.64 years. The mean age of the Turkish patients was 27.15±6.61 years and the mean age of the foreign women was 24.77±6.41 years.

The number of hospital admissions of female patients of Turkish and foreign origin according to years of admission is presented in Table 1.

HBsAg seroprevalence was determined as 1.1% for all patients. HBsAg seropositivity was found as 1.1% both in Turkish patients and foreign patients. HBsAg seroprevalence in women of Turkish origin was determined as 0.7%, 0.8%, 1.0%, 1.5% 1.5%, 2.1%, and 3.0% in the 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, and 45-49 age groups, respectively. In foreign women, it was 0.3%, 0.6%, 1.8%, 1.4%, 2.0%, 4.3%, and 4.7% in the abovementioned age groups. In both Turkish and foreign patients, the 15-19 years age group had the lowest seroprevalence rates, and the 45-49 years age group had the highest positivity (Table 2).

HBsAg seropositivity showed a significant increase with age. In the correlation analysis, there was a moderate relationship between age and HBsAg seropositivity at a significance level of $p<0.01$ (rho: 0.43). HBsAg seropositivity rates were not different between Turkish women and those of foreign origin in total, but for those aged 40 years or above, it was found to be higher in

women with foreign origin than in Turkish women (p=0.048). This is demonstrated in Figure 1.

Discussion

The ECDC report revealed that the highest endemicity (≥8%) for HBV was observed in South Asia, China, Indonesia, Nigeria, and Sub-Saharan Africa throughout the world. In the same report, Europe and the Middle East, in which Turkey is also located, were noted as mid-endemic (2-7%) (3). HBsAg seropositivity in women varies according to geographic region and ethnic groups (14).

In order to prevent vertical transmission, it is important to evaluate HBsAg seroprevalence in pregnant and reproductive age women (15). In this study, the HBsAg seroprevalence was found as 1.1% among women aged 15-49 years, who mostly comprised pregnant women. This ratio is consistent with the country results in low endemic regions (16).

The HBsAg seroprevalence determined in this study is consistent with other studies conducted on women of reproductive age in different regions of Turkey. In a retrospective study of pregnant women between 1995 and 2015, HBsAg seroprevalence was found to be 1.5% in 7605 pregnant women and HBsAg decreased from 2.6% to 0.8% over a 20-year period (17). In a study conducted in İstanbul, Turkey's most populous city, HBsAg seropositivity in pregnant women between 2008 and 2013 was determined as 1.2% (18). In another study conducted in pregnant women in İzmir in 2010-2011, the prevalence of HBsAg was found as 1.14% (19).

The CDC accept migrants as special groups in HBV epidemiology (20). More than 4 million refugees have migrated to Turkey during the civil war in Syria (12). In this study, the HBsAg seroprevalence in Syrian migrant women, who accounted for approximately one-fifth of the patients, was found as 1.1%, which is similar to Turkish women. In another study conducted in Turkish and Syrian pregnant women in 2015, the total HBsAg seropositivity was found as 1.4%, while this rate was 1.8% in Turkish pregnant women and 1.1% in Syrian pregnant women (21). In a study of pregnant women in Damascus, Syria, HBsAg seropositivity was found as 0.75% (22). HBsAg seropositivity in women undergoing premarital screening in Syria was found as 1.49% in 2011 and 0.68% in 2014 (23).

HBV vaccine was added to the national vaccination program in 1998 in Turkey. Additionally, a massive catch-up program was applied in middle and high school period, to those who were born after 1991. Thus, all people born after 1991 may practically be considered as vaccinated in terms of HBV (6). In our study, those who were considered to be vaccinated corresponded to the 15-19 and 20-24-year age groups. The seroprevalence of HBsAg in these two groups was 0.7% and 0.8%. For foreign women, the seroprevalence of HBsAg was found as 0.3% and 0.6% in the

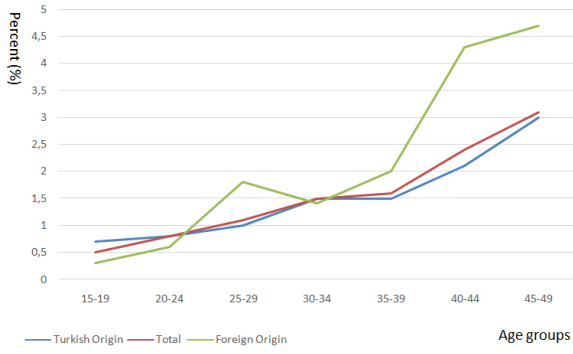

Figure 1. Hepatitis B surface antigen seropositivity by age groups

Table 1. Number of hospital admissions according to years of admission

	Turkish origin		Foreign origin	
Year of admission	**Number**	**%**	**Number**	**%**
2014	11.816	27.7	2214	19.2
2015	10.903	25.5	2572	22.3
2016	10.502	24.6	3022	26.2
2017	9.458	22.2	3714	32.3
Total	42.679	100.0	11.522	100.0

Table 2. Hepatitis B surface antigen seropositivity rates according to age groups

	HBsAg (+) Turkish origin		HBsAg (+) Foreign Origin	
Age groups (years)	**Number**	**%**	**Number**	**%**
15-19	34	0.7	7	0.3
20-24	97	0.8	23	0.6
25-29	105	1.0	45	1.8
30-34	124	1.5	22	1.4
35-39	69	1.5	16	2.0
40-44	28	2.1	10	4.3
45-49	15	3.0	2	4.7
Total	472	1.1	125	1.1

HBsAg: Hepatitis B surface antigen

15-19 and 20-24-year age groups, which may also be considered as mostly vaccinated because massive HBV vaccination began in Syria in 1994 (22). We believe this result is due to the fact that HBV vaccination programs in Turkey and Syria were put into practice at around the same time. In addition, HBsAg seroprevalence was found to be significantly higher in foreign women aged 40 years or above than in women of Turkish origin (Figure 1). However, there isinsufficient evidence in the literature to discuss why the vaccination rates in women of foreign origin are relatively lower after the fourth decade.

In our study, a significant correlation was found between age and HBsAg seropositivity. Seroprevalence decreased as age decreased (Figure 1). The lowest seroprevalence rates were found in the 15-19-year age group in Turkish and immigrant women (0.7% and 0.3%, respectively). In a study conducted on pregnant women by Furuncuoglu et al. (17), seroprevalence increased with increasing age. We believe that due to the natural flow of life, increasing age enables people to encounter more infectious agents.

Conclusion

Pregnant women make up a group that is capable of representing the reproductive age female population. Our results indicated that our region is low endemic in terms of HBsAg seroprevalence in women of reproductive age. Also, women of foreign origin are no different than Turkish women in this respect. However, in the upcoming years, the migrant population may be disadvantaged if they are not provided with adequate healthcare or do not receive adequate focus.

Ethics

Ethics committee approval: The study protocol was approved by Kahramanmaraş Sütçü İmam University Clinical Research Ethics Committe (approval number: 02, date: 06.02.2019).

Informed Consent: It wasn't obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.E., S.Ö., Design: A.E., A.R.Ş., R.A.O., Data Collection or Processing: A.E., S.Ö., Analysis or Interpretation: A.E., K.Y., R.A.O., Literature Search: A.E., K.Y., Writing: A.E., K.Y., A.R.Ş., R.A.O.

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Research Article

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Comparison of Qiagen and Iontek Hepatitis B Virus-DNA Polymerase Chain Reaction Quantitation Kits in Chronic Hepatitis B Patients Infected with Hepatitis B Virus Genotype D

Hepatit B Virüs Genotip D ile Enfekte Kronik Hepatit B'li Hastalarda Qiagen ve Iontek Hepatit B Virüs-DNA Polimeraz Zincir Reaksiyonu Kantitasyon Kitlerinin Karşılaştırılması

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ABSTRACT

Objectives: Hepatitis B virus (HBV)-DNA level is a good marker for viral replication and should be monitored at regular intervals in patients with chronic hepatitis B (CHB). The objective of this study is to compare the performance of Qiagen HBV-DNA kit with Iontek HBV-DNA kit and to determine the usability of Iontek.
Materials and Methods: Serum samples of 87 patients who had been identified as HBV genotype D previously, were sent to Kocaeli University for HBV-DNA quantitation. Serum HBV-DNA levels were determined by real-time polymerase chain reaction method using both systems simultaneously. The calculated viral load values were converted to logarithmic values and used for statistical comparison of two kits. T-test was used to study the statistical difference between two methods. The statistical comparison and the linearity between the two results were determined by Bland-Altman plot and Passing-Bablok analyses respectively.
Results: Log HBV-DNA results in Qiagen and Iontek kits were within the 95% confidence interval of the bias (-0.84; standard deviation: 0.67). There was no significant difference and the relationship between two variables was linear.
Conclusion: The comparative distribution analysis of Qiagen and Iontek kits indicated that a product produced in our country can be safely used in the treatment follow-up of patients with CHB. These type of studies may support the production of high value-added products in our country and also they can be utilized by other users in the world.
Keywords: Hepatitis B, real-time polymerase chain reaction, HBV-DNA

ÖZ

Amaç: Hepatit B virüs (HBV)-DNA düzeyi viral replikasyonun iyi bir göstergesidir ve kronik hepatit B'li (KHB) hastalarda düzenli aralıklarla izlenmelidir. Bu çalışmada amaç, Qiagen HBV-DNA viral yük kiti ve İontek HBV-DNA viral yük kitinin performans karşılaştırmasını yapmak ve İontek kitinin kullanılabilirliğini belirlemektir.
Gereç ve Yöntemler: Çalışmaya Kocaeli Üniversitesi'ne HBV-DNA kantitasyonları yapılmak üzere gönderilen ve önceden HBV genotip D olduğu tanımlanmış 87 hastanın serum örnekleri dahil edilmiştir. Serum HBV-DNA düzeyleri, her iki sistem de eş zamanlı kullanılarak, gerçek zamanlı polimeraz zincir reaksiyonu yöntemiyle belirlenmiştir. Elde edilen viral yük değerleri, logaritmik değer dağılımlarına çevrilmiş ve her iki kitin karşılaştırılmasında kullanılmıştır. İki metot arasındaki istatistiksel fark, t-testi kullanılarak araştırılmıştır. Her iki ölçüm arasındaki istatistiksel karşılaştırma Bland-Altman dağılım analizi ile belirlenirken, sonuçlar arasındaki doğrusallık Passing-Bablok dağılım analizi kullanılarak saptanmıştır.
Bulgular: Elde edilen sonuçlara göre Qiagen ve İontek kitlerinde, log HBV-DNA sonuçlarının %95 güven aralığı içinde yanlılık (-0,84, standart sapma: 0,67) uyumlu olduğu, anlamlı bir fark olmadığı ve iki değişken arasındaki ilişkinin doğrusal olduğu tespit edilmiştir.
Sonuç: Qiagen ve İontek HBV-DNA viral yük kitlerinin karşılaştırmalı dağılım analizleri bize ülkemizde üretilmiş bir ürünün, KHB'li hastaların tedavi takibinde güvenle kullanılabileceğini göstermektedir. Bu gibi çalışmalar, ülkemizde katma değeri yüksek ürünlerin üretilmesini desteklerken, bu tür ürünlerin dünyadaki diğer kullanıcılara da ulaştırılmasına imkan tanıyabilir.
Anahtar Kelimeler: Hepatit B, gerçek zamanlı polimeraz zincir reaksiyonu, HBV-DNA

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Introduction

The hepatitis B virus (HBV) affects more than 257 million people worldwide and is a potential life threat resulting in 880.000 deaths per year (1). The prevalence of HBV infection varies according to geographical regions around the world, but the highest seroprevalence of hepatitis B surface antigen (HBsAg) is reported in Africa (8.8%) and Western Pacific (5.3%) (1). The prevalence of chronic HBV ranges from 2% to 8% in Turkey and is located in intermediate- prevalence areas (2). It is estimated that HBV carriers in Turkey is 3.3 million and the overall HBsAg prevalence is 4.5% (3). The transmission of HBV can occur through various body fluids such as infected blood and blood products, from mother to baby, sexual contact with the unvaccinated individual, acupuncture, tattoo and/or saliva, vaginal secretions as well as seminal fluids (4). Today, it is tried to lower the risk of developing liver failure, cirrhosis and subsequent cancer with the follow-up and treatment of HBV infections (5).

Laboratory diagnosis of HBV infection can be performed by using different serological and molecular techniques as well as liver biopsy which plays an important role in treatment in patients with chronic hepatitis (CH) (6). Although liver biopsy is still considered as the gold standard for the determination of liver fibrosis and necro-inflammatory activity, laboratory methods are preferred because of temporary pain in the biopsy site, mild transient hypotension, as well as more serious complications such as bleeding, biliary peritonitis, bacteremia, sepsis, pneumothorax and rarely death (7). HBV infections can be identified by routine applications of serological and molecular markers (8). On the other hand, serum HBV-DNA levels that can be used in the treatment of chronic hepatitis B (CHB) can be measured by hybridization and polymerase chain reaction (PCR) based molecular techniques (9). Real-time PCR (rt-PCR) based molecular quantitation techniques such as Qiagen, Abbott, COBAS Ampliprep platforms are routinely used in our country (9). In order to determine viral load of HBV quantitatively, rt-PCR kits are also designed in our country.

In this study, we aimed to compare Qiagen HBV-DNA quantitative kit and Iontek HBV-DNA quantitative kit produced in our country by using sera of patients with chronic HBV infected with genotype D.

Materials and Methods

In this retrospective study, a total of 87 CHB patients infected with HBV genotype D were sent to the routine PCR unit of Kocaeli University Research and Application Hospital for HBV-DNA quantitation. HBV-DNA loads were obtained by using rt-PCR technique with Qiagen (Artus Hilden HBV QS RGQAR Qiagen, Germany) and Iontek (Iontek İstanbul, Turkey) kits simultaneously, according to the manufacturer's instructions and all tests were repeated twice for both kits. The obtained viral loads in IU/mL were converted into log IU/mL values that were used in the correlation analysis between Qiagen and Iontek assays.

The study was approved by the Clinical Research Ethics Committee of Kocaeli University (approval number: KAEK 2011/104). Since our study was retrospective, informed consent was not used.

Statistical Analysis

For the comparison Qiagen and Iontek HBV-DNA quantitation, HBV-DNA logarithmic (log) value distributions were determined and their mean and standard deviations (SD) were calculated. The statistical differences between HBV log values obtained from two kits, were calculated. The hypotheses constructed for the t-test used for this purpose were H0: the difference between the means was 0 and Ha: the difference between the means was different from 0. The confidence intervals (CI) between the obtained measurements were determined and the measurements were examined for linearity. The hypotheses designed for this purpose; H0; the relationship between the two variables is linear and Ha; The relationship between the two variables was determined as non-linear.

Significance of the difference between HBV-DNA log values of both kits was defined by p value (>0.5) according to t-test. The CI of the difference between the two measurements was calculated according to the Bland-Altman distribution. The linearity between the two measurements was determined by Passing-Bablok regression analysis.

For all statistical analyzes and figures, XLSTAT (Addinsoft Inc., New York, USA) statistical program was used.

Results

HBV-DNA loads were obtained by quantitative rt-PCR method in serum samples of 87 CHB patients infected with HBV genotype D. HBV loads were defined as IU/mL, but these values were converted to log values for all statistical analyzes. Log values obtained from the results of Qiagen and Iontek kits were used in this study.

HBV-DNA log values obtained with the kits of both manufacturers were compatible with each other (bias within 95% CI: -0.84; SD: 0.67). According to the serum HBV-DNA log value distributions, the most frequent viral load was detected in the range of 2.50-3.39, while the low frequency HBV-DNA loads were found in the values of 9.08-9.26 log. While the viral load was detected in 2 samples with 0.73 log values with the Iontek kit, no viral load of the same value was detected with the Qiagen kit. The distribution of frequency and log values of the patient samples is shown in Table 1. Qiagen and Iontek HBV-DNA quantitation log distribution graph is demonstrated in Figure 1.

The mean ± SD values for Qiagen and Iontek kits were found as 3.7950±2.0579 and 3.7127±1.9598 respectively. According to t-test, p value was found to be 0.0505>0.5.

Bland - Altman plot shows statistical comparison between two measurements. The bias and the standard error were found as -0.0824 and 0.3873. The CI for the difference between the two measurements was found to be (-0.8415; 0.6768). Bland-Altman distribution analysis is shown in Figure 2.

The Passing - Bablok test was used to determine the linearity between the two test results. In the Passing - Bablok regression analysis, p value was measured as 0.6190> 0.5. Qiagen and Iontek HBV-DNA log values were determined within 95% CI. Passing - Bablok linearity analysis is given in Figure 3.

Table 1. Frequency and value distribution of patient samples in the comparison of Qiagen and Iontek quantitation

	Qiagen		Iontek	
HBV-DNA loads (IU/mL)	**Frequency, n**	**Log-mean***	**Frequency, n**	**Log-mean***
10^1	-	-	2	0.73
10^1-<10^2	7	1.69	10	1.67
10^2-<10^3	32	2.50	24	2.54
10^3-<10^4	23	3.31	28	3.39
10^4-<10^5	7	4.19	5	4.39
10^5-<10^6	5	5.67	6	5.61
10^6-<10^7	3	6.38	2	6.31
10^7-<10^8	3	7.89	4	7.48
10^8-<10^9	5	8.59	5	8.26
10^9-<10^{10}	2	9.08	1	9.26
Total	87		87	

*HBV-DNA log values (bias: 95%; confidence interval: -0.84; standard deviation: 0.67)
HBV: Hepatitis B virus

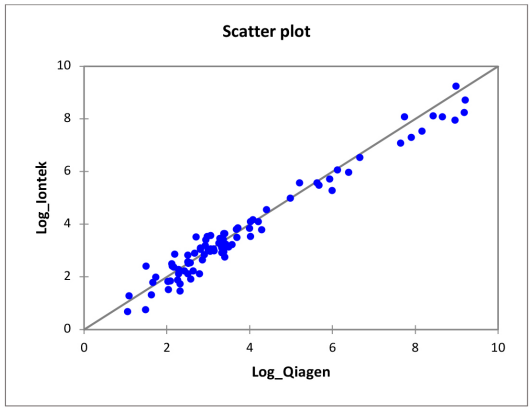

Figure 1. Qiagen and Iontek hepatitis B Virus-DNA quantitation log distribution graph

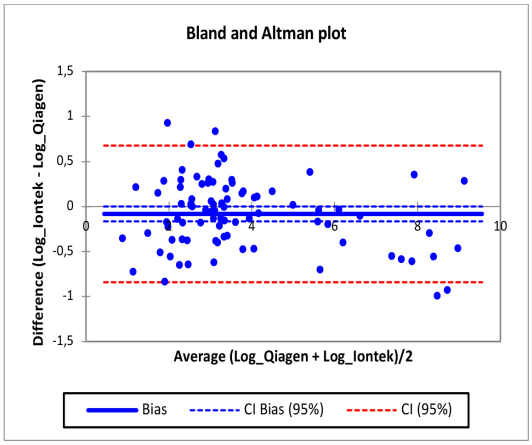

Figure 2. Bland Altman distribution. Statistical comparison of Qiagen and Iontek hepatitis B Virus-DNA quantitation within 95% confidence interval

CI: Confidence interval

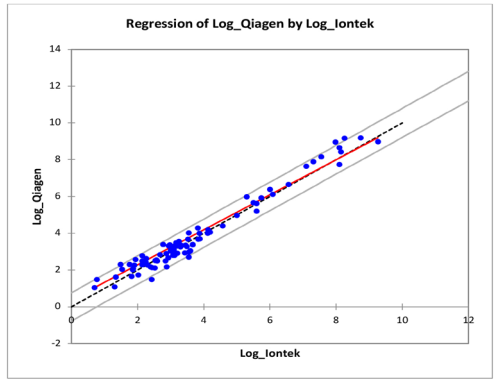

Figure 3. Passing - Bablok regression distribution. Linearity comparison in Qiagen and Iontek hepatitis B Virus-DNA quantitation

Discussion

HBV-DNA viral load analyzes used to confirm HBV infection and to evaluate antiviral response are considered to be a good indicator of viral replication and are often expressed using different units such as copies/mL or IU/mL (5,10,11,12). On the other hand, the World Health Organization recommends that serum HBV-DNA concentration to be expressed as standard IU/mL in monitoring the success of oral antiviral therapy in CHB patients (13). However, in order to express the performance characteristics of kits or methods as standard in comparative analyzes, viral load values are used by converting them to log values (14,15,16). In this study that focused on comparing Qiagen and Iontek HBV-DNA quantitation kits, HBV-DNA loads were defined in IU/mL, but these results were converted to log values for all statistical analyzes. In the HBV log value distribution graph, the correlation between the two variables was found to be consistent (Figure 1). There was no statistically significant difference between the results (p value: 0.0505). According to our findings, the hypothesis H0: (difference between the averages was 0) was accepted, whereas the Ha hypothesis (difference between the averages was different from 0) was rejected.

The reliability and reproducibility of any test kit and/or method must be measured before routine use. Therefore, the difference between the values obtained should be expected to be acceptable (17). In this study, distribution analyzes were used to understand the agreement between the two kits. For this purpose, Bland-Altman and Passing-Bablok analyzes were used. According to our findings, HBV-DNA log values obtained with Qiagen and Iontek kits were statistically compatible in Bland-Altman distribution within 95% CI (-0.84 and -0.67). In addition, the HBV-DNA log values obtained with the Qiagen and Iontek kits were linear according to the Passing Bablok distribution (p value; 0.6190). Bland Altman and Passing - Bablok distribution analyzes are the methods used to investigate the harmony between different methods or variables which are accepted as the gold standard in the statistical analysis of the comparison studies (18).

It is aimed to improve quality of life of people with CHB with lifelong follow-up and treatment. Often oral antivirals and rarely immunoregulatory agents (peg - interferon) are used in the treatment of CHB for this purpose (5,19). Oral antiviral therapy is based on lifelong HBV suppression and reduction of liver inflammation. However, serum HBV-DNA levels should be checked at regular intervals and treatment response should be monitored in patients with CHB during treatment (5). Many commercial kits are currently used to measure viral load by rt-PCR based technique (9). It will be beneficial for our country to produce analysis kits that can be used in molecular diagnosis. In many studies, Qiagen kits were compared with different platforms such as Roche, Abbott, DxN VERIS in terms of business model or performance characteristics (9,12,20). However, our knowledge about the performance characteristics of CE marked Iontek HBV-DNA quantitation kit that is produced in our country is limited. Our findings indicate that results of Iontek HBV-DNA rt-PCR kit are compatible with Qiagen rt-PCR kit results HBV-DNA in serum samples of patients with chronic HBV infected with HBV genotype D.

Conclusion

In conclusion, comparative distribution analysis of Qiagen and Iontek HBV-DNA viral load kits shows us that a product from our country can be used safely in the treatment follow-up of CHB patients. Such studies may be useful in supporting the production of high value-added products in our country while being delivered to other users in the world.

Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Kocaeli University (approval number: KAEK 2011/104).

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

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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

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Evaluating The Frequency of Autoantibodies in Patients with Chronic Hepatitis B

Kronik Hepatit B Hastalarında Otoantikor Sıklığının Değerlendirilmesi

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ABSTRACT

Objectives: The objective of this study is to evaluate the frequency of autoantibodies retrospectively in newly diagnosed chronic hepatitis B (CHB) patients.
Materials and Methods: The study was retrospectively conducted in between Jan 2010 and August 2015 in a research and training hospital in İstanbul with 122 patients (ages of 17-80) consulted to Gastroenterology and Infection Diseases and diagnosed with CHB and 117 healthy control group. In both groups, positive and negative rates of anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-mitochondrial antibody (AMA) and anti-liver-kidney mitochondrial antibody (anti-LKM) were compared.
Results: No AMA and LKM1 were observed in any patient or control groups. ANA result was positive in 9.8% of the patient group and 8.5% of the control group; and there was no statistically significant difference between them (p>0.05). ASMA result was positive in 5.7% of the patient group and 0.9% of the control group; and the difference between them was very close to significance but statistically not enough to be significant (p>0.05). Hepatosteatosis level of the patient group was significantly lower than the control group, statistically (p<0.05).
Conclusion: In this study, it is concluded that the low frequency of autoantibody may depend on the fact that it was only examined in CHB patients who are not receiving any treatment. Examination of autoantibodies in newly diagnosed chronic hepatitis B patients before and after treatment may provide insight in terms of occuring autoimmune phenomena cases and extrahepatic findings.
Keywords: Hepatosteatosis, chronic hepatitis B, autoantibody

ÖZ

Amaç: Bu çalışmada yeni tanı konulmuş kronik hepatit B (KHB) hastalarında otoantikor sıklığını retrospektif olarak değerlendirmek amaçlanmıştır.
Gereç ve Yöntemler: Çalışma Ocak 2010-Ağustos 2015 yılları arasında, İstanbul ilindeki bir eğitim araştırma hastanesinin gastroenteroloji ve enfeksiyon hastalıkları polikliniklerine başvuran, KHB tanısı alan 17-80 yaş arası 122 hasta ve 117 sağlıklı kontrol grubu ile retrospektif olarak gerçekleştirilmiştir. Her iki grupta anti-nükleer antikor (ANA), anti-düz kas antikoru (ASMA), anti-mitokondriyal antikorlar (AMA) ve anti-karaciğer-böbrek mitokondriyal antikoru (anti-LKM) pozitiflik ve negatiflik oranları karşılaştırılmıştır.
Bulgular: Hasta ve kontrol grubundaki hiçbir olguda AMA ve LKM1 görülmemiştir. Hasta gruptaki olguların %9,8'i ve kontrol grubundaki olguların da %8,5'inde ANA sonucu pozitif olup, aralarında istatistiksel olarak anlamlı bir farklılık bulunmamıştır (p>0,05). Hasta gruptaki olguların %5,7'si ve kontrol grubundaki olguların da %0,9'unda ASMA sonucu pozitif olup; aralarındaki farklılık anlamlılığa çok yakın olmasına karşın istatistiksel olarak anlamlı bulunmamıştır (p>0,05). Hasta grubun hepatosteatoz düzeyi, kontrol grubundan istatistiksel olarak anlamlı düzeyde düşük bulunmuştur (p<0,05).
Sonuç: Bu çalışmada gerçekleştirilen araştırmalar göstermektedir ki, otoantikor sıklığının düşük olması, yalnızca tedavi almayan KHB hastalarında bakılmasına bağlı olabilmektedir. Yeni tanı alan kronik hepatit B hastalarında tedavi öncesi ve sonrası otoantikor bakılmasının; ortaya çıkabilecek otoimmün olaylar ve ekstrahepatik bulgular açısından fikir verebileceği değerlendirilmiştir.
Anahtar Kelimeler: Hepatosteatoz, kronik hepatit B, otoantikor

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Introduction

Chronic Hepatitis B (CHB) infection is an important morbidity and mortality reason worldwide. Approximately 400 million people are infected with this virus and 1 million people die every year due to the complications associated with CHB infection such as liver cirrhosis, and hepatocellular carcinoma (1,2). Hepatitis B virus (HBV) have various antigenic molecules such as 42 nm partial double-stranded deoxyribonucleic acid (DNA) molecules surrounded with core proteins, hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg). The presence of HBsAg positivity in the blood for more than 6 months is defined as chronic HBV infection. CHB infection has a wide clinical spectrum from asymptomatic carriage to fulminant liver failure. Even though it is a hepatotropic virus, many other organ systems are also affected as a result of the autoimmunity that emerged as a result of its antigenic molecules. Many previous studies have revealed these extrahepatic involvements. Among them, the most commonly known are membranous glomerulonephritis and systemic necrotizing vasculitis (3,4).

Autoantibodies are the antibodies that are produced against the proteins, nucleic acids, carbohydrates, lipids and complex molecules of an organism. They benefit not only in diagnosis but also in progression. During CHB infection, autoantibody increase is commonly seen (5). It is known that these non-specific autoantibodies develop against some antigens of HBV and as a result of the inappropriate-excessive immune system activation of the host. As a result of the cross reaction between the viral and host antigens via the molecular similarity theory, it is revealed that high autoantibody may occur in the blood and in various tissues (6). These antibodies may cause a patient infected with HBV to be misdiagnosed with autoimmune hepatitis, toxic hepatitis, or systemic lupus erythematosus. High autoantibody in the individuals infected with HBV can be comorbid with a disease or it may be associated only with HBV (7). The aim of the present study is to determine the autoantibody frequency in newly diagnosed CHB patients.

Materials and Methods

In order to conduct the study, approval was taken from the Ethics Committee of the Ministry of Health Bağcılar Training and Research Hospital (approval number: 2015/414). Each of the patients included in the study signed an "informed consent form" that provides information about the study and states that the consent of the patient is taken. In the study, autoantibody frequencies of 122 patients who applied to the departments of Internal Medicine, Gastroenterology and Infectious Diseases outpatient clinic of a training and research hospital within the boundaries of İstanbul city between January 2010 and August 2015, were aged between 17 and 80 years, and newly diagnosed with CHB were retrospectively assessed. Demographic information of the patients was recorded from the patient files. Hepatic activity index (HAI) and fibrosis levels of the patients, who underwent liver biopsy, were recorded. HBsAg, anti-HBs, HBeAg, anti-HBe, HBV-DNA, alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin time, albumin, anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-mitochondrial antibody (AMA), and anti-liver-kidney mitochondrial antibody (anti-LKM) among the laboratory parameters were recorded and the frequency was statistically detected in CHB patients. One hundred seventeen healthy individuals who applied to the internal medicine and infectious diseases outpatient clinic and had no chronic diseases were included in the control group in the study. Healthy individuals were informed about the study and their consents were obtained. AMA, ANA, ASMA and anti-LKM examinations of the control group were requested and whole abdomen ultrasonographies were taken and hepatic lipidosis grades were detected. The patients who previously received interferon or oral anti-viral therapy, used alcohol or any drugs or toxic substance that may cause autoantibody positivity, were diagnosed with CHC, delta hepatitis or any viral or bacterial infections, liver cirrhosis or hepatocellular carcinoma, were diagnosed with rheumatologic diseases that involves liver and may cause autoantibody increase such as autoimmune hepatitis, primary biliary cirrhosis, autoimmune cholangitis, primary sclerosing cholangitis, had cardiac, renal and liver failures, and were pregnant were excluded from the study. 0.3 mL serum samples of ANA, ASMA, anti-LKM and AMA kits were sent and they were studied with Immune Fluorescent Technique in the Helios Helmed Integrated Optical System (AESKU. SYSTEMS) device. Hemogram was examined via the Multiangle Polarized Scatter Separation method in the Cell Dyn Ruby (ABBOTT) device. HBsAg, anti-HBs, anti-HVC, and anti-human immunodeficiency virus were studied by the microelisa method in Microelisa Analyser (DIASORIN). Biochemical tests such as ALT, AST, albumin, creatinine, and glucose were performed via photometric method in the Roche Hitachi-Cobas C systems device. Thyroid stimulating hormone, T4, triglyceride and total cholesterol were examined using the electrochemiluminescence immunoassay method in Roche Hitachi-Cobas C systems device.

Statistical Analysis

While assessing the results obtained in the study, IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for the statistical analyses. Shapiro-Wilk test was used to assess whether or not the parameters were normally distributed. In the data assessment, descriptive statistical methods (mean, standard deviation, frequency) were used. Additionally, Student's t-test was used to make comparison of normally distributed parameters between two groups in the quantitative data. Mann-Whitney U test was used to make comparison of non-normally distributed parameters between two groups. Chi-square test, Fisher's exact, chi-square test, and Yates' correction for Continuity were used for the comparison of qualitative data. Significance was assessed at the level of $p<0.05$.

Results

The study was conducted on a total of 239 cases, who were aged between 17 and 70. 97 (40.6%) of the participants were male and 142 (59.4%) were female. Mean age of the cases was 37.45±9.52 years. No statistically significant difference was found between female and male cases in the patient and control groups in terms of mean age and mean body mass index (BMI), gender distribution, smoking and alcohol use rates ($p>0.05$). (Table 1).

There was no statistically significant difference between the male and female cases in the patent group in terms of diabetes and hypertension prevalence (p>0.05). (Table 2). Anti-HBe was positive in 104 (85.2%) of those in the patient group and mean HBV-DNA level was 50495433±237566076.1, mean HAI score was 6.52±2.28, and mean fibrosis score was 1.80±1.12 (Table 3). AMA and LKM1 were not observed in any of the cases in the patient and control groups. ANA result was positive in 9.8% of the cases in the patient group and 8.5% of the cases in the control group. ASMA was positive in 5.7% of the cases in the patient group and 0.9% of the cases in the control group and no statistically significant difference was found between them (p>0.05). Mean blood glucose, AST, ALT and creatinine values of the patients were significantly higher than the control group (p<0.01; p<0.05). No significant difference was found between the groups in terms of haemoglobin, haematocrit, platelet, gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), albumin, globulin, total cholesterol, and triglyceride levels (p>0.05). It was found that ultrasonography (USG) hepatosteatosis level of the patient group was lower than the control group (p<0.01) (Table 4). No significant difference was found between the cases with positive and negative ANA and ASMA outcomes in the patient group in terms of age, BMI, USG, total cholesterol and triglyceride levels and gender distribution (p>0.05). No significant difference was found between the cases with positive and negative ANA outcomes in the control group in terms of age, BMI, USG, total cholesterol levels, and gender distributions (p>0.05). Triglyceride levels of the cases with negative ANA result were found to be significantly higher than the cases with positive ANA result (p<0.05) (Table 5).

No significant difference was observed between the HAI and fibrosis levels of the cases with positive and negative ANA and ASMA results in the patient group (p>0.05).

Table 1. Assessment of the groups in terms of demographic characteristics

	Patient (n=122)	Control (n=117)	Total (n=239)	p
Age (Mean ± SD)	37.61±8.73	37.28±10.32	37.45±9.52	[1]0.79
BMI (Mean ± SD)	27.08±4.81	27.11±5.11	27.10±4.95	[1]0.96
Male age (Mean ± SD)	38.52±8.84	38.19±11.30	38.36±10.05	[1]0.87
Male BMI (Mean ± SD)	26.74±4.31	27.64±4.44	27.17±4.37	[1]0.31
Female age (Mean ± SD)	36.97±8.66	36.67±9.65	36.82±9.13	[1]0.84
Female BMI (Mean ± SD)	27.33±5.15	26.76±5.52	27.05±5.33	[1]0.52
Gender n%				
Male	50 (41%)	47 (40.2%)	97 (40.6%)	[2]0.89
Female	72 (59%)	70 (59.8%)	142 (59.4%)	
Smoking n%	38 (31.1%)	25 (21.4%)	63 (26.4%)	[2]0.08
Alcohol n%	4 (3.3%)	2 (1.7%)	6 (2.5%)	[3]0.68

[1]Student t test, [2]chi-square test, [3]Fisher's exact test
SD: Standard deviation, BMI: Body mass index

Table 2. Assessment of diabetes and hypertension in the patient group in terms of gender

Patient group	Male (n=50)	Female (n=72)	Total (n=122)	p
	n (%)	n (%)	n (%)	
Diabetes	4 (8%)	4 (5.6%)	8 (6.6%)	0.715
Hypertension	3 (6%)	3 (4.2%)	6 (4.9%)	0.688

Fisher's exact test

Table 3. Assessment of distribution of the hepatitis B data in the patient group

Patient group		n	%
HBeAg (IU/mL)	Positive	18	14.8
	Negative	104	85.2
Anti-HBe (IU/mL)	Positive	104	85.2
	Negative	18	14.8
	Minimum	**Maximum**	**Mean ± SD (median)**
HBV-DNA (IU/mL)	1795	2100553841	50495433±237566076.1 (25340)
HAI	2	12	6.52±2.28 (6)
Fibrosis	0	6	1.80±1.12 (2)

HbeAg: Hepatitis B e antigen, HAI: Hepatic Activity index, SD: Standard deviation

HbeAg outcome was positive in 8.3% of the cases with positive ANA result and in 15.5% of the cases with negative ANA result in the patient group, and anti-HBe result was positive in 83.6% of the cases with negative ANA result and in 100% of the cases with positive ANA result and no significant difference was found between them (p>0.05). HbeAg result was positive in 15.7% of the cases with negative ASMA result and in 0% in the cases with positive ASMA result in the patient group. Anti Hbe result was positive in 84.3% of the cases with negative ASMA result and in 100% of the cases with positive ASMA result in the patient group and no significant difference was seen between them (p>0.05).

Discussion

There is a large number of evidence indicating that CHB and CHC infections may cause extrahepatic findings, autoantibody formation, and autoimmune diseases. Numerous studies have reported autoantibody frequency at different rates in the individuals infected with hepatitis C. In a study, the prevalence of autoantibodies was investigated in the CHB and CHC patients with positive HBV-DNA and HCV-RNA results who did not receive any treatment. A total of 63 patients were included in the study as 30 diagnosed with CHC and 33 diagnosed with CHB. RF was determined as 30%, ANA as 10%, and AMA as 6.7% in CHC patients; on the other

Table 4. Assessment of the autoantibody positivities, laboratory data and ultrasonography hepatosteatosis results of the groups

	Patient (n=122)	Control (n=117)	Total (n=239)	p
	n (%)	n (%)	n (%)	
AMA	0 (0%)	0 (0%)	0 (0%)	-
ANA	12 (9.8%)	10 (8.5%)	22 (9.2%)	0.90
ASMA	7 (5.7%)	1 (0.9%)	8 (3.3%)	0.06
LKM1	0 (0%)	0 (0%)	0 (0%)	-

Continuity (Yates) correction and Fisher's exact test were used.
AMA: Antimitochondrial antibody, ANA: Antinuclear antibody, ASMA: Anti-smooth muscle antibody

Table 4. Contuniued

	Patient (n=122)	Control (n=117)	Total (n=239)	p
	Mean±SD	Mean±SD	Mean±SD	
HgB (g/dL)	13.66±1.84	13.68±1.78	13.67±1.81	10.90
Htc (%)	41.84±5.3	41.44±4.93	41.64±5.11	10.53
Plt (K/mL)	243.66±65.47	256.63±58.78	250.01±62.49	10.10
Blood glucose (mg/dL) (median)	98.34±45.97 (89.5)	86.9±9.09 (86)	92.74±33.87 (88)	20.009**
AST (U/L) (median)	31.69±22.01 (27)	20.71±6 (20)	26.31±17.15 (22)	20.001**
ALT (U/L) (median)	42.45±33.72 (30.5)	22.55±14.2 (19)	32.71±27.85 (24)	20.001**
GGT (U/L) (median)	22.97±17.64 (17)	20.83±20.41 (14)	21.92±19.04 (16)	20.12
ALP (U/L)	71.41±21.65	74.46±23.13	72.9±22.39	10.29
Creatinine (mg/dL)	0.72±0.19	0.66±0.15	0.69±0.18	10.015*
Alb (g/dL)	4.51±0.45	4.5±0.36	4.51±0.41	10.91
Globulin (g/dL)	3.05±0.48	3.05±0.38	3.05±0.43	10.91
T. Cholesterol (mg/dL) (median)	175.53±33.22	174.32±42.07	174.94±37.74	10.8
Triglyceride (mg/dL) (median)	121.28±79.17 (101.5)	133.32±89.44 (114)	127.18±84.39 (106)	20.95

[1]Student's t-test, [2]Mann-Whitney U test, * p<0.05, **p<0.01
HgB: Hemoglobin B, HCT: Hematocrit, PLT: Platelet, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transpeptidase, ALP: Alkaline phosphatase

Table 4. Contuniued

	USG hepatosteatosis (grade)		p
	Mean ± SD	Median	
Patient (n=122)	0.52±0.72	0	0.004**
Control (n=117)	0.83±0.87	1	
Total (n=239)	0.67±0.81	0	

Mann-Whitney U test, **p<0.01, USG: Ultrasonography, SD: Standard deviation

hand, RF was found as 24.2% and ANA as 3% in CHB patients. LKM, SMA, and anti dsDNA were not observed in any of the both groups (8). Panasiuk (9), found that the rate of autoantibodies (ANA, AMA, ASMA) which were examined via the IFA method between the patients infected with hepatotropic viruses (HBV, HCV) and the patients with chronic liver disease was 23-28%. This rate

Table 5. Assessments regarding the ANA positivity in the control group and the ANA and ASMA in the patient group

Patient group	**ANA**		**p**
	Negative (n=110)	**Positive (n=12)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
Age	37.07±8.44 (37)	42.5±10.14 (38.5)	[1]0.12
BMI (kg/m²)	26.94±4.85 (26.52)	28.43±4.51 (28.03)	[1]0.22
USG hepatosteatosis	0.54±0.74 (0)	0.42±0.51 (0)	[1]0.80
T.Cholesterol (mg/dL)	175.71±33.9 (172)	173.92±27.29 (171.5)	[1]0.93
Triglyceride (mg/dL)	120.31±81.06 (100)	130.17±61.18 (131.5)	[1]0.26
Gender n%			
Male	44 (40%)	6 (50%)	[2]0.54
Female	66 (60%)	6 (50%)	

[1]Mann-Whitney U test, [2]Fisher's exact test.
ANA: Antinuclear antibody, BMI: Body mass index, SD: Standard deviation, USG: Ultrasonography

Table 5. Contuniued

Control group	**ANA**		**p**
	Negative (n=107)	**Positive (n=10)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
Age	37.42±10.37 (39)	35.8±10.18 (36.5)	[1]0.67
BMI (kg/m²)	27±4.79 (26.96)	28.25±8.11 (25.87)	[1]0.98
USG hepatosteatosis	0.79±0.85 (1)	1.3±1.06 (1)	[1]0.10
T. Cholesterol (mg/dL)	173.06±41.58 (172)	187.8±47.21 (193)	[1]0.38
Triglyceride (mg/dL)	137.79±91.6 (123)	85.5±38.78 (68.5)	[1]0.03*
Gender n%			
Male	44 (41.1%)	3 (30%)	[2]0.73
Female	63 (58.9%)	7 (70%)	

[1]Mann-Whitney U test, [2]Fisher's exact test, *p<0.05.
ANA: Antinuclear antibody, BMI: Body mass index, SD: Standard deviation, USG: Ultrasonography

Table 5. Contuniued

Patient group	**ASMA**		**p**
	Negative (n=115)	**Positive (n=7)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
Age	37.4±8.59 (37)	41±10.92 (37)	[1]0.52
BMI (kg/m²)	27.08±4.85 (26.64)	27.25±4.53 (26.72)	[1]0.83
USG hepatosteatosis	0.55±0.73 (0)	0.14±0.38 (0)	[1]0.13
T. Cholesterol (mg/dL)	175.07±33.8 (172)	183.14±21.7 (169)	[1]0.43
Triglyceride (mg/dL)	122.48±80.94 (101)	101.57±38.01 (108)	[1]0.83
Gender n%			
Male	48 (41.7%)	2 (28.6%)	[2]0.69
Female	67 (58.3%)	5 (71.4%)	

[1]Mann-Whitney U test, [2]Fisher's exact test.
ASMA: Anti-smooth muscle antibody, BMI: Body mass index, SD: Standard deviation, USG: Ultrasonography

was found as 25% in the individuals with non-infectious chronic liver disease. In the study by Volchkova et al. (10), to examine the autoimmune parameters in acute viral hepatitis, SMA and ANA were diagnostically found at significant titers in the patients with acute viral hepatitis A, B and C. Codes et al. (11), investigated ANA and ASMA autoantibodies in the patients with acute viral hepatitis and found that autoantibodies could be found in acute viral hepatitis but they did not have any prognostic significance. In their study, Tage-Jensen et al. (12), observed that among the circulating autoantibodies such as LMA, SMA, ANA and AMA, ANA was dominant in the acute-phase serums of the patients with late progressing chronic liver disease. In Turkey, Afşar et al. (13), determined the frequency of ANA as 65.3%, AMA as 3%, SMA as 7% and LKM as 1% in 98 patients infected with CHC and found the frequency of ANA as 66.6% and SMA as 12.6% in 102 patients injected with CHB and the autoantibody frequency in the control group including healthy individuals as 14.4%. In their study, Bayram et al. (14), investigated ANA by using indirect fluorescent antibody (IFA) method in serum samples and HBsAg and anti-HBc immunoglobulin G (IgG) by using enzyme immunoassay method. As a result of the study, it was found that ANA was positive in 46 (54.7%) of a total of 84 patients. HBsAg and anti-HBc IgG were positive in a total of 20.2% (17/84) of the study group as 23.7% (9/38) of the patients with negative ANA and 17.4% (8/46) of the patients with positive ANA (14). The incidence rate of HBV in the patients included in the study was not different between the groups with positive and negative ANA and was higher in total compared to the normal population. It is considered that this result

Table 6. Assessment of HAI and fibrosis, HBeAg and Anti HBe positivity according to the ANA and ASMA positivity in the patient group

Patient group	**ANA**		**p**
	Negative (n=110)	**Positive (n=12)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
HAI	6.48±2.30 (6)	6.92±2.19 (6.5)	0.47
Fibrosis	1.76±1.11 (2)	2.17±1.19 (2)	0.25

Mann-Whitney U test.
ANA: Antinuclear antibody, HAI: Hepatic activity index, SD: Standard deviation

Table 6. Contuniued

Patient group	**ASMA**		**p**
	Negative (n=115)	**Positive (n=7)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
HAI	6.57±2.26 (6)	5.71±2.75 (6)	0.45
Fibrosis	1.81±1.11 (2)	1.71±1.25 (1)	0.87

Mann-Whitney U test.
ASMA: Anti-smooth muscle antibody, HAI: Hepatic activity index, SD: Standard deviation

Table 6. Contuniued

Patient group	**ANA**		**p**
	Negative (n=110)	**Positive (n=12)**	
	n (%)	**n (%)**	
HBeAg (IU/mL)	17 (15.5%)	1 (8.3%)	1.000
Anti-HBe (IU/mL)	92 (83.6%)	12 (100%)	0.210

Fisher's exact test.
ANA: Antinuclear antibody, HbeAg: Hepatitis B e antigen

Table 6. Contuniued

Patient group	**ASMA**		**p**
	Negative (n=115)	**Positive (n=7)**	
	n (%)	**n (%)**	
HBeAg (IU/mL)	18 (15.7%)	0 (0%)	0.592
Anti-HBe (IU/mL)	97 (84.3%)	7 (100%)	0.592

Fisher's exact test.
ASMA: Anti-smooth muscle antibody, HbeAg: Hepatitis B e antigen

may be related to the exposure of the patients in the selected group to frequent invasive procedures for chemotherapy and/or for diagnostic purposes.

In their study, Unal et al. (15), assessed the prevalence of immunomodulator and also cellular and humoral immune parameters and the prevalence of autoantibodies before the antiviral treatment in the patients with chronic HBV infection and they found ANA positivity as 18.2%. According to the data of this study, the formation of ANA is a part of the natural course of chronic HBV infection and may indicate the importance of clinical follow-up with the predisposition towards the autoimmune diseases. In their study, Michalska et al. (16), showed that the patients infected with HBV and HCV may explicitly show the clinical features of autoimmune diseases and thus attention should be paid for the selection of the required treatment. In the present study, AMA and anti-LKM1 were not seen in any case in the patient and control groups. ANA result in 9.8% of the cases in the patient group and in the 8.5% of the cases in the control group was positive and no statistically significant difference was found between them. ASMA results was positive in 5.7% of the cases in the patient group and in 0.9% of the cases in the control group and the difference between was found very close to significance but it was not found statistically significant. In all the studies reviewed, the prevalence of the autoantibodies was reported as higher than the present study. Interferon therapy conducted in some of the patients was considered as a triggering factor. In all the studies investigating the frequency of autoantibodies in CHB and CHC, it is observed that HBV, especially HCV, has induced the antibody formation. Advanced studies can enable to clarify the role of these antibodies in chroniczation and activation of the disease. The studies have revealed that antiviral drugs and the use of interferon increase the autoantibody formation. Thus, pre-treatment autoantibody screening in newly diagnosed patients with CHB and CHC may give insight into the progression of the disease and the possible autoimmune events and extrahepatic findings. Furthermore, in the present study, the hepatosteatosis level was significantly higher in the control group compared to the patient group. Hepatosteatosis is defined as the amount of fat in the liver, especially triglycerides, more than 5% of the liver weight or filling of more than 5% of hepatocytes by lipid vacuoles in the histopathologic examination. Obesity, alcohol, diabetes, hyperlipidaemia, infection, inflammatory bowel diseases, some drugs and chemical substances may lead to hepatosteatosis (17). There is no explicative reason in approximately 5% of hepatosteatosis. Previous studies showed the comorbidity of CHC and hepatosteatosis frequently and it is thought that hepatosteatosis is caused by the HCV (18). In their study, Vere et al. (19), showed that steatosis was more frequent in the patients with CHC than the patients with CHB. In the same study, although the sensitivity and specificity were lower compared to biopsy, it was revealed that ultrasonographically detected steatosis was histopathologically associated with fibrosis. In the study conducted by Altıparmak et al. (20), in the patients with CHB, it was found that mean age, BMI, cholesterol and triglyceride levels were higher in the steatosis group; no significant difference was found between the groups with and without steatosis in terms of AST, ALT, ALP, GGT, and viral load, and is was considered that steatosis was associated with obesity and hyperlipidaemia rather than the effect of the virus. The fact that hepatosteatosis was higher in the control group than patient group in the present study support that this is not associated with the effect of HBV. The prevalence of at least one of ANA, AMA and ASMA in the patients with hepatic steatosis was 23-36% (21). Although the positive ANA result with non-alcoholic steatohepatitis (NASH) was shown, the prevalence and importance of ANA positivity has not been clearly known. In a previous study, laboratory data of a total of 55 patients histologically diagnosed with NASH were retrospectively assessed. ANA was found to be positive in 14 (25%) of 55 patients. When comparing the groups with positive and negative ANA results, no statistical difference was found between the groups in terms of age, gender distribution, BMI, ALT, AST, GGT, ALP, albumin, total cholesterol, triglyceride and ferritin (22). In the patients with NASH, there is no sufficient number of studies investigating the prevalence of ANA. Also in the patients with NASH, the importance of ANA positivity is not known explicitly. Cotler et al. (23), found ANA positivity as 34% in 74 patients with NASH. They did not find any difference between the patients with NASH having positive and negative ANA results in terms of the laboratory parameters but they found that ANA positivity was higher in females. In the study by Loria et al. (24), ANA was found to be positive in 18 (21.4%) of 84 patients with NASH. While the patients having ANA positivity were older than the patients with negative ANA, no difference was found between two groups in terms of biochemical parameters. ANA is positive in approximately 71% of the patients with autoimmune hepatitis. However, such a positivity of the autoantibody does not mean that autoimmune liver disease is present (99). Since any component of the liver cells can trigger the formation of autoantibody, serum autoantibodies were found as positive in approximately 7-52 of the patients with chronic liver diseases due to various reasons (100-101).

Study Limitations

We conducted a retrospective study of the records. For this reason, there are some limitations. Our other limitation is that, we did not investigate the pathologist observation difference.

Conclusion

Consequently, all the studies investigating the frequency of autoantibodies in CHB have revealed that HBV induces the formation of antibody. Also, these studies reported the prevalence of the autoantibodies higher than the present study. Interferon therapy and antiviral drug therapy conducted in some of the patients is considered as a triggering factor. In addition, in the present study, the number of individuals with hepatosteatosis in the control group may have increased the frequency of the autoantibodies in this group. It is required to conduct numerous prospective studies following autoantibody positivity in newly diagnosed patients before and after treatment.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee Ministry of Health Bağcılar Training and Research Hospital (approval number: 2015/414).

Informed consent: Each of the patients included in the study signed an "informed consent form" that provides information about the study and states that the consent of the patient is taken.

Peer-review: Internally peer-reviewed.

Author Contributions

Concept: E.Y., Design: E.Y., U.A., Data Collection or Processing: F.E.P., U.A., M.G.A, M.Ö., A.B.E., Ü.S.T., Analysis or Interpretation: F.E.P., A.B.E., Ü.S.T., H.Y., Literature Search: E.Y., A.B.E, Writing: U.A., H.Y.

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Research Article

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The Long-term (10.6 years) Outcome of Hepatitis C patients with Sustained Virologic Response Following Treatment with Pegylated Interferon + Ribavirin

Pegile Interferon + Ribavirin ile Tedavisi ile Kalıcı Virolojik Yanıt Elde Edilen Hepatit C Hastalarının Uzun Dönemli (10,6 yıl) Sonuçları

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ABSTRACT

Objectives: Directly-acting anti-viral agents for the treatment of hepatitis C have been revolutionised. In the meantime, hepatitis C patients with sustained virologic response (SVR) achieved with previous treatments have been forgotten. Hepatitis C patients with SVR achived by pegylated interferon + ribavirin (INF + RIB) treatment are followed and it is investigated whether cirrhosis, hepatocellular carcinoma (HCC) and/or decompensation developed or not in these patients.

Materials and Methods: One hundred thirty-five patients with hepatitis C virus who achieved SVR with pegylated INF alpha + RIB treatment between 2006 and 2010 are included in the study. At least twice a year, these patients were followed-up with ultrasonography, alpha fetoprotein and routine laboratory tests.

Results: Out of the patients, 97.8% were genotype 1 and 95% were evaluated with biopsy before the treatment. One hundred twenty non-cirrhotic patients and 15 patients with compensated cirrhosis were followed for a period of 10.6 years (distribution: 9-13 years). None of the non-cirrhotic patients developed cirrhosis or HCC. HCC was developed in one of 15 cirrhotic patients (6 years after the treatment), resulting in the death of the patient. There were no decompensation case.

Conclusion: It is evaluated that non-cirrhotic hepatitis C patients who achieved SVR with pegylated INF can be followed in a wider range of time. There should be a strict follow-up of cirrhotic patients, especially for HCC development.

Keywords: Hepatitis C, sustained virologic response, pegylated INF, cirrhosis, hepatocellular carcinoma

ÖZ

Amaç: Hepatit C'nin tedavisi için doğrudan etkili anti-viral ajanlar devrim yaratmıştır. Zamanla, önceki tedavilerle elde edilen kalıcı virolojik yanıtı (SVR) olan hepatit C hastaları unutulmuştur. Bu çalışmada Pegile interferon + ribavirin (INF + RIB) tedavisi ile SVR elde edilen hepatit C hastaları takip edilmiş ve bu hastalarda siroz, hepatoselüler karsinom (HCC) ve/veya dekompansasyon gelişip gelişmediği araştırılmıştır.

Gereç ve Yöntemler: Çalışmada, 2006-2010 yılları arasında pegile INF alfa + RIB tedavisi ile SVR elde edilen 135 HCV hasta incelenmiştir. Bu hastalar yılda en az iki kez ultrasonografi, alfa fetoprotein ve rutin laboratuvar testleri ile takip edilmiştir.

Bulgular: Hastalarımızın %97,8'i genotip 1 olup, %95'i tedavi öncesi biyopsi ile değerlendirilmiştir. Siroz olmayan 120 hasta ve kompanse sirozlu 15 hasta ortalama 10,6 yıl (dağılım: 9-13 yıl) takip edilmiştir. Siroz olmayan hastaların hiçbirinde siroz veya HCC gelişmemiştir. Sirozlu 15 hastanın 1'inde (tedaviden 6 yıl sonra) HCC gelişmiş ve bu hasta kaybedilmiştir. Dekompansasyon olgusu görülmemiştir.

Sonuç: Pegile INF ile SVR elde edilen, siroz olmayan hepatit C hastalarının daha geniş zaman aralıklarında izlenebileceği değerlendirilmiştir. SVR'li sirotik hastalarının ise, özellikle HCC gelişimi açısından sıkı takibi yapılmalıdır.

Anahtar Kelimeler: Hepatit C, kalıcı virolojik cevap, pegile interferon, siroz, hepatoselüler karsinom

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Introduction

The hepatitis C virus (HCV), commonly seen across the globe, is one of the leading causes of cirrhosis and hepatocellular carcinoma (HCC) (1). Interferon (INF)-based treatments have been used in HCV treatment for many years. INF treatments have a low success rate (40%-45%), especially in genotype-1 patients (2). Patients who did not respond to INF treatment either terminated with death by their liver-related complications over time, or eradicated HCV virus with new treatments. There have always been questions relating to the outcomes of patients who achieved sustained virologic response (SVR) using INF therapy. Previous studies have shown that in hepatitis C patients with SVR following treatment with INFs, the risk of cirrhosis and HCC is significantly reduced; inflammation and fibrosis are improved (3,4,5,6).

The main priority in hepatitis C patients is to eradicate the virus and achieve SVR (7). SVR is generally accepted as being the result of a negative HCV-RNA at 24 months after treatment. The next objective is to identify, prevent and treat liver complications that affect morbidity and mortality in patients with SVR. The fundamental issues here are progression to cirrhosis and development of HCC in non-cirrhotic patients with SVR. Another issue is decompensation and HCC develop risk in compensated cirrhotic patients with SVR? There have been previous studies seeking out answers to these questions. However, in most of these studies (4,8,9,10,11), the follow-up period was short and retrospective, and the number of prospective studies with long-term follow-up and only patients receiving pegylated-INF was very small (12,13).

Materials and Methods

Three hundred and sixty-nine HCV-RNA positive patients admitted between 2006 and 2010 were included in the study. Biopsy was performed in 95% of these patients. Biopsy was not performed in patients who showed apparent decompensated or compensated liver cirrhosis as detected during physical examination, laboratory tests, imaging examination and endoscopically. Liver biopsy was carried out in our clinic using a 16-gauge hepafix needle under ultrasound guidance. Histopathological examinations were evaluated by two experienced pathologists from the pathology department of our hospital. The Ishak scoring system was applied for histopathological evaluation (14). A haemogram, biochemical markers and other tests were investigated in the laboratory of our hospital. Hepatitis B and HIV were also investigated. HCV-RNA levels were measured in real time using the "polymerase chain reaction (PCR) technique with COBAS TaqMan 48 (Roche Diagnostic, USA)". HCV genotype was determined using the AMPLIQUALITY HCV-TS (AB Analitica, Italy) kit. The patient's age was accepted as the age at which he received treatment. Body mass index (BMI) and homeostatic model assessment for insulin resistance (HOMA-IR) were calculated with the following formulas before treatment: BMI=weight (kg) / height (m^2); HOMA-IR= fasting plasma insulin (µU/mL) X fasting plasma glucose (mg/dL) /405.

Treatment was given subcutaneously with Pegile INF alpha (α) 2a 180 µg weekly (Pegasys®) or Pegile IFN-α 2b (Pegintron®) at 1.5 µg/kg per week. Additionally, ribavirin was given at 800, 1000 or 1200 mg daily (depending on patient weight and genotype). Genotypes 1 and 4 received 48 weeks of treatment and genotypes 2 and 3 received 24 weeks of treatment.

The following patients were excluded from the study (Figure 1): 84 patients who showed no primary response to treatment; 63 patients who developed a relapse after treatment; 15 patients who could not tolerate treatment or became decompensated during treatment; 17 patients who could not be treated due to decompensated liver cirrhosis; 12 patients who were on a hemodialysis program; and 43 patients who were not followed-up regularly. 24 weeks after the end of treatment, patients with negative HCV-RNA values were considered as SVR. We proceded with the study with 120 non-sirotic and 15 compensated cirrhotic patients meeting these conditions (Figure 1).

In addition to routine laboratory tests, screening with ultrasound and alpha fetoprotein (AFP) levels is recommended in individuals at risk for HCC (1). magnetic resonance imaging (MRI) and/or computed tomography (CT) for the diagnosis of HCC in suspected patients and acceptance as HCC for those with typical imaging findings are recommended in the guidelines (15,16). After the treatment, we evaluated our patients at least twice a year and looked at AFP levels and ultrasonography (USG) in addition to routine laboratory tests. While HCV-RNA levels were measured at 3, 6, 12, 18, and 24 months over the first 2 years after treatment, it was evaluated just once per year after the first 2-year follow-up. MRI and/or CT scans and endoscopy were performed in suspicious patients. When at least one of variceal bleeding, ascites, or encephalopathy was present, decompensated cirrhosis were accepted as being present.

The study protocol was prepared in accordance with the Helsinki Declaration. This study was approved by the Local Ethics Committee Mersin University (approval number: B.30.2. MEU.0.01.00/1871). Written and oral consent was obtained from the patients.

Statistical Analysis

Categorical variables were recorded in percentage, and continuous variables as mean (± standard deviation) or as median. Shapiro Wilk-W test was used to evaluate the normal distribution of the variables. While the Student's t-test used for continuous variables with normal distribution, and Mann-Whitney test was

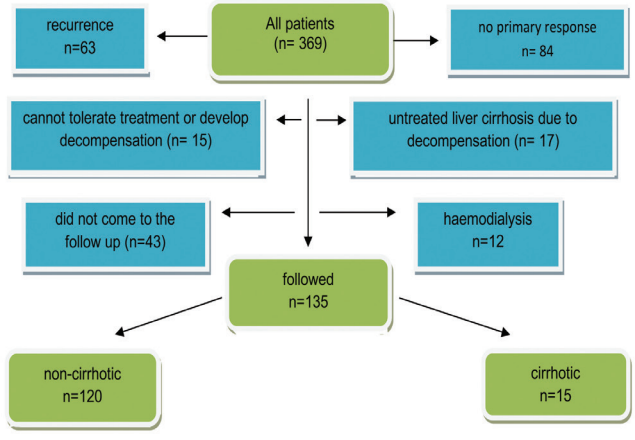

Figure 1. Flow chart

used for continous variables without normal distribution; chi-square test was used for categorical variables. The data were analysed using SPSS version 21.0.0 for Windows (IBM Corp™, Armonk, NY).

Results

The mean age was 49.3±9.82 years (22-67 years). Eighty patients were female and 55 patients were male. The mean follow-up was 10.6 years (9-13 years). With the exception of 3 patients (in the cirrhotic group: one patient, of genotype 2; in the non-cirrhotic group: one patient each for genotypes 2 and 4), all remaining patients (97.8%) were genotype 1. While 114 of the genotype1 patients were genotype 1b, 4 of them were genotype 1a; no subgroup could be detected in 14 patients. 82 patients received Pegile INF alpha (α) + 2a + Ribavirin (RIB) and 53 patients received Pegile INF-α 2b + RIB. A total of 14 patients were using alcohol and were social drinkers (one to two times a month, one to two glasses). Of these 14 patients, 10 were non-cirrhotic and 4 were cirrhotic. None of our patients were alcohol-dependent. Hepatitis B was present in one of our non-cirrhotic patients. HIV was not detected in any of our patients. 11.7% (14/120) of our non-cirrhotic patients had diabetes mellitus (DM); 3.4% (n=4) had hypothyroidism; and 0.8% (n=1) had hyperthyroidism. 40% (6/15) of the cirrhotic patients had DM; and 13.3% (n=2) had hypothyroidism. Demographic data and results from laboratory testing of the patients before treatment are shown in Table 1.

It seemed that while alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and AFP values were higher in the cirrhotic group, the number of platelets, leukocytes and erythrocytes was higher in the non-cirrhotic group (Table 1). However, due to the difference between the number of patients in these two groups, these was not suitable for any beneficial statistical evaluation.

Liver biopsy was not carried out in 7 of 15 patients with cirrhosis due to obvious cirrhosis in the pre-treatment evaluation. A fibrosis score of 6 was determined in 3 of 8 patients diagnosed with cirrhosis (Ishak fibrosis 5-6) by biopsy. Histological activity index (HAI) scores for these patients ranged from 5 to 13. All non-

Table 1. Demographic and laboratory data of patients

	Total group (n=135)	Non-cirrhotic group (n=120)	Cirrhotic group (n=15)
Age (year)	49.3±9.82	48.39±9.86	56.6±5.43
Sex (female)	80 (59.3)	71 (59.2%)	9 (60%)
BMI	26.9±4.34	26.52±4.12	30.47±4.76
Duration of follow-up (years)	10.6±1.03	10.49±0.87	11.47±1.63
peg-IFN 2α + RBV	82 (61%)	76 (63%)	6 (40%)
peg-IFN 2β + RBV	53 (39%)	44 (37%)	9 (60%)
HCV-RNA (x103 IU/mL)	1310±2648	1276±2464	1612±3933
Genotip 1	132 (97.8%)	118 (98.3%)	14 (93.3%)
Plt (x103/μL)	203.4±68.5	211.9±66.1	135.5±46.2
Htc (%)	40.62±4.22	40.37±4.2	37.2±4.86
Wbc (μL)	6786±1942	6949±1883	5483±1908
AFP (IU/mL)	4.09±2.17	3.79±1.86	6.48±2.89
ALT (U/L)	76.94±64.34	73.18±64.24	107±56.86
AST (U/L)	58.36±39.33	54.75±37.99	97.67±46.7
GGT (U/L)	47.5±44.8	43.58±42.97	78.93±46.75
ALP (U/L)	87.76±35.9	86.03±34.77	101.67±41.41
Bilirubin (mg/dL)	1.34±0.56	1.12±0.42	3.12±1.67
INR	1.12±0.08	1.08±0.07	1.42±0.15
FBG (mg/dL)	105.39±37.73	101.48±29.35	136.67±69.42
INSULIN (μU/mL)	12.55±7.41	12.43±7.36	13.47±7.75
HOMA-IR	3.45±3.03	3.27±2.8	4.88±4.15
TC (mg/dL)	175.8±40.77	176.77±41.06	168.07±37.46
LDL (mg/dL)	100.05±34.11	100.84±34.5	93.73±30.12
HDL (mg/dL)	48.31±12.97	48.59±13.13	46.07±11.4
TG (mg/dL)	137.21±67.36	136.69 65.25	141.33 82.16
TSH (μIU/mL)	2.23±1.78	2.22±1.66	2.72±2.59

AFP: Alpha fetoprotein, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, FBG: Fasting blood glucose, GGT: Gamma glutamyl transpeptidase, HDL: High-density lipoprotein, Htc: Hematocrit, LDL: Low-density lipoprotein, peg-IFN 2α: Pegylated interferon-2α, peg-IFN 2β: Pegylated interferon-2β, Plt: Platelet, RBV: Ribavirin, TB: Total bilirubin, TC: Total cholesterol, TG: Triglycerides, TSH: Thyroid stimulating hormone, Wbc: Leukocyte

cirrhotic patients underwent biopsy before treatment. 80% had mild fibrosis (0-2). Significant inflammation (HAI: 8-12) was present in 10.8%. 20% had moderate to severe steatosis. Fibrosis, HAI scores and steatosis rates of non-cirrhotic patients are shown in Table 2.

During the treatment, HCV-RNA levels were negative in 92% (124/135) of the patients after 3 months. The remaining 11 patients were determined to be below 10.000 IU/mL; all of these were negative after 6 months of treatment. No recurrence was observed in any patient who was followed up for a mean of 10.6 years after treatment.

In the follow-up of cirrhotic patients, one patient developed HCC. The AFP level of the patient was 7.09 IU/mL before treatment, increasing to 654 IU/mL in 7 years. Abdominal USG showed localised hypoechoic areas in the liver. MRI was performed and infiltrative HCC invading the portal vein was detected. This patient died 9 months after diagnosis. In addition, another cirrhotic patient with diabetes died of coronary artery disease at 4 years after treatment. With the exception of these, 13 cirrhotic patients had stable follow-up. Decompensation did not develop. It was found that the most recent AST, ALT, GGT, AFP levels of these patients were improved as compared to pre-treatment values (Table 3).

In the follow-up of 120 Hepatitis C patients with SVR, cirrhosis and HCC were not detected in any of the patients. Four of these patients died due to extra-hepatic causes.

Discussion

Directly-acting antiviral drugs for the treatment of chronic hepatitis C have been revolutionized. Treatment success rates are over 95% (17,18,19). High success has also been achieved in HCV-induced decompensated liver patients who were not able to receive interferon therapy (20). In addition, these drugs are used safely in patients who have not been able to obtain SVR in interferon treatments previously, and almost complete success is achieved (21). It seems that eradication of HCV is not an issue anymore. Subsequently, the real question has been: "should we monitor HCV patients with SVR in terms of whether cirrhosis, HCC, relapse or decompensation develops". These questions have also begun to be asked in direct acting-oral antiviral drugs, and can be seen in studies in this direction (22,23). Previously, there have been studies in this direction, but the number of long-term studies is limited.

In this study, with a mean follow-up period of 10.6 years in 135 hepatitis C patients (15 of whom presented with compensated-cirrhosis), no recurrence was detected in any of the patients. Some previous studies (mean follow-up of 2-5 years) support our results (5,24,25,26). Even though there are publications indicating that late relapse may occur, the probability of this is reported to be around 1% in most cases (8,11,27,28). After treatment, we regularly checked the HCV-RNA level at least once per year using a sensitive method up to 50 IU/mL.

Table 2. Histopathological evaluation of non-cirrhotic patients

Ishak fibrosis	Number of patients	Ishak HAI	Number of patients	Steatosis	Number of patients
0	15	0-4	36	NO (0%)	55
1	43	5-8	71	Mild (1-33%)	41
2	38	8-12	13	Moderate (34-66%)	17
3	16	12-18	0	High (67-100%)	7
4	8	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-

HAI: Histological activity index

Table 3. Cirrhotic patients' final values compared with pre-treatment values*

	Cirrhotic patient (n=13)		
	Pre-teatment value	Last value	p
ALT (U/L)	107.2±60.9	31.3±15.6	0.0001
AST (U/L)	95.6±48.7	33.2±13.8	0.0001
GGT (U/L)	64.8±31.9	50.2±34.4	0.0105
Albumin (g/dL)	3.58±0.42	4±0.49	0.0011
AFP (IU/mL)	6.29±3.01	2.74±1.19	0.0063
Plt (x10^3/µL)	139±43.6	149.6±69.3	0.0261
Htc (%)	37.5±5.3	37.1±4.41	0.3068
Wbc (µL)	5495±1782	5536±1689	0.3026

AFP: Alpha fetoprotein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transpeptidase, Htc: Hematocrit, Plt: Platelet, Wbc: Leukocyte, *: Two patients who died were excluded from the evaluation

None of our non-cirrhotic patients developed cirrhosis or HCC. In a study of 137 non-cirrhotic hepatitis C patients who achieved SVR with interferon-based treatments, no HCC or cirrhosis was detected after approximately 8.6 years (range: 2-19.9 years) (12). In another study including 150 patients with SVR (pretreatment and 5 years later, biopsy was performed), it was determined that in more than 90%, improvements in fibrosis scores or inflammation were found (5). In this study too, the development of HCC or cirrhosis was not detected in non-cirrhotic patients. Although some studies have reported cirrhosis, the rate of this is low (>5%) (9,29). Only one of our patients had concomitant hepatitis B, but no additional liver disease. Diseases of comorbidity such as DM and hypothyroidism were limited in number, and most importantly, there were no alcohol-dependent patients. We believe that cirrhosis does not develop due to these factors in our study.

The 5-year decompensation rate in non-SVR compensated cirrhotic patients ranges between 18%-25% (30,31). In cirrhotic patients with acquired SVR, the decompensation rate decreases significantly (32). In our study, no decompensation occurred in cirrhotic patients. In a study of 103 patients with SVR (two patients were cirrhotic) with a 23-year follow-up period, no decompensation was found (13). In a joint study of 5 hepatology units in Europe and Canada, they found 30% (142/479) SVRs after interferon-based treatment in patients with cirrhosis or advanced fibrosis (Isaac fibrosis score 4-6). The mean follow-up period for these patients was 2.1 years (0.8 to 4.9 years), and no patients developed decompensation (4). In another study involving 8 centers from Europe, the 5-year decompensation rate was found to be 1% [95% confidence interval (CI), 0.0% 32.3%] (33). In our patients, we believe that the most important factor behind the lack of development of decompensation, as we mentioned above, was that there were no alcohol-dependent patients or patients suffering from further hepatic diseases.

HCC is one of the most common complications and causes of death in patients with cirrhosis due to hepatitis C (34). This risk is reduced by HCV eradication. In our study, none of the non-cirrhotic patients developed HCC, while only one (6.7%) of 15 compensated cirrhotic patients developed HCC. In a study involving cirrhotic hepatitis C patients, with an average follow-up of 32 months, it was determined that 3% of cirrhotic patients with SVR and 17% of non-SVR patients developed HCC (35). In a study with an average follow-up of 46.7 months, HCC developed in 1% of hepatitis C patients with SVR, while HCC developed in 5.5% of patients with non-SVR (9). In this study, a proportional decrease was observed in non-cirrhotic patients. Some studies with different follow-up times have shown that the risk of HCC development is less than 10% in cirrhotic patients (5,12,13). In a meta-analysis covering those obtained from IFN-based SVR, the annual risk of HCC formation was calculated as 1.14% (95% CI 0.86-1.52) (36).

In our study, one of the limitations was the low rate of cirrhosis patients. This low rate may cause limitations in generalizing the results to the population.

Conclusion

Although there are many studies following hepatitis C patients who had previously taken interferon therapy and achieved SVR, our study shows some different features. Our study offers some advantages, such as: 95% of our patients being diagnosed by biopsy; 97.8% of patients being genotype 1 (greater than 90% of them were genotype 1b); patients received only pegileinterferon+ribavirin treatment; no cases of HIV, HBV (except one patient) or alcohol dependence; long-term follow-up period (10.6 years); and the fact the study was conducted at a single center.

None of the non-cirrhotic patients that we followed up had cirrhosis or HCC. Although no decompensation was observed in the cirrhotic patients, 6.7% developed HCC. In the light of previous studies, we believe that non-cirrhotic hepatitis C patients with interferon therapy may be followed up less frequently than those with cirrhotic patients, and that cirrhotic patients should be followed closely especially in terms of HCC.

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Ethics

Ethics Committee Approval: This study was approved by the Local Ethics Committee Mersin University (approval number: B.30.2. MEU.0.01.00/1871)

Informed Consent: Verbal and written informed consent received.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: O.Ö., S.Y., Design: O.Ö., S.Y., Data Collection or Processing: O.Ö., S.Y., Analysis: O.Ö., S.Y., Literature Search: O.Ö., Writing: O.Ö., S.Y.

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Research Article

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Prevalence of Hepatitis B and C Infection in Patients with Rheumatoid Arthritis in Marrakesh

Marakeş'te Romatizmal Hastalıkları Olan Hastalarda Hepatit B ve C Enfeksiyon Sıklığı

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ABSTRACT

Objectives: Although there is no difference in frequency of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection in patients with rheumatoid arthritis (RA) in general population, multicenter studies are needed across the country to support this idea. The aim of this study is to invastigate the prevalence of HBV and HCV infections in RA patients.
Materials and Methods: This study is carried out for 3 years and is a retrospective and descriptive study. In this study, we recorded the clinical and immunological characteristics of the patients as well as immunosuppressive treatments and immune status responses to HBV and HCV.
Results: One hundred fifty-three RA patients were included in this study. (83.5%) of the patients were female and the mean age was 50±13. 6.53% had a history of tuberculosis disease infeciton. The average duration of disease progression was 4.1 years. These were erosive RA present in 68% of cases and seropositive in 83.5%. Activity was severe in 8.7% of cases. Out of 153 patients, 3 were hepatitis B surface antigen positive, 3 were anti-HBs antibodies positive, and 19 were anti-HBc antibodies positive.
Conclusion: New cases will be identified in screening, even in populations where viral hepatitis is not endemic. In patients with immunodeficiency, early diagnosis is essential given the severity of hepatitis B and C infection.
Keywords: HVB, HVC, rheumatoid arthritis

ÖZ

Amaç: Genel popülasyondaki romatoid artrit'li (RA) hastalarda hepatit B virüsü (HBV) ve hepatit C virüsü (HCV) enfeksiyonlarının görülme sıklığı farklı olmamasına rağmen, bu fikri desteklemek için ülke çapında çok merkezli çalışmalara ihtiyaç vardır. Bu çalışmanın amacı RA hastalarında HBV ve HCV enfeksiyonlarının prevalansını araştırmaktır.
Gereç ve Yöntemler: Bu çalışma, 3 yıl boyunca yürütülmüş olup geriye dönük ve tanımlayıcı bir çalışmadır. Çalışmamızda, hastaların klinik ve immünolojik özellikleri ile birlikte, immünosüpresif tedaviler ve HBV ve HCV'ye karşı immün yanıtları kaydedilmiştir.

Bulgular: Bu çalışmaya 153 RA hastası dahil edildi. Hastaların %83,5'ü kadındı ve yaş ortalamaları 50±13 idi. %6,53'ünde tüberküloz enfeksiyonu öyküsü vardı. Ortalama hastalık ilerlemesi süresi 4,1 yıldı. Bunlar olguların %68'inde erozif RA mevcuttu ve %83,5'i seropozitifti. Olguların %8,7'sinde aktivite şiddetliydi. Yüz elli üç hastanın 3'ünde hepatit B yüzey antijeni pozitif, 3'ünde anti-HBs antikoru pozitif, 19'unda anti-HBc antikoru pozitifti.
Sonuç: Viral hepatitin endemik olmadığı popülasyonlarda bile taramada yeni olgular tanımlanacaktır. İmmün yetmezliği olan hastalarda hepatit B ve C enfeksiyonunun ciddiyeti göz önüne alındığında, erken tanı şarttır.
Anahtar Kelimeler: HVB, HVC, romatoid artrit

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Introduction

Rheumatoid arthritis (RA) is systemic inflammatory rheumatic diseases with a complex and partially understood etiology. Several pathogens have been debated to trigger the initial immune response necessary for development of RA in a genetically susceptible host (1). Numerous viruses have been associated with the development of inflammatory arthritis, including the hepatitis viruses [hepatitis B virus (HBV) and HCV], human immunodeficiency virus, parvovirus B19, human T-cell lymphotropic virus-1, and alpha viruses (2).

Hepatitis infections are widespread diseases in the world, and an estimated 2 billion people have been infected with HBV and 170 million people have been infected with HCV (3). Immunosuppressive therapy, especially tumor necrosis factor-alpha inhibitors and anti-B cell therapy can induce viral reactivation in patients with concurrent HBV infection (4). Therefore, screening for HBV and HCV infection is recommended for patients who receive immunosuppressive therapy (2,4).

The prevalence of HBV and HCV infections in the general population may differ according to geographic regions. In Morocco, HBV and HCV prevalence was reported to be 1.81% and 1.58%, respectively (5). Although the frequency of HBV and HCV infections is not expected to be different in RA (6) patients from the general population, multicenter countrywide studies are required to support this idea. The purpose of this study was to explore the prevalence of HBV and HCV infections in RA patients.

Materials and Methods

Study Population and Design

This is a retrospective and descriptive study, carried out over a period of 3 years between January 2015 and July 2018, conducted in collaboration between the Bacteriology-Virology laboratory and the rheumatology department of the Mohammed VI University Hospital of Marrakesh. This study included 153 cases of RA, diagnosed with RA according to the ACR (RA classification criteria) eular 2010 criteria.

Clinical and laboratory data [serum aminotransferase aspartate aminotransferase (AST), alanine aminotransferase (ALT), hepatitis B surface antigen (HBsAg), anti-HBc, anti-HBs, anti-HCV, hepatitis B e antigen (HBeAg), anti-HBe, HBV-DNA, and HCV-RNA] were evaluated according to patient medical records. ALT, AST levels >40 IU/mL were considered high transaminase levels.

This study has been approved by the Ethics Committee of Mohammed VI Hospital in Marrakech (approval number: 28/04, date: 14/04/2018). Informed consent was obtained from the patients.

Serological Tests

Serological tests were detected using chemiluminescence microparticle immunoassay on the Abbott Architect i1000. Patients with positive HBsAg or anti-HCV results were additionally tested for HBV-DNA or HCV-RNA in serum. HBV-DNA and HCV-RNA were tested by a real-time polymerase chain reaction (RT-PCR)-based method.

Statistical Analysis

Data analysis was performed using version 14 SPSS (Statistical Package for the Social Science Program Inc., Chicago. Illinois, USA). Descriptive statistical analyses were presented as the range, mean and standard deviation for quantitative variables.

Results

In this study, 153 RA patients were included, (83.5%) were female and the average age was 50±13 (range: 38-88 years old), 6.53% of patients had a history of tuberculosis disease (10 cases).

The average duration of disease progression was 4.1 years. These were erosive RA in 68% of cases and seropositive in 83.5%. Activity was severe in 8.7% of cases, while 26.6% of patients were in remission according to Disease Activity Index 28 vs.

Therapeutically, 71.8% were on Methotrexate while 16.5% on Rituximab and 1% on Tociluzimab. Long-term corticosteroid therapy was prescribed in 81.6% of patients.

Among a total of 153 patients, HBsAg was positive in 3 (1.96%) patients, and negative in 150 (98.04%) patients, Anti-HBs antibodies were positive 24 (15.68%) patients, anti-HBC antibodies was positive in 19 (12.41%) patients.

The interpretation of Hepatitis B serologic test results show that: 3 Patients had Hepatitis B infection, 10 patients had successful vaccination, 14 patients had resolved hepatitis B infection (immune to reinfection), and in 2 patients anti-HBc was only positive and the other markers were negative.

In the HBsAg (+) and anti-HBc (+) group, (2/3) of patients had a negative HBeAg (-) and a positive anti-HBe (+), and (1/3) of patients had a positive HBeAg (+) and a negative anti-HBe (-). All patients had a positive HBV-DNA results and a high AST and/or ALT levels, Table 1.

In the anti-HBc (+), HBsAg (-) and anti-HBs (-) group: all patient had a negative HBeAg and a negative anti-HBe. But (1/2) of patients had a positive HBV-DNA results and high ALT and AST level, Table 1.

The screen for hepatitis C (anti-HCV) was negative in all patients.

Discussion

Hepatitis virus infections are an important issue because of the difficulties in the diagnostic and therapeutic approach of rheumatic diseases. HBV and HCV infections may present with several rheumatic manifestations and may have a role in the etiopathogenesis of autoimmune diseases (5). Otherwise, immunosuppressive drugs are commonly used in the

Table 1. Completeness of hepatitis screening

	Number	Percentage
HBsAg (+)	3	1.96
HBsAg (-)	150	98.04
Anti-HBc (+)	19	12.41
Anti-HBc (-)	134	87.58
Anti-HBs (+)	24	15.68
Anti-HBs (-)	129	84.31
Anti-HCV (+)	0	0
Anti-HCV (-)	153	100

HBsAg: Hepatitis B surface antigen, HBc: hepatitis B core, HCV: Hepatitis C virus

management of rheumatic diseases and were shown to induce viral reactivation in HBV- and HCV - positive patients, and in most instances, flares are asymptomatic. Several case reports have documented HBV reactivation in inactive HBV carriers treated with methotrexat and biologic agents, including infliximab (7,8), etanercept (9), adalimumab (10), and rituximab (11,12). Therefore, ACR recommends screening for HBV and HCV before non-biologic or biologic immunosuppressive therapy (4,12).

HBsAg is the best assay screening for HBV infection but is sometimes negative and other marker such as anti-HBc should be examined (13). In this study prevalence of anti-HBC is higher than prevalence of HBsAg for meticulous evaluation of HBV infection in RA patients, because isolated anti-HBc positive is a risk factor for reactivation of HBV (14).

Isolated positive anti-HBc can be seen in three conditions that the first is acute infections, second chronic hepatitis which may be progressive and third improved and therefore type 1 and type 2 after immunosuppressive therapy are at risk for reactivation and fulminant hepatitis (15).

Patients who had anti-HBc positive treated with immunosuppressive drugs, reactivation of hepatitis may be occured so evaluation every 6-month for hepatitis had indicated until don't delayed is prophylaxis (16).

Before chemotherapy, patients should be screened for serological HBV markers and HBV-DNA levels in case of HBsAg and/or anti-HBc positivity. HBV vaccination is recommended in seronegative patients. In HBsAg-positive patients, pre-emptive treatment should be initiated whatever the HBV-DNA levels and continued for 6-12 months after immunosuppressive therapy is done. Entecavir or tenofovir are preferable in patients with an initial viral load 42000 IU/mL. ALT and HBV-DNA levels should be closely monitored in anti-HBc positive patients with or without anti-HBs, and antiviral therapy should be started when HBV reactivation is confirmed. Anti-HBs titers should be monitored closely in anti-HBs positive patients because a decrease in anti-HBs can precede seroreversion. Therapy is not needed as long as anti-HBs titers are protective (17).

Detection of serum anti-HCV antibodies is an indicative of past or active infection, but viraemia assesses by reverse transcription RT-PCR is a better sensitive indicator of chronic HCV infection than serology (18).

In this study we didn't find any case of hepatitis C, hence, some patients infected with HCV could have been missed in this study, because patients were systematically screened for anti-HCV antibodies with a search for HCV-RNA only in cases of seropositivity. However, this methodology was used because the prevalence of patients negative for HCV by serology, but positive by RT-PCR seems to be very low. This joins the results of Cacoub et al. (19), who did not find any patient with RA in a sample of 1614 patients with chronic HCV infection.

Only the use of rituximab and high dose steroids were significantly associated with high risk of developing HCV. Rituximab as a single agent or in combination with steroids or other chemotherapeutic agents is the drug associated with the highest risk of HBV reactivation, in persons with chronic or past HBV infection (20). Rituximab had also been described to be an important cause of HCVr. Rituximab-based therapy causes a profound depletion of Bcells and a marked reduction of T-cells, mainly CD4+ cells, and has been reported to cause reactivation of other viruses, including cytomegalovirus and herpes simplex virus (20). Steroids are known to cause a rapid depletion and apoptosis of circulating T-cells. Previous studies revealed that steroids stimulate HCV replication *in vitro,* and there are clinical reports that patients treated with high dose steroids had an increase in HCV-RNA levels (20).

Hepatitis B and C infections are widespread diseases in the world, and their prevalence in the general population differs according to geographic regions ranging from over 10% in Asia to under 0.5% in the United states and Northern Europe (21). In another point, hepatitis infection may present with numerous extrahepatic manifestations, and patients often apply to different specialities according to the predominant clinical feature. Patients' joint symptoms [(of the most common extre-articular findings) may be believed to be associated with HBV or HCV in certain times. So, the real prevalence of these infection was reported as 0.12% and 0.86% in early arthritis]. In addition, HCV prevalence was reported as 0.65% in 309 RA patients in France (21). these values were not greater than expected based on data from the general population in the same geographic area. In another study from China, the prevalence of HBsAg was shown as 12.8% in the general population, 9.6% in RA patients (2,22,23).

In our country, HBV and HCV prevalence was reported as 1.81% and 1.58% in the general population (24). In our study, we found similar HBsAg prevalence in RA patients compared to the general population. On the other hand, we showed a lower prevalence of anti-HCV antibodies seropositivity in RA patients according to our national data.

Study Limitations

There are also several limitations to our study, foremost being its retrospective nature, Baseline HBV and HCV testing was missing in some patients, who were excluded from the study, the second limitation we did not screen our patients for HCV-RNA, resulting in underestimation of the incidence of HCV, third the small number of patients.

Conclusion

Even in populations where viral hepatitis is not endemic, new cases will be identified on screening. Given the seriousness of hepatitis B and C infection in immuno-compromised patients, early identification is essential. For the best outcome, anti-viral treatment should be initiated prior to antirheumatic treatment. Greater awareness of asymptomatic current or past hepatitis B or C infection is necessary of all cases are to be recognized. This premise applies not only for RA but also for all chronic inflammatory diseases treated using immunosuppressive drugs.

Ethics

Ethics Committee Approval: This study has been approved by the Ethics Committee of Mohammed VI Hospital in Marrakech (approval number: 28/04, date: 14/04/2018).

Informed Consent: Informed consent was obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Data Collection and/ or Processing: A.R., N.S., Analysis and/or Interpretation: T.R., A.H., Literature Search: A.R., I.B., N.S., Writing Manuscript: A.R.

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Research Article

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Effect of Advanced Fibrosis Presence on Adherence to Hepatocellular Carcinoma Surveillance in Chronic Hepatitis C Patients with Sustained Virologic Response

Kalıcı Viral Yanıtı Elde Edilen Kronik Hepatit C Hastalarında İleri Fibrozis Varlığının Hepatoselüler Karsinom Tarama Uyumuna Etkisi

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ABSTRACT

Objectives: Although hepatocellular carcinoma (HCC) screening is accepted as standard care in patients with chronic hepatitis C (CHC) diagnosis, sustained viral response (SVR) has been obtained by treatment and it is not certain what the condition is in the group of patients with advanced fibrosis who are at increased risk for developing HCC before treatment. In this cohort of patients, the practice of HCC was intended to be evaluated in real life conditions.

Materials and Methods: Between 2007-2019, the information cards of the patients diagnosed with CHC were retrospectively examined. Patients with advanced fibrosis,prior the treatment, who had obtained SVR were enrolled in the study. HCC screening was defined as alpha-fetoprotein testing and liver imaging combined. HCC surveillance every 6 months or more was defined as compliance with screening guidelines.

Results: The number of patients in the study was 83 (n=32, cirrhosis). During the follow-up period, the median was 35 (13-124) months, 24 (6.1%) patients were diagnosed with HCC. 48.2% (n=40) of the patients observed screening guidelines, while 22.9% (n=19) did not follow the guidelines; 28.9% (n=24) did not have screening.

Conclusion: HCC screening in CHC patients with advanced fibrosis is not carried out in accordance with the guidelines.

Keywords: Hepatocellular carcinoma, advanced fibrosis, hepatocellular cancer surveillance, chronic hepatitis C

ÖZ

Amaç: Kronik hepatit C (KHC) tanılı hastalarda, hepatoselüler karsinom (HCC) taraması standart bakım olarak kabul edilmesine rağmen, tedavi ile kalıcı viral yanıt (KVY) elde edilmiş olup, tedavi öncesi HCC gelişimi açısından artmış risk taşıyan ileri fibrozisli hasta grubunda durumun ne olduğu konusu kesinlik kazanmamıştır. Bu hasta kohortunda, HCC taraması pratiğinin, gerçek yaşam koşullarında değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: 2007-2019 tarihleri arasında, tanı almış KHC tanılı hastaların bilgi kartları retrospektif olarak incelendi. Tedavi öncesinde ileri fibrozisi olan ve KVY elde edilen hastalar çalışmaya alındı. HCC taraması tanımı, alfa-fetoprotein tetkiki ve karaciğer görüntülemesinin birlikte yapılması olarak yapıldı. HCC taramasının 6 ayda bir veya daha sık yapılması tarama kurallarına uyulması olarak tanımlandı.

Bulgular: Çalışmaya dahil edilen hasta sayısı 83 idi (n=32, siroz). Ortanca 35 (13-124) ay olan takip süresi boyunca, 24 (%6,1) hasta HCC tanısı aldı. Hastaların %48,2'sinde (n=40) tarama kurallarına uyulduğu, %22,9'unda (n=19) kurallara uyulmadığı ve hastaların %28,9'unda (n=24) ise tarama yapılmadığı görüldü.

Sonuç: İleri fibrozisi olan KHC hastalarında HCC taraması, kılavuz önerilerine uygun yapılmamaktadır.

Anahtar Kelimeler: Hepatoselüler karsinoma, ileri fibrosis, hepatoselüler kanser taraması, kronik hepatit C

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Introduction

Hepatitis C virus (HCV), a single-stranded RNA virus in the Flaviviridae family, is one of the major causes of chronic liver disease, cirrhosis, and hepatocellular carcinoma (1). Identified first in 1989, the global prevalence of this virus is estimated at approximately 2.5% (2). In a study performed on 5460 people in 2015 in Turkey that is considered as one of the low endemic regions for HCV by the World Health Organization, seroprevalence of HCV was 1%, and the genotype 1b was reported as the most frequently detected subtype (3).

Of the patients infected with HCV, 15-40% recover and the remainders develop chronic hepatitis C (CHC) infection (4). CHC progresses to cirrhosis in 20-30% of untreated CHC patients, and 1-4% of the cirrhotic patients develop hepatocellular carcinoma per year (5). Liver cancer is the second most common cause of cancer-related deaths across the world and the 6th most common cancer (6).

CHC infection rarely leads to the development of hepatocellular carcinoma (HCC) without advanced fibrosis or cirrhosis and it differs from CHB infection in this respect (4,7). The vast majority of the cases with HCC, which is generally observed in CHC patients with the developed cirrhosis and is the most common cause of the liver-related deaths in this patient group, is unfortunately detected at the advanced stage, therefore these patients cannot benefit from the curative treatment options that can be used at the early stage (2). Indeed, a study performed by Stravitz et al. (8) suggests that the quality of HCC surveillance may have a highly important impact on diagnosis, treatment and survival.

The patients who achieved a sustained virologic response (SVR) with the use of conventional combination of pegylated-interferon+ribavirin (PI + R) or the direct-acting agents (DAA), which have been introduced in the recent years, are considered to be cured since their late relapse rates are highly low (9). However, although the risk of the HCV-related fatal complications is reduced after the achievement of a SVR, it is not completely ruled out. Even though non-cirrhotic patients with negative HCV-RNA results at Week 48 can be excluded from the follow-up, it is recommended, due to the ongoing HCC risk, to continue to follow the patients with advanced fibrosis up even after the achievement of a SVR (10).

Current HCC surveillance guidelines emphasize that it is appropriate to perform a surveillance using serum alpha-fetoprotein (AFP) test and liver ultrasonography (USG) together (because of that the individual uses of the tests have their own specific problems, and the sensitivity and specificity of AFP is low) every 6 months in patients at risk (10).

Although HCC surveillance is accepted as the standard care and the effect of the surveillance performed in accordance with the recommended guidelines on the patient survival has been demonstrated, the extent, to which the surveillance guidelines and recommendations are adhered in real life, should be investigated. Even though there is a limited number of studies related to the surveillance adherence of this patient group in the literature, a sustained virologic response has been achieved with the treatment. However, the condition in the patient group at an increased pre-treatment risk for the development of HCC remains unclear.

In this study, we aimed to evaluate the practice of HCC surveillance in real-life conditions in the cohort of the CHC patients with advanced fibrosis who achieved a SVR with treatment.

Materials and Methods

Patient Population

In this retrospective cohort study, the information cards of the patients diagnosed with CHC, for which antiviral treatment (including direct-acting antivirals) was initiated by our Gastroenterology Department of our University between May 2007 and May 2019, were retrospectively reviewed. Patients that achieved a SVR with treatment and were followed up for more than 12 months after this response were evaluated taking their demographic and clinical information into consideration. Patients who had advanced fibrosis before the treatment were included in the study. HCV-RNA results, liver function tests, liver synthesis capacity tests, hemogram, AFP and liver biopsy results and hepatobiliary system imaging results [Abdominal USG, magnetic resonance imaging (MRI) and computed tomography (CT)] of the patients older than 18 years in this group along with the treatments they received (including the conventional PI + R and DAA) were reviewed. Patients who are under 18 years, did not achieve a SVR with treatment, were diagnosed with HCC before the treatment or follow-up or received curative treatment accordingly, were diagnosed with HCC within the first 6 months of the follow-up, had a history of liver transplant, were co-infected with HBV and/or HIV, and have missing data were excluded from the study.

Definitions Used in the Study

Based on the liver biopsy performed before the treatment, the patients who were reported at stage 3 or 4 (F3 or F4, respectively) were accepted as patients with non-cirrhotic advanced fibrosis while the patients who were reported at stage 5 or 6 (F5 or F6, respectively) were accepted as patients with cirrhosis (cirrhotic advanced fibrosis) using the Ishak scale (11).

Cirrhosis diagnosis was made based on the detection of cirrhosis in liver biopsy (F5 or F6) and for the patients with unsuitable conditions for biopsy, the detection of radiological and biochemical findings consistent with cirrhosis (cirrhosis without biopsy). HCC surveillance was defined as the combination of AFP examination and liver imaging (abdominal USG and/or abdominal CT or MRI).

HCC surveillance every 6 months or more frequent was defined as "adherence to the surveillance", the surveillance every 7-12 months was defined as "suboptimal adherence to the surveillance", the surveillance every 13-24 months was defined as "non-adherence to the surveillance" and other situations were defined as "no surveillance".

Study Objectives

It was investigated how often HCC surveillance was performed, whether it was in accordance with the current guidelines, or it differed among patients with advanced fibrosis with or without cirrhosis or among different treatment groups. Factors affecting the surveillance were evaluated. The ethics committee approval for the study was obtained from the Local Ethics Committee of Kocaeli University (approval number: 327, date: 2019). Informed consent was obtained from all individual participants included in the study.

Statistical Analysis

All statistical analyses were performed using IBM SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA). Descriptive statistics were reported as proportions (%) for categorical variables and mean ± standard deviation or median interquartile range for continuous variables. Comparative analysis between groups was performed using the ki-kare test for categorical variables. For continuous variables, the Student's t-test was used to evaluate normally distributed continuous variables, and the Wilcoxon rank-sum test was used to evaluate continuous variables that were not normally distributed. Statistical significance was defined as a two-tailed p value <0.05.

Results

In the examination of the information cards of the patients who were diagnosed with CHC and followed by the Department of Gastroenterology of our university during the abovementioned period, there were 393 patients that achieved a SVR with an antiviral treatment, 95 of them with direct-acting antiviral treatment. Of the patients, 57% (n=224) were male and the mean age was 53.7±17.2. Of these patients, 94.9% (n=373) had genotype 1, 1.5% (n=6) had genotype 2 and 3.6% (n=14) had other genotypes.

The rate of patients with advanced fibrosis, including cirrhosis, was 21.1% (n=83) (according to the Ishak scale, cirrhosis 38.6% n=32; F5 n=16; F6 n=6; cirrhosis without biopsy n=10). The mean age was 55.8±21.4 in this patient group (57 men, 26 women; 89.1% (n=74) genotype 1). Demographic and clinical characteristics of the study patients are presented in Table 1.

Eleven (34.4%) of the patients with cirrhosis were decompensated (12 of the patients (37.5%) had a history of decompensation). MELD score was 11±2.6 in this patient group. Based on the evaluation of all cirrhotic patients, the number and proportion of patients in the Child A, B, C group according to the Child-Pugh-Turcotte classification were 21 (65.6%), 9 (28.1%) and 2 (6.3%), respectively (Table 2).

During the median follow-up period of 35 (13-124) months, 24 (6.1%) patients were diagnosed with HCC.

Adherence to Hepatocellular Carcinoma Surveillance Guidelines

The surveillance guidelines were adhered in 48.2% (n=40) of the patients while they were not adhered in 22.9% (n=19) (screening was performed in 11 patients (13.3%) every 7-12 months and in 8 patients (9.6%) every 13-24 months), and no surveillance has been performed in 28.9% (n=24) of the patients. HCC surveillance rates are presented in Table 3.

Table 1. Clinical and demographic features of patients with advanced fibrosis before treatment

Number of patients with advanced fibrosis (n)	83
Number of patients with liver biopsy (n)	73
Age	55.8±21.4
Sex (male/female) (n)	57/26
Genotype n (%)	
1 (A/B)	74 (6/68) (89.1)
2	3 (3.6)
3	4 (4.8)
4	2 (2.4)
Cirrhosis (with liver biopsy) (n)	22
Cirrhosis (without liver biopsy) (n)	10*
Histology (n) Ishak et al. (11)	73
F3/F4/F5/F6	25/26/16/6
Patients with advanced fibrosis/cirrhosis (n)	51/32
Follow-up duration (months); mean (range)	35 (13-124)
Number of patients with SVR (n)	83
Treatment regimes (Peg-IF+RBV/DAAs)	(46/37)

SVR: Sustained viral response, Peg-IF: Pegile interferon; RBV: Ribavirin; DAAs: Direct acting antivirals

Table 2. Features of patients with cirrhosis before treatment

Number of patients with cirrhosis (n)	32
Biopsy (+)/(-)	22/10
Child-pugh score (n) (%)	
A	21 (51.0)
B	9 (35.3)
C	2 (13.7)
MELD score	11±2.6
Number of patients with decompansated (n)	11
Number of patients with history of decompensation (n)	12

MELD: Model for end stage liver disease

Table 3. HCC Surveillance Adherence in Patients with Advanced Fibrosis

Advanced fibrosis, (with and without Cirrhosis) (n=83) HCC Surveillance adherence; n (%)	**1-6 months**	**7-12 months**	**12-24 months**	**25 - > months**
USG+AFP	40 (48.2)	11 (13.3)	8 (9.6)	24 (28.9)
AFP	60 (72.3)	10 (12.0)	4 (4.8)	9 (10.8)
Advanced Fibrosis (with Cirrhosis) (n=32) HCC Surveillance adherence; n (%)	**1-6 months**	**7-12 months**	**12-24 months**	**25 - > months**
USG+AFP	20 (62.5)	6 (18.75)	4 (12.5)	2 (6.25)
AFP	24 (75)	6 (18.75)	2 (6.25)	-

HCC: Hepatocellular carcinoma, USG: Upper abdominal ultrasonography and liver assessment, AFP: Alpha-fetoprotein

Based on the single evaluation of the request of AFP examination, it was requested in 60 patients every 1-6 months and in 14 patients every 7-24 months, and not requested in 9 patients (Table 3).

Moreover, the adherence rates were lower in patients treated with non-DAA antiviral regimens compared to the patients treated with DAA [for full adherence, 28.3% (n=13) vs 73% (n=27); p<0.001] (Table 4).

Effects of Patient Characteristics on Adherence to Surveillance Guidelines

In the evaluation of patient characteristics in terms of adherence to surveillance guidelines, the rate of adherence to HCC surveillance guidelines was statistically significantly higher in the patients who are elderly (59±6 vs 52±3; p<0.001), are with decompensation or a history of decompensation [86.9% (20/23) vs 33.3% (20/60); p<0.001] and visit outpatient clinic more frequently (median 3.8±0.6 vs 1.8±0.2; p<0.001) compared to the patients without these characteristics.

Relationship Between the Presence of Advanced Fibrosis and Adherence to Surveillance Guidelines

Upon the achievement of SVR, the patients with cirrhosis had a higher rate of annual outpatient visits compared to the patients with non-cirrhotic advanced fibrosis (mean: 2.8±2.2/year vs 1.7±1.3 visit/year; p=0.014), two or more liver imaging (n=22, 68.8% vs n=21, %41.2; p=0.026) and relatively higher rate of adherence to HCC surveillance guidelines (n=20, 62.5% vs n=20, 39.2%; p=0.066) compared to patients with non-cirrhotic advanced fibrosis.

It was found out that one fourth of the patients with non-cirrhotic advanced fibrosis did not come to their outpatient clinic visits, approximately one third of the patients did not have a liver imaging test (31.4%; 16/51) and the optimal HCC surveillance was not performed in almost two-thirds (60.8%; 31/51) of the patients (Table 3).

Frequency of Development of Hepatocellular Carcinoma by Adherence to Surveillance Guidelines and Characteristics

During the follow-up period, a total of 24 patients developed HCC (n=14, 58.3% and n=10, 41.6% respectively in the patient groups with and without adherence to the surveillance guidelines). In the group with adherence to the surveillance guidelines, the tumor size was smaller (2.1±2.4 vs 6.5±1.9 cm; p<0.001), there were more patients in Stage 0 and A according to Barcelona Clinical Liver Cancer staging (n=10, 32.3% vs n=1, 2.9%; p=0.005), and there were more patients meeting the Milan Liver transplant criteria (n=11, 41.2% vs n=2, 5.9%; p=0.011). In the comparison in terms of AFP values, there were no values above 1000 ng/mL at the time of diagnosis in any patient in the group with adherence (Table 5).

Treatments in the Patients Developing Hepatocellular Carcinoma and Follow-Up Results

Of the patients that developed HCC, 6 were treated using locoregional treatment methods and curative resection was performed in 6 patients. Seven patients have undergone liver transplantation. Four patients died. The last patient was followed up without any treatment, as he refused all treatment options. In the group with adherence, curative resection was performed in 5 patients and 6 patients received liver transplant treatment while in the group without adherence curative resection was performed in one patient and one patient received a transplant. While one patient died in the group with adherence, 3 patients died in the group without adherence (Table 5).

Discussion

The purpose of the chronic HCV treatment is to protect the patient from the complications of chronic HCV infection by obtaining a sustained virologic response. Although SVR is achieved with treatment, patients should continue their outpatient visits for

Table 4. Antiviral Treatment Regimes and HCC Surveillance Adherence Relationship (USG+AFP)

HCC Surveillance adherence n (%) (months)	1-6	7-12	12-24	25 - >
DAAs (n=37)	27 (73.0)	5 (13.5)	2 (5.4)	3 (8.1)
PEG-IF/RBV (n=46)	13 (28.3)	5 (10.9)	7 (15.2)	21 (45.7)

HCC: Hepatocellular carcinoma, DAAs: Direct acting antivirals, PEG-IF/RBV: Pegylated interferon/ribavirin

Table 5. HCC development frequency and features according to adherence with surveillance

Number of patients with HCC (n=24)	Adherence with surveillance (n=14)	Adherence without surveillance (n=10)
Tumor size (cm); mean ± SD	2.1±2.4	6.5±1.9
BCLC (stage) (n)		
0	1	0
A	9	1
Meeting with milan transplantation criteria (n)	11	2
AFP (IU/mL); Mean ± SD	310±245	650±165
AFP (IU/mL) >1000 IU/mL (n)	0	4
Liver transplantation/resection (n)	6/5	1/1
Locoregional therapy (n)	3	3

HCC: Hepatocellular carcinoma, n: Number, BCLC: Barcelona clinical liver cancer (staging system), AFP: Alpha-fetoprotein, SD: Standard deviation

the follow-up of complications that may be developed, such as chronic liver disease or hepatocellular carcinoma. Therefore, the follow-up of the patients with advanced fibrosis with or without cirrhosis at 6-month intervals should be planned.

A total of 83 patients with SVR were included in our study. While 32 of them had cirrhosis in the pre-treatment period, 51 of them had non-cirrhotic advanced fibrosis. Based on the results of study, the patients with cirrhosis have significantly greater number of outpatient visits and imaging examinations during the follow-up period after the achievement of SVR compared to those without cirrhosis. However, although the majority of the cirrhotic patients continued their follow-up procedures after the achievement of SVR, 10% of the patients did not continue even their outpatient visits. Adherence of the non-cirrhotic CHC patients with advanced fibrosis to the surveillance protocols is lower compared to cirrhotic patients. When patients learn that they are cured at the end of the treatment, they think that they are not necessary to be followed up for a disease that no longer exists, and because of this thought a significant proportion of patients either discontinue the follow-up or skip their checks. However, some patients apply due to the development of HCC years after the achievement of SVR (12). In order to further reduce this rate, starting from the stages of diagnosis and treatment, it is of great importance to take sufficient time to the patients in outpatient visits, provide detailed information on the importance of the treatment and the follow-up after the treatment, and emphasize the potential complications over and over again.

It is a matter of concern that the rate of non-optimal surveillance for HCC is 22.9% (n=19) and the rate of non-surveyed patients is 28.9% (n=24). It was observed that only 61.4% (n=51) of the patients were screened every 12 months and only 48.2% (n=40) were screened every 6 months (USG+AFP). Although a study reporting the surveillance rates by specialty suggests that the gastroenterologists (100%) perform more frequently HCC surveillances on patients at risk compared to nephrologists (71%), primary care physicians (84.2%) or internists (88.4%) (p=0.016), the results of our study remained far from the aforementioned rate of 100% (13). This shows a poor adherence to the current guidelines and demonstrates that although HCC surveillance guidelines are regularly updated and the national and international liver diseases meetings and post-graduate training courses continue to draw attention to this issue, the concern of non-adherence to the guidelines in everyday practices still continues.

The results of our study are in line with the results of the study suggesting that HCC follow-up procedures are not sufficient in patients with CHB infection and sharp decreases have been observed in adherence over time during the 5-year follow-up period even in patients who were initially properly followed-up (14). The results of our study also coincide with the low adherence rates reported in other HCC surveillance studies in high-risk populations (15,16).

Moreover, the follow-up rates in patients treated with DAAs were significantly higher than the ones in the patients treated with non-DAA treatments. It is thought that the oral use of DAAs in a shorter period of time facilitates the treatment and the high success rate of DAA treatment reinforces the patients' trust in the treatment, and this has an effect on the adherence of the patients to the follow-up procedure after the treatment.

Development of HCC was detected in 24 (6.1%) patients during the follow-up period in our study and 4 of them died before they even had any chance of treatment. Development of HCC is the most important complication of CHC in the long term and it is of vital importance. The greatest importance in determining the follow-up algorithms of the patients is attached to HCC surveillance. In a study performed in Austria in 2018, 551 CHC patients with SVR were followed for approximately 15 months, HCC development was detected in 4.1% of these patients and the mortality rate was reported as 2.2% (17).

Considering the longer follow-up period in our study, HCC development and mortality rate were lower in our study group. The fact that 71% of the patients included in that study had cirrhosis at the beginning of the study may have directly influenced the greater number of HCC development and higher mortality rates compared to our study. Moreover, although the rate of patients with genotype 3, which are thought to have a higher risk of developing HCC, is below 1% in our study, the said rate being 7.3% in that study seems to influence this issue.

As shown in various studies, the absence of optimal surveillance efforts affects the estimated life expectancies of the patients due to the inability to receive appropriate treatments (18,19,20). Indeed, a study examining the HCC surveillance in cirrhotic patients revealed that the mean 3-year survival in this patient group showed a direct and strong correlation with the quality of the surveillance (40% for optimal surveillance; 27% for suboptimal surveillance and 13% if no surveillance is performed, $p<0.005$) (8).

Our study showed that the diagnosis of HCC in adhered patients was made when the tumors were significantly smaller which allowed patients that adhere to the surveillance guidelines to receive significantly greater amount of curative treatment. Our results also confirm the results of other studies indicating that early detection of HCC increases the likelihood of receiving curative treatment (21,22,23).

Since our study was performed retrospectively and was not designed to reveal whether there was a difference between the physician and the patient in terms of adherence to the surveillance guidelines, it was not reviewed whether the test and the imaging evaluations were requested by the physicians at appropriate time intervals and the patients adhered to these requests. However, it was found that the rate of performing the HCC surveillance optimally was higher in patients who had frequent outpatient visits. This may be the result of the fact that physicians are more frequently reminded of these examinations in patients with frequent visits or that patients more adhere to the physician's recommendations by remembering the importance of HCC surveillance.

Another point to be emphasized is that the patients' adherence to the physicians' recommendations may affect the rate of adherence to the HCC surveillance guidelines. Many studies address the obstacles reported by patients that decrease the adherence to the HCC surveillance guidelines. These include the length of time between the clinical visit and the performance time of radiological imaging, living far from the hospital and the limited number of clinical visits. Indeed, some patients believe that they do not need any surveillance if they are on a healthy diet, or if

they do not have any complaints or their initial examinations gave results within the limits (24,25). Yet our study was not designed to evaluate this aspect.

Study Limitations

It is reported that significant progress has been made in recent years in the treatment of CHC infection, which is one of the most important causes of chronic liver disease and HCC around the world and in our country, and thanks to the newly developed DAAs, a much easier and effective treatment can be administered and the global eradication of HCV may be possible. However, even if a SVR is achieved, patients with an increased risk of complications and HCC development, especially due to the presence of advanced fibrosis**,** should be followed and screened for HCC development at regular intervals using laboratory and imaging examinations. The gradual increase in the pool of advanced fibrosis patients that achieved a sustained virologic response due to effective treatments and the longevity of the life expectancy of this patient group lead to the rapid growth of the risky population in terms of HCC development. Our study is important since it is one of the rare studies with high patient numbers on this increasingly important subject. However, there are some limitations. First of all, its retrospective design may have caused loss of information and problems of objective evaluation. However, the use of clinical information and the detailed review of the physicians' notes allowed to evaluate the external examinations and imaging, and prevent to make any mistake in the classification of cirrhosis and HCC. There is no control group in our study and the nature of the disease does not allow any randomized, controlled, prospective study to be performed. In addition, we think that the occurrence of errors in terms of patient selection has been significantly reduced due to the fact that the study was performed in real-life conditions, although patients from a single reference center have been included.

Conclusion

HCC surveillance in patients with advanced fibrosis is not performed in accordance with the guidelines, more particularly in non-cirrhotic patients. Increased adherence to surveillance guidelines seems to facilitate the detection of HCC development at an early stage, and positively affect the survival and enable more curative treatments to be performed in the meanwhile. Since the most effective indicator of the higher adherence seems to be the increased number of visits, it is thought that the ensuring continuity in outpatient visits, maintaining more than two visits per year, and taking sufficient time at the visits to provide more information may increase the adherence of both physician and patient to the surveillance guidelines and decrease the morbidity and mortality rates.

Ethics

Ethics Committee Approval: The ethics committee approval for the study was obtained from the Local Ethics Committee of Kocaeli University (approval number: 327, date: 2019).

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.Ş., S.H., Concept: G.Ş., Design: G.Ş., Data Collection or Processing: G.Ş., S.H., Analysis or Interpretation: G.Ş., Literature Search: S.H., Writing: G.Ş.

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Research Article

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Which is More Important and Insidious in Dialysis Patients? Occult Hepatitis B or Occult Hepatitis C?

Diyaliz Hastalarında Hangisi Daha Önemli ve Sinsidir? Okült Hepatit B veya Okült Hepatit C mi?

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ABSTRACT

Objectives: The aim of this study was to investigate the presence of occult hepatitis B infection (OBI) and Occult hepatitis C infection (OCI) in hemodialysis patients and to determine whether there is an activation in the follow-up or not.

Materials and Methods: Demographic data, causes of renal failure, access to hemodialysis, duration of hemodialysis, alanine aminotransferase (ALT) levels, hepatitis indicators of 100 HD patients with normal ALT levels were recorded in this study. Serum anti-hepatitis B core antibody (anti-HBc) immunoglobulin G (IgG) was tested with ELISA (Architecht, Abbott). Serum hepatitis B virus (HBV)-DNA, HCV-RNA [in peripheral blood mononuclear cells (PBMNC)] were studied with "real-time" polymerase chain reaction method.

Results: Anti-HBc IgG positivity was detected in 27% of patients, but with no isolated anti-HBc IgG positivity. In 4% of the patients, HBV-DNA positivity and OBI infection were detected. None of the patients showed HCV-RNA positivity in serum and in PBMNC, therefore OCI was not detected. None of the patients developed OBI or OCI activation in five-years follow-up. Renal transplantation was performed in one of the OBI patients and lifelong prophylaxis was planned with oral antiviral medication.

Conclusion: Presence of OCI is lower than OBI in hemodialysis patients.

Keywords: Occult Hepatitis B, occult hepatitis C, hemodialysis

ÖZ

Amaç: Bu çalışmanın amacı hemodiyaliz hastalarında okült hepatit B enfeksiyonu (OBE) ve okült hepatit C enfeksiyonu (OCE) varlığını araştırmak ve izlemde aktivasyon olup olmadığını tespit etmektir.

Gereç ve Yöntemler: Çalışmaya, normal alanin aminotransferaz (ALT) düzeyine sahip 100 HD hastasının demografik verileri, böbrek yetmezliği nedenleri, hemodiyalize erişim, hemodiyaliz süresi, ALT düzeyi ve hepatit göstergeleri kaydedilmiştir. Serum anti-hepatit B çekirdek antikoru (anti-HBc) immünoglobulin G (IgG), ELISA (Architecht, Abbott) ile test edilmiştir. Serum HBV-DNA, HCV-RNA [periferik kan mononükleer hücrelerinde (PKMNH)] "gerçek zamanlı" polimeraz zincir reaksiyonu yöntemi ile araştırılmıştır.

Bulgular: Hastaların %27'sinde anti-HBc IgG pozitifliği saptanmıştır, ancak izole anti-HBc IgG pozitifliği bulunmamaktadır. Hastaların %4'ünde HBV-DNA pozitifliği ve OBE tespit edilmiştir. Hiçbir hastada serum ve PBMNH'de HCV-RNA pozitifliği bulunmamıştır, bu nedenle OCE saptanmamıştır. Beş yıllık takip süresince hiçbir hastada OBE veya OCE aktivasyonu gelişmemiştir. OBE hastalarından birinde böbrek nakli yapılmış ve oral antiviral ile yaşam boyu profilaksi planlanmıştır.

Sonuç: Hemodiyaliz hastalarında OCE varlığı OBE'den düşüktür.

Anahtar Kelimeler: Okült hepatit B, okült hepatit C, hemodiyaliz

Zanapalıoğlu Gazel Ö, Şener A. Which is More Important and Insidious in Dialysis Patients? Occult Hepatitis B or Occult Hepatitis C?. Viral Hepat J. 2020;26:39-42.

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Introductıon

In patients with chronic renal failure (CRF), infections are important causes of morbidity and mortality. These patients are particularly at risk of parenterally transmitted viral hepatitis (1). Hepatitis B and C viruses (HBV and HCV) are primarily transmitted parenterally in dialysis patients. Chronic hepatitis B (CHB) Chronic hepatitis C and (CHC) are more common infectious agents in patients with CRF compared to the normal population. These infections are also causes morbidity and mortality in patients with CRF and in patients undergoing renal transplantation (2,3). According to the Turkish Nephrology Association; hepatitis B surface antigen (HBsAg) positivity was 2.65% and anti-HCV positivity was 3.94% in hemodialysis patients in Turkey at 2017 (3).

HBV infection recovery defined as HbsAg dissaperance with HBV-DNA negativity in case of anti-Hbs positivity. The evaluation of serological markers for determining the infection is important but may be insufficient. Sensitive polymerase chain reaction (PCR) techniques have shown a low level of HBV-DNA in some patients who have spontaneously and serologically lost their HBsAg in serum and/or liver. Therefore, this condition, which defines chronic HBV infection (by PCR) with undetectable HBsAg levels, is called occult HBV infection (OBI) (4). OBI is divided into two groups according to anti-HBc and anti-HBs positivity.

The actual cause of approximately 10% of liver enzyme abnormalities is unknown. In the last decade, OCI has been defined with studies have been conducted to identify patients with chronic liver disease whose etiology has not been clarified. Firstly HCV-RNA was detected in liver cells when anti HCV and HCV-RNA were negative in serum. Thereafter HCV-RNA was found in liver and in peripheral blood mononuclear cells (PBMNC) with undiagnosed high liver function tests. Viral RNA can be detected in PBMNC over 70% of patients with OCI (5,6).

OCI, firstly defined by Castillo et al. (6) HCV-RNA is detected in liver cells, while serum anti-HCV and HCV-RNA was negative. In the following years, Fabrizi and Martin (7,8) defined OCI, in patients with elevated liver enzyme; serum anti HCV and HCV-RNA were negative, whereas HCV-RNA was detected in liver cells and PBMNC (7,8). Recent studies report two different types of OCI; seronegative and positive. In both types of OCI, HCV-RNA is positive in liver cells of patients, and viral RNA can be detected in PBMNC with serum ultracentrifugation (9,10).

The aim of this study was to investigate the presence of OBI and OCI in hemodialysis patients in Çanakkale, and follow up the reactivation of OBI and OCI.

Materials and Methods

This study was approved by the Çanakkale Onsekiz Mart University Ethics Commitee (approval number: 2014/03, date: 05.02.2014). We included 100 patients over 18 years of age and written informed consent was obtained from the patients. Patients were selected who had normal alanine aminotransferase (ALT) levels and shows seronegativity for HbsAg and anti-HCV antibody tests. The demographic data, ALT levels, hemodialysis periods, hepatitis B vaccination history, HBsAg, anti-Hbs and anti-HCV indicators were recorded.

Peripheral venous blood samples were collected from 5 mL each of 3 separated biochemistry tubes for anti-HBc Immunoglobulin G (IgG), HBV-DNA, HCV-RNA, and an amount of 9 mL blood in EDTA tube for PBMNC separation.

Anti-HBc IgG test was carried out with the Architect anti-HBC II Reagent kit. Blood samples for HBV-DNA and HCV-RNA isolation were centrifuged at 1500 rpm for 15 minutes. The obtained sera were stored at -20 °C until isolation of DNA and RNA.

Whole blood (9 mL) was taken into the EDTA tube for further differentiation of PBMNC. Histopaque (R)-1077 (9 mL) was added to 50 mL falcon tube. Gently drop whole blood with sterile pasteur pipette from the edge of the falcon tube onto the Histopaque®-1077. According to the manufacturer's recommendations, it was centrifuged at 400 G cycle for 30 minutes. After centrifugation, the cells in the cloud appearing in the middle of the tube were identified as PBMNC, and these cells were taken to the microvida lid cryo tubes by taking 3 mL with the help of micropipette. RNA was stored at -20 °C until isolation.

Prepared serum and PBMNCs after DNA/RNA isolation using HBV-DNA and HCV-RNA isolation kit (Magnesia®-2448 nucleic acid isolation and PCR setup robot) in Anatolia Diagnostic and Biotechnology R&D laboratory Montaina® 4896 real time (RT)-PCR Bosphore® HBV/HCV quantification (analytical sensitivity is 25 IU/mL and its linear range is 1x10 duy-1x 10 v IU/mL) was performed using Kit V1.The Bosphore® HBV Quantification Kit v1 (analytical sensitivity of 10 IU/mL and a linear range of 1x10 analytic-1x10 Kit IU/mL).

OBI was defined as HBV-DNA positivity in patients with HBsAg negative and with normal ALT levels.

OCI was defined as HCV-RNA positivity in patients with anti-HCV negative in patients with normal ALT levels.

Statistical Analysis

SPSS 20.0 package program used for data collection, recording and analysis.

Results

The study included 100 patients with normal ALT levels and HBsAg negative, anti-HCV negative in one dialysis center in Çanakkale province. Demographic data of the patients included in the study are given in Table 1. Fiftyeight (58%) of the patients were male and 42 (42%) were female. The mean age was 63.5±12.5 years. Eighty-five (85%) patients underwent dialysis through arteriovenous fistula. Other patients underwent dialysis with a

Table 1. General characteristics of patients

Age (year), ± SD	**63.5±12.5**
Gender (female/male)	42/58
Comorbid diases (other than CRF), n (%)	88 (88%)
Hemodialysis way	
Catheter, n (%)	15 (15%)
A/V fistula, n (%)	85 (85%)
Total hemodialysis time (month), ± SD	67.6±51.3
ALT, ± SD	10.5±7.2

ALT: Alanine aminotransferase, normal range: 0-55 IU/mL, A/V: Arteriovenous, SD: Standart deviation

Table 2. Occult Hepatitis B diagnosed patients' characteristics

Patient Number	Age	Gender	HD way	Total HD time (month)	HBV DNA level (IU/mL)	Anti Hbs titer (mIU/mL)
(42)	33	M	AVF	59	61.4	1000
(58)	61	M	AVF	112	56.9	270
(98)	67	F	AVF	93	60	220
(100)	67	M	AVF	27	48.6	21

HD: Hemodialysis, AVF: Arteriovenous fistula, M: Male, F: Female

permanent hemodialysis catheter. The mean duration of dialysis was 67.6 months. 95% of the patients had dialysis 3 days a week and 5% had 2 days dialysis. When the serological tests of the patients are examined; 27 patients (27%) were showed anti-HBc IgG and anti-HBs positivity together. None of the patients had anti-HBc IgG positivity alone. HBV-DNA was positive in 4 (4/100, 4%) of all patients. OBI diagnosed patients' characteristics were shown at Table 2. When the hepatitis B vaccination history were examined, it was seen that 55% had at least three doses.

There was not any clinical and laboratory activation of hepatitis B in five-years follow-up of these four patients. Serum and PBCMN were investigated by PCR for OCI and HCV-RNA was not detected in any of the patients. The general characteristics of 27 patients with anti-HBc IgG positive are in Table 2. The mean age of the patients was 57±16.2 years. The mean duration of hemodialysis was 72.7±37.5 months. ALT levels were within normal limits and the mean was 9.7±2.3 IU/mL.

Discussion

Although HBsAg positivity was decreased in hemodialysis patients, HBV viremia OBI was shown by PCR tests. The prevalence of OBI varies from 1% to 87% in different regions of the world (11).

The incidence of OBI is different in every country, for example it has been reported between 0%-36.4% in blood donors in our country (12). According to other studies, OBI was reported actullay between 3.4% and 19% in hemodialysis patients (13). In this study, we investigated the presence of OBI and OCI with RT-PCR in hemodialysis patients. In our study, the incidence of OBI was found 4%. But OCI was not obtained in our study group. In Egypt, Helaly et al. (14) found anti-HBc IgG positivity in all patients who had detected OBI. Therefore, in the presence of anti-HBc IgG positivity, patients should be investigated for possible OBI by molecular methods (14). But in our study none of the OBI patients did not showed this antibody positivity for core antigen. In our opinion, the main cause of this stiutaion is; core antigen production was irregular in hepatocytes. If the replication continues regularly, you can detect antibody response, but if not antibody shows negativity. In hemodialysis patients this irregular sythesis might be in maximum level because of the CRF.

In the study conducted by Fontenele et al. (15) in Brazil, 79% of 301 patients with CRF who had hemodialysis showed anti-HBs positivity and isolated anti-HBs positivity was detected in only 35% of the patients. They found OBI in three patients with anti-HBs positivity alone. Anti-HBs positivity was found in 95% of our patients and 68% of patients have been showed anti-HBs positivity alone. All of OBI patients showed anti-HBs positivity alone.

Although the exact cause is unknown, the presence of anti-HBs in hemodialysis patients with OBI suggests that inadequate neutralization of virus and routine serological profiles alone are not always sufficient to define the status of HBV infection.

There are also important studies on the clinical consequences of OBI in organ transplant patients. Each hemodialysis patient is a candidate for kidney transplantation. Before solid organ transplantation, recipients should be screened for serological tests (HBsAg, anti-HBs, anti-HBc IgM, IgG or total, anti-HCV) for possible hepatitis. In hemodialysis patients receiving immunosuppressive therapy before and after transplantation, viral activation of HCV and HBV might have seen and also cause fulminant hepatitis. Screening with appropriate methods before solid organ transplantation will increase transplant success (16). Therefore, it is suggested that these patients should be evaluated in terms of HBV-DNA search by molecular methods.

One of the OBI patient has been undergone renal transplantation. Oral antiviral prophylaxis has been began and hepatitis B viral activation hasn't seen as other OBI patients in five-year follow-up.

Study Limitations

Our study population was not enough to make a general recommendation for management of OBI in all dialysis patients.

Conclusion

In our study, we found the incidance of OBI 4% in seronegative hemodialysis patients, but OCI was not obtained. When the infectious properties of these patients are also taken into consideration, it is inevitable that HBV negative patients will be infected by dialysis. It is an important risk factor that may adversely affect morbidity and mortality in these patients whose quality of life has decreased significantly due to CRF. Since our key diagnostic method for detection of OBI is HBV-DNA, it is essential to standardize the technique and used method. During the follow-up in dialysis units, once a year, viral DNA analysis with PCR-based method can be helpful in preventing the problems that may occur in the expected for organ transplantation.

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Ethics

Ethics Committee Approval: This study was approved by the Çanakkale Onsekiz Mart University Ethics Commitee (approval number: 2014/03, date: 05.02.2014).

Informed Consent: Written informed consent was obtained from the patients.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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