VIRAL HEPATITIS SOCIETY

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# Viral Hepatitis Journal

## VİRAL HEPATİT DERGİSİ

### RESEARCH ARTICLES



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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.
The journal's editorial policies are based on "ICMJE Recommendations" (2016, http://www.icmje.org/) rules.
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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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**Scientific and Ethics Responsibility**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

• Author number for review articles should not exceed three.

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

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

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

Manuscripts should be written with Microsoft Word.

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

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

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

**ARTICLE SECTIONS**

The text file should include the title in Turkish, keywords, the title in English, keywords in English, the text of the article, references, tables (only one table for one page) and figure legends (if any), respectively. Within the text file, the names of the authors, any

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information about the institutions, the figures and images should be excluded.

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

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

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

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

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

**Keywords:**

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

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

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

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

Original researches should have the following sections;

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

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

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

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

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

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

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

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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 key words. The main text should consist of introduction, case report, discussion, acknowledgment, conclusion and references. The entire text should not exceed 5 pages (A4, formatted as specified above).

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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.

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

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Letters to the Editor should be short commentaries related to current developments in viral hepatitis and their scientific and social aspects, or may be submitted to ask questions or offer further contributions in response to work that has been published in the Viral Hepatitis Journal. Letters do not include a title or an abstract; they should not exceed 1,000 words and can have up to 5 references.

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

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

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

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

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

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

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

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

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Articles must be complete. They must include the following:

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• Title Page

• Article sections

• Turkish and English titles

• Abstract (250 words) (Turkish and English)

• Keywords (minimum 3; maximum 6)

• Article divided into appropriate sections

• Complete and accurate references and citations

• List of references styled according to "journal requirements"

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

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## EDITORIAL

Dear colleagues,

We are here again with the second issue of 2017 that includes new interesting subjects. This issue starts with review article titled "TNF-alpha 308 SNP *Rs3091256* GG Genotype is Strongly Associated with Fibrosis in Patients with Chronic Hepatitis C ", and continues with the research articles titled "Evaluation of the Seroprevalence of Hepatit A and Vaccination Status in Children Aged Two and Sixteen Years.", "Does Vitamin D Level Affect the Response to Antiviral Treatment in Egyptian Patients with Chronic Hepatitis C?", "Can HBsAg Be Used as a Viral Replication Marker in Chronic Hepatitis B Patients?" And the last one is "Investigation of Mean Platelet Volume, Platelet Distribution Width and Erythrocyte Distribution Width in Patients with Hepatitis B Virus Infection." Our primary aim is to update the readers with the recent developments. With this purpose in mind, we expect your contributions with original articles, reviews, case reports and letters to the editor. To meet in new issues. This journal is indexed in Emerging Sources Citation Index (ESCI).

**Prof. Dr. Fehmi TABAK**
**Doç. Dr. Ebubekir Şenateş**

**Prof. Dr. Rahmet Güner**
**Prof. Dr. Tansu Yamazhan**

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Viral Hepatitis Journal 2017;23(2):39-45

# *TNF-alpha 308 SNP Rs3091256 GG Genotype is Strongly Associated with Fibrosis in Patients with Chronic Hepatitis C*

## TNF-alfa 308 SNP *Rs3091256* GG Genotipi Hepatit C Virüs Hastalarının Karaciğer Fibrozis Evreleri ile İlişkilidir

Özgür GÜNAL[1], Didem YALÇIN[2], Betül ÇELİK[3], Aydın RÜSTEMOĞLU[4], Osman DEMİR[5], Şener BARUT[6], Ömer ATEŞ[4], Sırrı KILIÇ[1]

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***ABSTRACT***

**Objective:** We aimed to review the influence of host genetic factors on the clinical course, treatment response as well as fibrosis progression in patients with viral hepatitis C genotype 1.
**Materials and Methods:** Ninety-five patients with chronic hepatitis C virus (HCV) infection and 97 controls were enrolled. The patients received pegylated interferon (Peg-IFN)+ribavirin therapy for 48 weeks and were followed up for the next 48 weeks. Aspartat aminotransferase/platelet ratio (APRI) was used to detect liver fibrosis DNA specimens were extracted from the peripheral blood mononuclear cells and the tumor necrosis factor-alpha (TNF-α) 308 *rs3091256* was genotyped by the polymerase chain reaction-restriction fragment length polymorphism method.
**Results:** All patients included in the study were infected with HCV genotype 1. of the 95 HCV-positive patients, spontaneous viral clearence was observed in 25.5%, rapid viral response in 44.2%, early viral response in 91.8%, and sustained viral response was found in 73.3% of patients. The allele and genotype were not significant between patients and controls. There was no significant difference in virologic response as well. However, TNF-α-308 single nucleotide polymorphisms (SNP) *rs3091256* GG genotype was strongly associated with fibrosis and alanine aminotransferase (ALT) levels (p=0.006 and p=0.017, respectively).
**Conclusion:** TNF-α-308 polymorphisms may reveal different results among countries. Patients having SNP *rs3091256* GG are prone to have higher ALT levels and fibrosis score but have better treatment outcome.
**Keywords:** Hepatitis C, tumor necrosis factor alpha, polymorphisms, interferon, treatment

***ÖZ***

**Amaç:** Viral hepatit C genotip 1 hastalarında genetik faktörlerin klinik gidiş, tedavi cevabı ve fibrozis ilerlemesi üzerindeki etkisini gözden geçirmektir.
**Gereç ve Yöntemler:** Çalışmaya 95 kronik hepatit C virüs (HCV) hastası ve 97 sağlıklı gönüllü dahil edildi. Hastalar 48 hafta süreyle pegylated interferon (Peg-IFN)+ribavirin tedavisi kullandı ve sonraki 48 hafta boyunca takip edildi. Karaciğer fibrozis evresini belirlemek için aspartat aminotransferaz/platelet ratio (APRI) kullanıldı. DNA örnekleri periferik kan mononükleer hücrelerden izole edildi ve tümör nekroz faktörü-alfa (TNF-α) 308 rs3091256, polimeraz zincir reaksiyonu-kısıtlama fragmanı uzunluğu polimorfizmi yöntemi ile genotiplendi.
**Bulgular:** Tüm hastalar HCV genotip 1 ile enfekte idi. HCV hastalarının (95), %25,5 spontan viral klirensi, %44,2'si hızlı viral yanıt, %91,8'i erken viral yanıt ve %73,3'ünde kalıcı viral yanıt gözlendi. Hastalar ve kontroller arasında allel ve genotip açısından anlamlı fark yoktu. Virolojik yanıt da belirgin değildi. Bununla birlikte, TNF-α-308 tek nükleotit polimorfizmi (SNP) *rs3091256* GG genotipi fibroz ve alanin aminotransferaz (ALT) seviyeleri ile kuvvetli bir şekilde ilişkiliydi (sırasıyla p=0,006 ve p=0,017).
**Sonuç:** TNF-α-308 polimorfizmleri, ülkeler arasında farklı sonuçlar ortaya çıkarabilir. SNP *rs3091256* GG'ye sahip hastalar, daha yüksek ALT ve fibroz skoru göstermekle birlikte, bu popülasyonda daha iyi tedavi sonucuna sahiptir.
**Anahtar Kelimeler:** Hepatit C, tümör nekroz faktörü-alfa, polimorfizm, interferon, tedavi

**Günal Ö, Yalçın D, Çelik B, Rüstemoğlu A, Demir O, Barut Ş, Ateş Ö, Kılıç S. TNF-alpha 308 SNP Rs3091256 GG Genotype is Strongly Associated with Fibrosis in Patients with Chronic Hepatitis C. Viral Hepat J. 2017;23:39-45.**

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**Table 1.** Clinical variables of the patients

| Variables | | Statistics |
|---|---|---|
| Patients | Active hepatitis | 70 (73.7) |
| | Spontaneous clearence | 25 (26.3) |
| RVY | hvy + | 27 (42.2) |
| | hvy - | 37 (57.8) |
| EVY | evy + | 58 (90.6) |
| | evy - | 6 (9.4) |
| Primary Non-responder | yes | 6 (9.4) |
| | no | 58 (90.6) |
| Relaps | yes | 15 (23.4) |
| | no | 49 (76.6) |
| SVR | yes | 46 (71.9) |
| | no | 18 (28.1) |
| Sex | female | 63 (63.6) |
| | male | 36 (36.4) |
| Treatment | IFN-α | 35 (47.3) |
| | IFN-β | 29 (39.2) |
| | No treatment | 10 (13.5) |
| TNF | GG | 78 (82.1) |
| | GA | 14 (14.7) |
| | AA | 3 (3.2) |
| TNF_Gene | GG | 78 (82.1) |
| | GA+AA | 17 (17.9) |
| IL28B860 | CC | 23 (23.2) |
| | CT | 58 (58.6) |
| | TT | 18 (18.2) |
| IL28B275 | GG | 18 (18.6) |
| | GA | 53 (54.6) |
| | AA | 26 (26.8) |
| IL28B917 | GG | 10 (10.1) |
| | GT | 49 (49.5) |
| | TT | 40 (40.4) |
| CCR5 | NN | 93 (93.9) |
| | ND | 6 (6.1) |
| APRI score | 0-0.49 | 43 (58.1) |
| | 0.50+ | 31 (41.9) |
| APRI score | 0.0 | 27 (36.5) |
| | No fibrosis | 44 (59.5) |
| | Extensiv fibrosis | 3 (4.1) |
| Age | | 55.91±9.84 |
| HCV RNA | | 784.545±1345.413 |
| HAI (ISHAK) | | 9.04±3.33 |
| Fibrosing score (ISHAK) | | 1.83±1.05 |
| WBC | | 6357.87±2192.49 |
| HB | | 12.98±1.61 |
| PLT | | 220.29±69.81 |
| AST | | 38.7±28.07 |
| ALT | | 44.85±42.02 |
| APRI | | 0.56±0.42 |

Used to n (%) for qualitative variables and mean ± standard deviation for quantitative variables
RVY: Rapid virologycal response, EVY: Early virologic reponse, SVR: Sustained virologic response, APRI: Aspartate aminotransferase to platelet ratio index, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TNF: Tumor necrosis factor, HAI: Hepatic activity index, WBC: White blood cells, HB: Hemoglobin, PLT: Platelets, HCV: Hepatitis C virus IFN-α: Interferon alpha, IFN-β: Interferon beta

found to be significantly higher in CHC patients with TNF-α-308 GG polymorphism compared to those with GA+AA polymorphism (p=0.006) (Table 3).

To evaluate the clinical applicability of the outlined SNP, we calculated the predictive odds ratios for the SNP between rapid virological response (RVR), early virological response (EVR) and SVR (Table 4). There were 24 patients who had spontaneous viral clearance. RVR was seen in 27 patients. EVR in 56 patients and SVR was observed in 44 patients. All the genotypes or alleles predicted the positive response to treatment in the overall study population.

## Discussion

HCV infection continues to be a major health problem worldwide. Viruses are the most common cause of diseases such as chronic hepatitis and liver cirrhosis. HCV is divided into seven major genotypes (21). After infection with HCV, a large proportion of patients develop CHC, with a very few spontaneous clearance. (6). There are viral and host factors that are important in the development of chronic infection. Baseline viral load, RVR and host characteristics (e.g. alcohol consumption, steatosis, liver fibrosis, metabolic syndrome, ethnicity, and host genetic polymorphisms, especially IL28B, are the examples that have impact on virological response of the host (2). The most common HCV genotype worldwide is genotype 1 (46% of all HCV cases), while genotype 3 is the second most common (30%). However, the distribution of these genotypes varies between countries (22).

The first target for HCV is human hepatocytes. The immune system first activates the natural immune system. As a result, local IFN production begins and HCV genome replication and spreading in the liver parenchyma is disrupted (23). The mechanism of effective clearance of HCV from the human body is likely related to both environmental and host genetic factors. For example, it has been observed that treatment success in patients of European ancestry is better than in patients of African ancestry (24). In the current study, we performed an analysis to examine the association between the SNP in the promoter region of TNF-α-308 *rs3091256* and fibrosis score, ALT level, spontaneous viral clearance and treatment response to HCV infection.

There are varying results from studies on TNF-α-308 gene polymorphism in the literature. Although some studies have reported a significant association between TNF polymorphism and response to hepatitis C treatment, some studies reported

## Introduction

Hepatitis C virus (HCV) is a major etiologic factor for the development of chronic liver diseases (cirrhosis, hepatocellular carcinoma, etc.). Hepatocytes are the primary target cells supporting HCV replication. After HCV infection, the innate immune system begins to respond to the virus and after 4 to 8 weeks, the CD8 + T cells recognize viral peptides that bind to human leukocyte antigen class I molecules in virus-infected hepatocytes (1).

This initiates signaling pathways leading to the synthesis of interferon (IFN), tumor necrosis factor (TNF) and a variety of other cytokines. In the acute phase of the infection, the virus is removed from the T-cell-mediated antiviral mechanisms. The rate of spontaneous viral clearance in acut HCV infection is aproximately 26% (range: 15-40%) (2,3,4,5). In patients who cannot clear the virus from hepatocytes in the first phase, HCV remains for years as long as it is not being treated. The effective treatment of chronic HCV infection (CHC) is based on a combination of pegylated-IFN (Peg-IFN) and ribavirin (RBV) (6). IFN, especially IFN- λ3, interacts with its acceptor, a heterodimer (IFN-lambdaR1 x IL-10R2). In IFN-based treatments, sustained viral response (SVR) rate is 40% (2,7).

The most important parameter in the therapeutic success in HCV infection is based on the HCV genotype (e.g., genotype 1 is the most difficult to treat) (2). In IFN-based treatments in the genotype 2, 3 and 5, the rate of SVR is 70-90% while in genotype 1 and 4, it is not more than 50% (1,8). HCV genotype 1 (91.8%) was the most common genotype in multicentre studie performed by Gürbüz et al. (9) in our country, while genotype 2 (4%) was detected in the second frequency.

Besides HCV genotype, serum alanine aminotransferase (ALT) level, histological grading and cytokine response of the host may affect HCV infection, viral clearance, and treatment (10,11). Among the cytokines, the most attention was devoted to TNF-alpha (TNF-α). Serum TNF-α level elevates in CHC patients (12) and SVR has been found to be associated with the baseline increased production of TNF-α (11). A positive correlation has been found between serum TNF-α levels and hepatic necroinflammatory score as well (13).

It was demonstrated that HCV can directly induce the expression of TNF-α in hepatocytes (14). Induction of TNF-α by HCV is dependent on Toll-like receptor (TLR) 7 and TLR8. Form recognition receptors seen in many cell types that participate in the innate immune response associated with viral infections and viral antigens are called TLRs (15). TNF binds to two receptors, TNFR1 and TNFR2; the first is structurally expressed in most cells, the second is inducible and has a more limited expression pattern (16). Upon receptor binding, TNF-α signals through a variety of cytosolic proteins, including TRADD (TNFR1-associated death domain protein) (17) and TNF receptor-associated factor 2 (18), leading to I_B degradation and the subsequent release and nuclear translocation of nuclear factor (NF)-κB. Binding of NF-κB to gene promoters initiates transcription of numerous proinflammatory cytokines, including TNF-α, IL-6, IL-8, and CXCL-10 (19,20) which suppress HCV replication.

The present study was designed to investigate the frequency of single-nucleotide polymorphism (SNP) of the TNF-308 locus in a population in Turkey, a region with a high prevalence of HCV infection. High prevalence of genotype 1b is more likely associated with fibrosing score, ALT level, spontaneous viral clearance and efficacy of treatment of HCV genotype 1.

## Materials and Methods

A total of 95 anti-HCV-positive genotype 1b and 2 patients (70 HCV RNA+ chronic active hepatitis and 25 HCV RNA-negative and spontaneous clearance) and 97 healthy control subjects (57 female, 40 male) were included in the study. The Ethics Committee of Gaziosmanpaşa University approved the present study and all participants provided written informed consent for the study (Grant number: 11-BADK-111).

Genomic DNA was extracted from blood samples using an Invitrogen Genomic DNA Isolation Mini Kit K1820-02 (Invitrogen Life Technologies, Carlsbad, CA, USA). Polymerase chain reaction (PCR) was performed in a total volume of 25 µL, using 100 ng genomic DNA with 20 pmol each primers, 0.2 mM each dNTP, 1X buffer, 2 mM $MgCl_2$ and 1 U Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA). Cycling was performed in a techne TC-4000 thermal cycler (Bibby Scientific Limited, Staffordshire, UK) as follows: amplification consisted of a 2-minute denaturation step at 96 °C; 35 cycles of one minute at 94 °C, one minute at 60 °C, one minute at 72 °C and final extension of 7 minutes at 72 °C followed by cooling to 4 °C.

Genotype analysis of TNF-α-308 (*rs3091256*) polymorphism was performed using restriction fragment length polymorphism. PCR products were digested with Nco1 restriction enzyme. The digested PCR products were resolved by electrophoresis on 2.5% agarose gels containing 0.5 µg/mL ethidium bromide. Restriction fragments were visualized with the use of a Vilber-Lourmat Gel Quantification and Documentation System QUANTUM-ST4 (Vilber Lourmat BP 66 Torcy, France). Aspartate aminotransferase/platelet ratio index (APRI) was used to determine liver fibrosis stage.

### Statistical Analysis

Descriptive analyses were performed to provide information on general characteristics of the study population. Independent samples t-test was used to compare the continuous data between the groups. The continuous data were presented as mean ± standard deviation. Chi-square test was used to compare the categorical data between/among groups. Categorical variables were presented as a count and percentage. A p-value of less than 0.05 was considered statistically significant. Analyses were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS Inc., IBM Co., Somers, NY).

## Results

Ninety-five patients (62 female, 33 male) received Peg-IFN+RBV for 48 weeks and followed up for the next 48 weeks. APRI was used to determine liver fibrosis stage. There was no significant difference between the patient and the control groups in terms of age, gender and viral genotype. Clinical variables are given in Table 1. There was no statistically significant difference between CHC patients and healthy controls in terms of TNF-α-308 (*rs3091256*) polymorphism genotype distribution (p=0.362, Table 2). On the other hand, ALT values were found to be higher in CHC patients with TNF-α-308 GG polymorphism compared to CHC patients with GA+AA polymorphism (p=0.017). Moreover, APRI score was

the opposite. In their study, Dai et al. (24) from Taiwan suggested that TNF polymorphism at position-308 may be a predictor of treatment failure in patients treated with a combination of IFN-α and RBV and in another study from Brazil, the TNF-α-308 a allele was found to be a predictor of null virological response (25,26), but other investigations have failed to confirm such findings. An Egyptian study found that at the TNF-α 308 position, the G/G allele was most common (78.5%) in the study population compared to controls (27).

In this study, no significant difference was observed in the frequency of the TNFα-308 (*rs3091256*) polymorphism. Our results are consistent with those reported by Barrett et al. (28) who did not find the SNP at 308 to be associated with viral recovery or persistence. In studies conducted in different countries, there was

**Table 2.** Summary of genotyping between patients completed antiviral treatment for chronic hepatitis C virus and control

| Genotype/allele | Patients (95) | p for HWE | Control (97) | p for HWE | p | OR |
|---|---|---|---|---|---|---|
| GG | 78 (0.8210) | | 82 (0.8454) | | 0.701 | 0.84 (0.39-1.79) |
| GA | 14 (0.1474) | | 15 (0.1546) | | 1.000 | 0.94 (0.43-2.07) |
| AA | 3 (0.0316) | **0.0339** | 0 | 0.492 | 0.119 | 7.38 (0.38-142.61) |
| G | 170 (0.8947) | | 179 (0.9227) | | 0.378 | 0.71 (0.35-1.43) |
| A | 20 (0.1053) | | 15 (0.0773) | | | |

OR: Odds ratio, HWE: Hardy-Weinberg equilibrium

**Table 3.** Comparison of qualitative and quantitative variables between to GG and GA+A

| Variables | | TNF | | p |
|---|---|---|---|---|
| | | GA+AA | GG | |
| Non-responder | Yes | 4 (7.8) | 2 (18.2) | 0.287[a] |
| | No | 47 (92.2) | 9 (81.8) | |
| Relapse | Yes | 12 (23.5) | 2 (18.2) | 0.525[a] |
| | No | 39 (76.5) | 9 (81.8) | |
| SVR | Yes | 37 (72.5) | 8 (72.7) | 0.652[a] |
| | No | 14 (27.5) | 3 (27.3) | |
| APRI | | 0.61±0.46 (n=58) | 0.39±0.17 (n=13) | **0.006**[b] |
| ALT | | 47.90±45.53 (n=78) | 31.29±18.27 (n=17) | **0.017**[b] |

Used to n (%) for qualitative variables and mean ± standard deviation for quantitative variables
[a]: Fisher's exact test, [b]: Independent samples t-test
SVR: Sustained virologic response, APRI: Aspartate aminotransferase to platelet ratio index, ALT: Alanine aminotransferase, TNF: Tumor necrosis factor

**Table 4.** Genotype and allele frequencieas for tumor necrosis factor-alpha 308 *rs3091256*

| | | Genotypes | | | Alleles | |
|---|---|---|---|---|---|---|
| | | AA | G | A | GG | GA |
| Patients | CHC (n=70) | 58 (82.86%) | 10 (14.29%) | 2 (2.86%) | 126 (90.00%) | 14 (10.00%) |
| p | | 1.000 | 1.000 | 1.000 | 1.000 | |
| OR, 95% CI | | 0.97, 0.29-3.27 | 1.17, 0.30-4.54 | 0.68, 0.06-7.50 | 1.05, 0.36-3.05 | |
| RVR | Yes (27) | 25 (92.59%) | 2 (7.41%) | 0 | 52 (96.30%) | 2 (3.70%) |
| | No (34) | 26 (76.47%) | 7 (20.59%) | 1 (2.94%) | 59 (86.76%) | 9 (13.24%) |
| p | | 0.162 | 0.276 | 1.000 | 0.110 | |
| OR, 95% CI | | 3.85, 0.77-19.33 | 0.31, 0.06-1.58 | 0.41, 0.02-9.81 | 3.97, 0.83-18.93 | |
| EVR | Yes (56) | 47 (83.93%) | 8 (14.29%) | 1 (1.79%) | 102 (91.07%) | 10 (8.93%) |
| | No (5) | 4 (80.00%) | 1 (20.00%) | 0 | 9 (90.00%) | 1 (10.00%) |
| p | | 1.000 | 0.563 | 1.000 | 1.000 | |
| OR, 95% CI | | 1.31, 0.16-10.49 | 0.67, 0.08-5.42 | 0.30, 0.01-6.56 | 1.13, 0.14-8.93 | |
| SVR | Yes (44) | 36 (81.82%) | 7 (15.91%) | 1 (2.27%) | 79 (89.77%) | 9 (10.23%) |
| | No (16) | 14 (87.50%) | 2 (12.50%) | 0 | 30 (93.75%) | 2 (6.25%) |
| p | | 0.715 | 1.000 | 1.000 | 0.725 | |
| OR, 95% CI | | 0.64, 0.13-3.26 | 1.32, 0.26-6.84 | 1.14, 0.05-27.06 | 0.59, 0.12-2.80 | |

RVR: Rapid virologycal response, EVR: Early virologic reponse, SVR: Sustained virologic response, CHC: Chronic hepatitis C, OR: Odds ratio, CI: Confidence interval

no correlation between *TNF* gene polymorphisms and histological severity or response to antiviral treatment (29). In a meta-analysis of studies performed at different centers, it has been shown that there is no significant association between TNF-α-308, -238 gene polymorphisms and susceptibility to infection among different HCV subgroups. (30). Besides, the distributions of TNF-α-308, -238 A/G alleles were also not significantly different between the persistent infection group and the spontaneous clearance group. It is well known that certain diseases such as psoriasis and concomitant HCV infection are succesfully treated with anti-TNF therapy without signs of reactivation of HCV (31,32). Therefore, we conclude that TNF-α-308 (*rs3091256*) polymorphism may not really have any effect on treatment response.

TNF-α may affect hepatic fibrogenesis by stimulating hepatic stellate cells (33). After TNF-α activation, Kupffer cells secrete TGF-beta1, an important fibrogenic molecule. The relationship between cirrhosis development and TNF promoter has been investigated extensively (34,35,36,37). Although Romero-Gómez et al. (38) found no association between polymorphism in -308 and the severity of fibrosis in HCV and Abdel-Latif found (11) in both fibrotic and cirrhotic cases, no significant correlation was observed in levels of matrix metalloproteinases (MMP)-2, MMP-9, and TNF-α between fibrotic and cirrhotic cases (39). TNF-α has been found higher in cirrhotic patients compared to CHC patients with no or mild fibrosis (40). Consistent with these results, our study also confirmed the association between TNF-α-308 GG polymorphism and fibrosis score (p=0.006). This may be explained by higher constitutive and inducible transcriptional activity of TNF. Nevertheless, in a meta-analysis of 11 different studies, no association was found between TNF-α-308G> A polymorphism and liver cirrhosis risk in both Caucasians and Asian populations (41).

No correlation has been shown between 308 promoter polymorphisms and necroinflammatory histological activity. Although one study compared ALT levels between SVR patients and non-responders and found no statistically significant difference (10), the other found a statistically significant difference between healthy controls and those with cirrhosis and hepatocellular carcinoma (30), these studies did not include TNF polymorphism. Abbas et al. (41) studied HCV genotype 3 and found no association between ALT level and TNF-α-308 polymorphism. Besides, only 5% of their patients had TNF-α-308 GG promoter. In our study, we found a statistically significant relationship between TNF-a-308 GG polymorphism and high ALT levels in HCV genotype 1 patients (p=0.017) and although not statistically significant, 73.3% of our study population had SVR. In a study from Turkey, liver infiltrating lymphomononuclear cells were stimulated with TNF-α and histology activity index and HCV genotype revealed a negative correlation between TNF-α levels and elevated ALT levels in patients infected with 1b (42).

After circulating HCV particles reach the basolateral surfaces of hepatocytes, where the virus first binds to several receptors, the virus attaches to hepatocytes, it fuses the membrane and enters the cytosol and starts to replicate (43). Liver damage from HCV depends on both host's immune system-mediated reactions and viral cytopathic effects (44). The CD95 frequency was significantly higher in HCV antigen-positive hepatocytes compared to uninfected cells (45). TNF might play a role in hepatic necrosis and inflammation. Serum ALT level correlates with liver damage and we here propose that elevated ALT levels is the hallmark of hepatocyte injury eventully leading to fibrosis as well as elimination of virus in CHC (the more inflammation, the more viral eradication) (46).

### Study Limitations

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

## Conclusion

In conclusion, this is the first article in which ALT level and liver fibrosis are associated with TNF-α-308 GG polymorphism treated with IFN. The discrepancies in TNF genetic polymorphism and treatment responses among studies may be due to differences between ethnic groups.

### Ethics

**Ethics Committee Approval:** The Ethics Committee of Gaziosmanpaşa University approved the present study (approval number: 11-BADK-111).

**Informed Consent:** Informed consent forms were obtained from all the patients who participated in the study.

**Peer-review:** Internally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practise: Ö.G., A.R., Ö.A., Ş.B., Concept: Ö.G., D.Y., B.Ç., Desing: Ö.G., D.Y., B.Ç., Data Collection or Processing: Ö.G., A.R., Analysis: O.D., S.K., Literature Search: Ö.G., D.Y., B.Ç., Ö.T., Writing: Ö.G., D.Y., B.Ç.

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

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## References

1. Cybula M, Szemraj J. The role of hepcidin and polymorphisms in the regulatory region of the IL-28B gene in HCV infections. Postepy Hig Med Dosw (Online). 2013;67:1273-1282.
2. Buti M, Esteban R. Hepatitis C virus genotype 3: a genotype that is not 'easy-to-treat'. Expert Rev Gastroenterol Hepatol. 2015;9:375-385.
3. Di Bisceglie AM. Natural history of hepatitis C: its impact on clinical management. Hepatology. 2000;31:1014-1018.
4. Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, Schraut WW, Schirren CA, Waechtler M, Backmund M, Pape GR. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology. 2003;125:80-88.
5. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat. 2006;13:34-41.
6. Saito T, Ueno Y. Transmission of hepatitis C virus: self-limiting hepatitis or chronic hepatitis? World J Gastroenterol. 2013;19:6957-6961.
7. Manns MP, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. Gut. 2006;55:1350-1359.
8. Stättermayer AF, Ferenci P. Effect of IL28B genotype on hepatitis B and C virus infection. Curr Opin Virol. 2015;14:50-55.

9. Gürbüz Y, Tülek NE, Tütüncü EE, Koruk ST, Aygen B, Demirtürk N., et al. Evaluation of Dual Therapy in Real Life Setting in Treatment-Naïve Turkish Patients with HCVInfection: A Multicenter, Retrospective Study. Balkan Med J. 2016 Jan;33(1):18-26.
10. Par G, Szereday L, Berki T, Palinkas L, Halasz M, Miseta A, Hegedus G, Szekeres-Bartho J, Vincze A, Hunyady B, Par A. Increased baseline proinflammatory cytokine production in chronic hepatitis C patients with rapid virological response to peginterferon plus ribavirin. PLoS One. 2013;8:e67770.
11. Abdel-Latif MS. Plasma Levels of Matrix Metalloproteinase (MMP)-2, MMP-9 and Tumor Necrosis Factor-α in Chronic Hepatitis C Virus Patients. Open Microbiol J. 2015;9:136-140.
12. Walsh KM, Timms P, Campbell S, MacSween RN, Morris AJ. Plasma levels of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinases-1 and-2 (TIMP-1 and TIMP-2) as noninvasive markers of liver disease inchronic hepatitis C: comparison using ROC analysis. Dig Dis Sci. 1999;44:624-630.
13. Lee J, Tian Y, Chan ST, Kim JY, Cho C, Ou JH. TNF-α Induced by Hepatitis C Virus via TLR7 and TLR8 in Hepatocytes Supports Interferon Signaling via an Autocrine Mechanism. PLoS Pathog. 2015;11:e1004937.
14. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev. 2009;22:240-273.
15. Puimège L, Van Hauwermeiren F, Steeland S, Van Ryckeghem S, Vandewalle J, Lodens S, Dejager L, Vandevyver S, Staelens J, Timmermans S, Vandenbroucke RE, Libert C. Glucocorticoid-induced microRNA-511 protects against TNF by down-regulating TNFR1. EMBO Mol Med. 2015;7:1004-1017.
16. Jones SJ, Ledgerwood EC, Prins JB, Galbraith J, Johnson DR, Pober JS, Bradley J. TNF recruits TRADD to the plasma membrane but not the trans-Golgi network, the principal subcellular location of TNF-R1. J Immunol. 1999;162:1042-1048.
17. Takeuchi M, Rothe M, Goeddel DV. Anatomy of TRAF2. Distinct domains for nuclear factor-kappaB activation and association with tumor necrosis factor signaling proteins. J Biol Chem. 1996;271:19935-19942.
18. Yang J, Lin Y, Guo Z, Cheng J, Huang J, Deng L, Liao W, Chen Z, Liu Z, Su B. The essential role of MEKK3 in TNF-induced NF-κB activation. Nat Immunol. 2001;2:620-624.
19. Blonska M, Shambharkar PB. Kobayashi M, Zhang D, Sakurai H, Su B, Lin X. TAK1 is recruited to the tumor necrosis factor-α (TNF-α) receptor 1 complex in a receptor-interacting protein (RIP)-dependent manner and cooperates with MEKK3 leading to NF-κB activation. J Biol Chem. 2005;280:43056-43063.
20. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59:318-327.
21. Venegas M, Brahm J, Villanueva RA. Genomic determinants of hepatitis C virus antiviral therapy outcomes: toward individualized treatment. Ann Hepatol. 2012;11:827-837.
22. Horner SM, Gale M Jr. Regulation of hepatic innate immunity by hepatitis C virus. Nat Med. 2013;19:879-888.
23. Kanwal F, White DL, Tavakoli-Tabasi S, Jiao L, Lin D, Ramsey DJ, Spiegelman A, Kuzniarek J, El-Serag HB. Many patients with interleukin 28B genotypes associated with response to therapy are ineligible for treatment because of comorbidities. Clin Gastroenterol Hepatol. 2014;12:327-333.
24. Dai CY, Chuang WL, Chang WY, Chen SC, Lee LP, Hsieh MY, Hou NJ, Lin ZY, Huang JF, Hsieh MY, Wang LY, Yu ML. Tumor necrosis factor-alpha promoter polymorphism at position -308 predicts response to combination therapy in hepatitis C virus infection. J Infect Dis. 2006;193:98-101.
25. Grandi T, Silva CM, Amaral KM, Picon PD, Costi C, Fré NN, Fiegenbaum M, Gregianini TS, Niel C, Rossetti ML. Tumour necrosis factor 308 and-238 promoter polymorphisms are predictors ofa null virological response in the treatment of Brazilian hepatitis C patients. Mem Inst Oswaldo Cruz. 2014;109:345-351.
26. Brinkman BM, Zuijdgeest D, Kaijzel EL, Breedveld FC, Verweij CL. Relevance of the tumor necrosis factor alpha (TNFα) -308 promoter polymorphism in TNFα gene regulation. J Inflamm. 1996:46:32-41.
27. Talaat RM, Esmail AA, Elwakil R, Gurgis AA, Nasr MI. Tumor necrosis factor-alpha -308G/A polymorphism and risk of hepatocellular carcinoma in hepatitis C virus-infected patients. Chin J Cancer. 2012;31:29-35.
28. Barrett S, Collins M, Kenny C, Ryan E, Keane CO, Crowe J. Polymorphisms in tumor necrosis factor-alpha, transforming growth factor beta, interleukin-10, interleukin-6, interferon-gamma, and outcome of hepatitis C virus infection. J Med Virol. 2003;71:212-218.
29. Höhler T, Kruger A, Gerken G, Schneider PM, Meyer zum Büschenfelde KH, Rittner C. Tumor necrosis factor alpha promoter polymorphism at position-238 is associated with chronic active hepatitis C. J Med Virol. 1998;54:173-177.
30. Amini M, Poustchi H. Hepatitis C virus spontaneous clearance: immunology and genetic variance. Viral Immunol. 2012;25:241-248.
31. He J, Pei X, Xu W, Wang C, Zhang X, Wu J, Zhao W. The relationship between tumor necrosis factor-α polymorphisms and hepatitis C virus infection: a systematic review and meta-analysis. Ren Fail. 2011;33:915-922.
32. Salvi M, Macaluso L, Luci C, Mattozzi C, Paolino G, Aprea Y, Calvieri S, Richetta AG. Safety and efficacy of anti-tumor necrosis factors α in patients with psoriasis and chronic hepatitis C. World J Clin Cases. 2016;4:49-55.
33. Friedman SL. Molecular mechanisms of hepatic fibrosis and principles of therapy. J Gastroenterol. 1997:32:424-430.
34. Bataller R, North KE, Brenner DA. Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. Hepatology. 2003;37:493-503.
35. Yee LJ, Tang J, Herrera J, Kaslow RA, van Leeuwen DJ. Tumor necrosis factor gene polymorphisms in patients with cirrhosis from chronic hepatitis C virus infection. Genes Immun. 2000;1:386-390.
36. Radwan MI, Pasha HF, Mohamed RH, Hussien HI, El-Khshab MN. Influence of transforming growth factor-b1 and tumor necrosis factor-a genes polymorphisms on the development of cirrhosis and hepatocellular carcinoma in chronic hepatitis C patients. Cytokine. 2012;60:271-276.
37. Dai CY, Chuang WL, Lee LP, Chen SC, Hou NJ, Lin ZY, Hsieh MY, Hsieh MY, Wang LY, Chang WY, Yu ML. Associations of tumour necrosis factor alpha promoter polymorphisms at position -308 and -238 with clinical characteristics of chronic hepatitis C. J Viral Hepat. 2006;13:770-774.
38. Romero-Gómez M, Montes-Cano MA, Otero-Fernández MA, Torres B, Sánchez-Muñoz D, Aguilar F, Barroso N, Gómez-Izquierdo L, Castellano-Megias VM, Núñez-Roldán A, Aguilar-Reina J, González-Escribano MF. SLC11A1 promoter gene polymorphisms and fibrosis progression in chronic hepatitis C. Gut. 2004;53:446-450.
39. Andersen ES, Ruhwald M, Moessner B, Christensen PB, Andersen O, Eugen-Olsen J, Weis N. Twelve potential fibrosis markers to differentiate mild liver fibrosis from cirrhosis in patients infected with chronic hepatitis C genotype 1. Eur J Clin Microbiol Infect Dis. 2011;30:761-766.
40. Chen D, Liu JL, Liu Y, Zhu J, Wang SW. Lack of an association between -308G>A polymorphism of the TNF-α gene and liver cirrhosis risk based on ameta-analysis. Genet Mol Res. 2011;10:2765-2774.

41. Abbas Z, Moatter T, Hussainy A, Jafri W. Effect of cytokine gene polymorphism on histological activity index, viral load and response to treatment inpatients with chronic hepatitis C genotype 3. World J Gastroenterol. 2005;11:6656-6661.
42. Akyüz F, Polat N, Kaymakoglu S, Aksoy N, Demir K, Beşişik F, Badur S, Cakaloglu Y, Okten A. Intrahepatic and peripheral T-cell responses in genotype 1b hepatitis C virus-infected patients with persistently normal and elevated aminotransferase levels. World J Gastroenterol. 2005;11:7188-7191.
43. Dubuisson J, Cosset FL. Virology and cell biology of the hepatitis C virus life cycle – An update. J Hepatol 2014;61(Suppl 1):3-13.
44. Mengshol JA, Golden-Mason L, Rosen HR. Mechanisms of Disease: HCV-induced liver injury. Nat Clin Pract Gastroenterol Hepatol. 2007;4:622-634.
45. Hiramatsu N, Hayashi N, Katayama K, Mochizuki K, Kawanishi Y, Kasahara A, Fusamoto H, Kamada T. Immunohistochemical detection of Fas antigen in liver tissue of patients with chronic hepatitis C. Hepatology. 1994;19:1354-1359.
46. Shahid M, Idrees M, Nasir B, Raja AJ, Raza SM, Amin I, et al. Correlation of biochemical markers and HCV RNA titers with fibrosis stages and grades in chronic HCV-3a patients. Eur J Gastroenterol Hepatol. 2014;26(7):788-94.

Research Article

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# *Evaluation of the Seroprevalence of Hepatit A and Vaccination Status in Children Aged Two and Sixteen Years*

## İki-On Altı Yaş Arası Çocukların Hepatit A Seroprevalansı ve Aşılanma Durumunun Değerlendirilmesi

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***ABSTRACT***

**Objective:** In this study; the aim was to evaluate the seroprevalence of hepatitis A virus (HAV) in 2-16 year age group, and the rate of hepatitis A vaccination.

**Materials and Methods:** This study was conducted on 400 children aged between 2 and 16 years with no chronic diseases who attended the pediatrics outpatient clinic in Health Sciences University, Haydarpaşa Numune Training and Research Hospital. After obtaining informed consent from the parents, blood samples were taken for investigating serological markers for hepatitis A in the microbiology laboratory using the ELISA method. The parents were asked whether or not their children had been vaccinated against hepatitis A.

**Results:** In this study 44.3% of the participants included were girls and 55.8% were boys. The mean age of the children was 10.8±4.18 years. 27.3% of patients were anti-HAV IgG-positive, and 11% had been vaccinated against hepatitis A. When we compared preschool and school age patients, anti-HAV IgG positivity was detected in all children who were vaccinated in the preschool group; while 5.9% of unvaccinated children were anti-HAV IgG-positive and 94.1% were negative. It was found that school age children were unvaccinated, and anti-HAV IgG was positive in 19.6% of the children and negative in 80.4% of the children.

**Conclusion:** In our study, although the prevalence of hepatitis A was found to be low compared to the eastern and southeastern cities of our country, it is still higher than in the developed countries. In order to prevent hepatitis A infection, it is necessary to improve the socio-economic conditions of the country, to create better sanitary conditions and hygienic practices, and raise awareness of the infection.

**Keywords:** Hepatitis A virus, vaccination, seroprevalence

***ÖZ***

**Amaç:** Bu çalışmada 2-16 yaş grubu olgularda, hepatit A virüsünün (HAV) seroprevalansının ve hepatit A aşısının yaptırılma oranlarının değerlendirilmesi amaçlandı.

**Gereç ve Yöntemler:** Sağlık Bilimleri Üniversitesi, Haydarpaşa Numune Eğitim ve Araştırma Hastanesi, Çocuk Polikliniği'ne başvuran 2-16 yaş arası, kronik hastalığı olmayan 400 çocuk araştırma kapsamına alındı. Ailelerin onayı alındıktan sonra alınan kan örneği tıbbi mikrobiyoloji laboratuvarına gönderilip ELISA yöntemi ile hepatit A serolojik belirteçleri çalışıldı. Hastalara, hepatit A aşısını yaptırıp yaptırmadıkları soruldu.

**Bulgular:** Çalışmaya alınan çocukların 177'si (%44,3) kız, 223'ü (%55,8) erkek olup ortalama yaşları 10,8+/-4,18 yıldı. Olguların 109'unun (%27,3) anti-HAV IgG'si pozitifti ve 44'ü (%11) hepatit A aşısı yaptırmıştı. Okul öncesi ve okul dönemi olarak karşılaştırdığımızda okul öncesi grupta aşı olan tüm çocuklarda anti-HAV IgG pozitifliği görülürken; aşı olmayan çocukların %5,9'unda anti-HAV IgG pozitif, %94,1'inde negatiftir. Okul dönemindeki çocuklarda aşı varlığı görülmezken, çocukların %19,6'sında anti-HAV IgG pozitif iken, %80,4'ünde negatiftir.

**Sonuç:** Çalışmamızda, hepatit A prevalansı ülkemizin Doğu ve Güneydoğu illerine göre düşük bulunsa da halen gelişmiş ülkelerin seroprevalansına göre yüksek seyretmektedir. Bunu önlemek için ülkenin sosyo-ekonomik şartlarının düzeltilmesi ve toplumun bilinçlendirilmesi gerekmektedir. Sağlık Bakanlığı Ulusal Aşı Programı kapsamındaki hepatit A aşısı ile HAV enfeksiyonu sıklığı azalacaktır.

**Anahtar Kelimeler:** Hepatit A virüsü, aşılama, seroprevalans

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## Introduction

Hepatitis A virus (HAV) appears at earlier ages in developing countries depending on socio-economic and hygienic conditions. When hepatitis A infection occurs in early childhood, it progresses in an asymptomatic manner. The symptoms and complications increase with age (1,2). The age in which individuals face HAV and the frequency of HAV infection are directly related with the socio-economic conditions of the country and the region (3,4,5). Low socioeconomic level, living in crowded areas, being in rural areas and low educational status of parents increase the HAV prevalence (6,7).

HAV, which is highly infectious, is a member of the picornaviridae family. Infection is spread via the fecal-oral route as well as through ingestion of contaminated food and water (3). In diagnosing the acute disease, anti-HAV IgM positivity in serum is adequate. Anti-HAV IgM indicates an ongoing or a past infection. Anti-HAV IgG starts to be positive a few weeks after the infection persists for years after infection and confer life-long immunity (8).

Hepatitis A can be prevented through, passive hepatitis A immunization. The vaccine is administered twice in the 0, 6$^{th}$ or 12$^{th}$ months of life (9). HAV vaccination was added to the routine schedules by the Ministry of Health of Turkey in 2012.

## Materials and Methods

The study group consisted of 400 children aged 2-16 years who were admitted to the pediatric outpatient clinic in Health Sciences University, Haydarpaşa Training and Research Hospital. The study started after obtaining consent from parents of the children. Special care was given to select children who did not have any chronic diseases. The study was approved by the Local Ethics Committee of Health Sciences University, Haydarpaşa Numune Training and Research Hospital (approval no: HNEAH-KAEK 2016/67), and was conducted between July 2016 and September 2016.

Data on name, family name, age, gender and telephone number was recorded, and the parents were asked whether or not their children had been vaccinated against hepatitis A.

Blood samples of the children were centrifuged at the medical microbiology laboratory. The hepatitis A serologic markers (anti-HAV Ig M, anti-HAV IgG) were determined using enzyme-linked immunosorbent assay and the measurements were made in mIU/mL (Architect Systems and Abbott Diagnostics Division, the USA). The results were evaluated in the light of the recommendations of the manufacturer company.

### Statistical Analysis

To evaluate the findings obtained in the study, the NCSS 2007 (Kaysville, Utah, USA) program was used for statistical analyses. Definitive statistical methods were used to evaluate the study data (average values, standard deviation, median, frequency, rates, minimum, maximum values, etc.). Pearson's chi-square test, Fisher's exact test and Yates's correction for continuity were used in comparing the qualitative data. The results were evaluated at a 95% confidence interval and a p value of less than 0.05 was considered statistically significant.

## Results

Our study was conducted on 400 cases [55.8% of whom were males (n=223), and 44.3% (n=177) were females]. The age of the patients varied between 2 and 16, and the mean age was 10.08±4.18 years. In this study 19.5% (n=78) of the children were aged between 2 and 5 years, which is the preschool period, and 80.5% (n=322) of them were aged between 6 and 16 years, which is schooling period (Table 1).

Overall, 98.3% (n=393) of children were anti-HAV IgM-negative and 1.8% (n=7) were positive. 72.8% (n=291) were anti-HAV IgG-negative and 27.3% (n=109) were positive. 89% (n=356) of children were unvaccinated and 11% had been vaccinated against hepatitis A (Table 2).

41% (n=32) of the preschool children were negative for anti-HAV IgG and 59% (n=46) were positive. 80.4% of the children who were at schooling period (n=259) were negative and 19.6% (n=63) were positive (Table 3).

43.6% of the preschool children (n=34) were unvaccinated while 56.4% (n=44) had been vaccinated. All the school age children (n=322) were unvaccinated (Table 4).

All preschool children who were vaccinated (n=44) and 5.9% of children who were unvaccinated (n=2) were anti-HAV IgG-positive

**Table 1.** The distribution of the definitive characteristics

| | | |
|---|---|---|
| Gender; n (%) | Female | 177 (44.3) |
| | Male | 223 (55.8) |
| Age (years) | min-max (median) | 2-16 (11) |
| | Ave ± SD | 10.08±4.18 |
| Age groups; n (%) | Pre-school (age 2-5) | 78 (19.5) |
| | School period (age 6-16) | 322 (80.5) |
| Type of the patient; n (%) | Outpatient | 395 (98.8) |
| | Hospitalized | 5 (1.3) |

SD: Standard deviation, min: Minimum, max: Maximum

**Table 2.** The distribution of the anti-Hepatitis A virus IgM, anti-Hepatitis A virus IgG and vaccination results

| | | |
|---|---|---|
| Anti-HAV IgM; n (%) | Negative | 393 (98.3) |
| | Positive | 7 (1.8) |
| Anti-HAV IgG; n (%) | Negative | 291 (72.8) |
| | Positive | 109 (27.3) |
| Vaccination; n (%) | Negative | 356 (89) |
| | Positive | 44 (11) |

HAV: Hepatitis A virus, IgM: Immunoglobulin M, IgG: Immunoglobulin G

**Table 3.** Evaluation of anti-Hepatitis A virus IgG results according to the groups

| | | Preschool (2-5 years) (n=78) | School period (6-16 years) (n=322) | |
|---|---|---|---|---|
| | | n (%) | n (%) | p |
| Anti-HAV IgG | Negative | 32 (41) | 259 (80.4) | [a]**0,001**** |
| | Positive | 46 (59) | 63 (19.6) | |

[a]Pearson chi-square test, **p<0.01, HAV: Hepatitis A virus, IgG: Immunoglobulin G

and 94.1% (n=32) were negative. The school age children (n=322) had not been vaccinated; 19.6% (n=63) of them were anti-HAV IgG-positive and 80.4% (n=259) were negative.

## Discussion

The age to face HAV infection and the prevalence of hepatitis A vary among different parts of the World due to different socio-economic conditions (10,11). The seroprevalence studies conducted at various parts of our country have shown that there were differences between regions based on the socio-economic conditions, and there were differences even between some areas within the same region or between the urban and rural areas.

Erdoğan et al. (6) conducted a study with 0-19 age group children in the city of Edirne and determined that the anti-HAV positivity in 2-5 age group, 6-10 age group 11-14 age group and 15-19 age group was 4.4%, 25%, 37.3%, and 43.2%, respectively. Yapicioglu et al. (12) conducted a study on children aged 2-6 years in Adana, and determined a HAV IgG positivity of 28.8%. In their study performed on 1-6 age group children in the Konya region, Atabek et al. (13) found that the anti-HAV IgG positivity rate in the urban and rural areas was 25.7% 67.8%, respectively. Karaman et al. (14) reported an anti-HAV IgG positivity rate of 54.9% among 1-15 age group children in the Van region.

As a result of socio-economic development, access to clean water sources, application of hygiene principles and with the help of the hepatitis A vaccination practices, which started as of late 2012, the number of the cases decreased from 3624 in 2012 to 707 in 2015; and the prevalence decreased from 4.8 per in 100.000 2012 to 0.9 per 100.000 in 2015 (15).

In this study, which was conducted with 400 children aged 2-16 years, who were admitted to our hospital, the anti-HAV IgG seropositivity was determined as 27.3%. While 41% of the preschool children were negative for anti-HAV IgG, 59% were positive. It was found that 80.4% of school age children were anti-HAV IgG-negative and 19.6% were positive. We associate these results with hepatitis A vaccination included in the routine schedules by the Ministry of Health of Turkey in 2012 and administered in preschool children aged 18 and 24 months given in 2 doses.

The hepatitis A vaccination rate was determined as 11% in our study. We believe that the low rate of vaccination found in this study was associated with the fact that hepatitis A vaccination that was included in the routine schedules in 2012 and 19.5% of the 400 children included in our study were aged 2-5 years; the vaccination status was declared by the mothers, who might have forgotten, and with the vaccination status cards which might be ignored to be filled out in some situations (Table 4).

**Table 4.** Evaluation of vaccination results according to the groups

| | | Preschool (age 2-5) (n=78) | School period (age 6-16) (n=322) | |
|---|---|---|---|---|
| | | n (%) | n (%) | p |
| Vaccination | Negative | 34 (43.6) | 322 (100) | [a]**0.001**** |
| | Positive | 44 (56.4) | 0 (0) | |

[a]Pearson chi-square test, **p<0.01

Immunity against HAV being acquired with vaccination or by having the infection itself, which are not serologic markers in distinction, and considering only anti-HAV IgG positivity in determining hepatitis A immunity are among the limitations of our study.

Especially, when the seropositivity rate found in our study is compared with that in the eastern and southeastern regions of Turkey, this rate is clearly low (7,14). We consider that this may stem from the fact that the socio-economic conditions, infrastructure, conscious levels of families and the application of hygiene conditions being better in Istanbul when compared with that in the eastern and southeastern regions.

According to the results of the present study, the seropositivity rate in Istanbul was found to be lower than reported in the previous years (16). We may associate this situation with the fact that the socio-economic level in the whole country is increasing, hygiene conditions are improving, bottled water is used, and the educational level within families is increasing.

## Conclusion

In the present study, although the hepatitis A prevalence was found to be lower when compared with the eastern and southeastern regions of Turkey, it is still higher than that in developed countries. For prevention of hepatitis A, the socio-economic conditions of the country must be improved, better sanitary conditions must be provided, and the awareness of the infection must be raised. For the purpose of acquiring immunity against the disease without being infected with it, hepatitis A vaccination, which is included in the National Vaccination Program of the Ministry of Health, must be applied in an efficient manner.

### Ethics

**Ethics Committee Approval:** The study was approved by Local Ethics Committee of Health Sciences University, Haydarpaşa Numune Training and Research Hospital (approval no: HNEAH-KAEK 2016/67).

**Informed Consent:** Informed consent was taken from the parents.

**Peer-review:** Externally and internally peer-reviewed.

### Authoring Contributions

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

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

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## References

1. Tosun S, Ayaz H, Deveci S, Aksu S. Çocuk ve erişkinlerde hepatit A virusu ile karşılaşma durumunun değerlendirilmesi. Antalya: X.Ulusal Viral Hepatit Kongre Kitabı; 2010; p. 121.
2. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. Vaccine. 2010;28:6653-6657.

3. NadaYazigiand William F. Balistreri: Viralhepatitis. In: Kleigman RM, Behrman RE, Jenson HB, Stanson BF, (eds.), Nelson textbook of Pediatrics 20th Edition; 2015; p. 1680-1690.
4. Badur S. Viral Hepatitler (HAV, HBV, HDV). İçinde: Ustaçelebi Ş, Abacıoğlu H, Badur S, (eds.), Moleküler, Klinik, Tanısal Viroloji. Ankara: Güneş Kitabevi; 2004; p.175-183.
5. Jacobsen KH, Koopman JS. Declining hepatitis A seroprevalence a global review and analysis. Epidemiol Infect. 2004;132:1005-1022.
6. Erdoğan MS, Oktun M, Tatman-Otkun M, Akata F, Türe M. The epidemiology of hepatitis a virus infection in children, in Edirne, Turkey. Eur J Epidemiol. 2004;19:267-273.
7. Taşyaran MA, Akdağ R, Akyüz M, Parlak M, Ceviz N, Yılmaz S. Erzurum bölgesi çocuklarında fekal oral bulaşan hepatit viruslarının seroprevalansı. Klinik Dergi. 1994;7:74-75.
8. Shah U, Habib Z, Kleinman RE. Liver failure attributable to hepatitis A virus infection in a developing country. Pediatrics. 2000;105:436-438.
9. Malay S, Tizer K, Lutwick LI. Current Update of Pediatric Hepatitis Vaccine Use. Pediatr Clin North Am. 2000;47:395-406.
10. Mostafavi N, Kelishadi R, Kazemi E, Ataei B, Yaran M, Motlagh ME, Qorbani M, Heshmat R, Tajadini MH, Ghaffari Hoseini S. Comparison of the prevalence and risk factors of hepatitis A in 10 to 18-year-old adolescents of sixteen IranianProvinces: The CASPIAN-III Study. Hepat Mon. 2016;16:e36437.
11. Stephen MF, Ian DG. Hepatitis A Virus. In: Mandell GL, Bennett JE, Dolin R, (eds.), Principles and Practice of Infectious Disease. Churchill Livingstones: Newyork: 2000;p. 1920-1940.
12. Yapicioglu H, Alhan E, Yildizdas D, Yaman A, Bozdemir N. Prevalence of hepatitis A in children and adolescents in Adana, Turkey. Indian Pediatr. 2002;39:936-941.
13. Atabek ME, Fındık D, Gulyuz A, Erkul I. Prevalence of anti-HAV and anti- HEV antibodies in Konya, Turkey. Health Policy. 2004;67:265-269.
14. Karaman S, Karaman K, Kızılyıldız BS, Ceylan N, Kaba S, Parlak M, Beger B, Ceylan A. Seroprevalance of hepatitis a and associated factors among 1-15 year old children in Eastern Turkey. Int J Clin Exp. 2015;8:19394-19399.
15. http://www.medikalakademi.com.tr/hepatit-a-asisi-sayesinde-turkiyede-hastalıkgorulme-sikligi-5-kat-azaldi.Hepatit A Aşısı Sayesinde Türkiye'de Hastalık Görülme Sıklığı 5 Kat Azaldı. Medikal Akademi; 2016.
16. Aldeniz C, Çavuşoglu S, Altunay H. İstanbul'da A ve E hepatitlerinin seroprevalansı. Viral Hepat J. 1998:31-36.

Research Article

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# *Does Vitamin D Level Affect the Response to Antiviral Treatment in Egyptian Patients with Chronic Hepatitis C?*

## Kronik Hepatit C'li Mısırlı Hastalarda D Vitamini Düzeyi Antiviral Tedavi Cevabını Etkiler mi?

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***ABSTRACT***

**Objective:** Vitamin D deficiency is common in patients with chronic liver disease. Several studies demonstrated that its levels are inversely related to the disease severity and documented improvement of the disease following supplementation especially regarding to hepatitis C virus (HCV) infection. To study level of vitamin D in Egyptian patients with chronic HCV infection and to investigate its correlation with activity and fibrosis scores of their liver biopsies, as well as the relationship of vitamin D levels with patients' response to antiviral therapy.

**Materials and Methods:** The study included 60 Egyptian patients with chronic HCV infection who were scheduled for antiviral medications (pegylated-interferon and ribavirin) for 48 weeks and 50 healthy age- and sex-matched individuals non-reactive for HCV antibodies as a control group. Serum 25-hydroxyvitamin D was measured in all patients and controls and compared with patients' liver biopsy results and their virological response (after 48 weeks treatment) assessed by polymerase chain reaction for HCV.

**Results:** Serum vitamin D levels were inversely correlated with activity and fibrosis scores in liver biopsy. On the other hand, 63.3% of cases had good response to interferon treatment and 36.7% of them had no response without significant difference in serum vitamin D levels between responders and non-responders (39.2±23.6 and 37.1±13.2 ng/mL, respectively).

**Conclusion:** Vitamin D levels could affect liver necro-inflammatory process in Egyptian patients with chronic hepatitis C infection, but did not show significant effect on response to antiviral therapy.

**Keywords:** Chronic hepatitis C, virological response, vitamin D

***ÖZ***

**Amaç:** Kronik karaciğer hastalığı olanlarda D vitamini eksikliği yüksek prevalans göstermektedir. D vitamini düzeyleri hastalığın şiddeti ile ters ilişki göstermektedir. Girişimsel çalışmalar suplemantasyon sonrası kronik karaciğer hastalığında iyileşme raporlamaktadır. Benzer şekilde hepatit C virüs (HCV) enfeksiyonuna ilişkin sağlık durumunda düzelme bildirilmiştir. Kronik HCV enfeksiyonu olan Mısırlı hastalardaki vitamin D düzeyini belirlemek ve vitamin düzeyi ile karaciğer biyopsisi aktivite ve fibrozis skorları arasındaki ilişkiyi araştırmak, ayrıca antiviral tedaviye hastanın cevabı ile ilişkisini incelemektir.

**Gereç ve Yöntemler:** Bu çalışma kronik HCV enfeksiyonu olan 60 Mısırlı hastada gerçekleştirilmiştir. Bu hastalar 48 hafta antiviral tedavi (pegile-interferon ve ribavirin) almıştır. Yaş ve cinsiyet uyumlu, HCV antikorları açısından reaktif olmayan 50 sağlıklı kişi de kontrol grubunu oluşturmuştur. Serum 25 hidroksivitamin D hem hastalar, hem de kontrol grubunda ölçülmüştür. Sonuçlar hastaların karaciğer biyopsi sonuçları ve HCV polimeraz zincir reaksiyonu ile değerlendirilen virolojik cevapları (48 haftalık tedavi sonrası) ile karşılaştırılmıştır.

**Bulgular:** Serum D vitamini düzeyleri karaciğer biyopsisi aktivite ve fibrozis skorları ile ters ilişkili bulunmuştur. Olguların %63,3'ü interferon tedavisine iyi yanıt vermiştir. Tedaviye cevap ile vitamin D düzeyi ilişkisi ise korele bulunmamıştır (Tedaviye cevap veren ve vermeyen olgulardaki D vitamin düzeyi ortalamaları sırasıyla 39,2±23,6 ng/mL, 37,1±13,2 ng/mL).

**Sonuç:** Kronik hepatit C enfeksiyonlu Mısırlı hastalarda D vitamini düzeyleri karaciğerin nekro-enflamatuvar süreçlerini etkilemiştir, ancak antiviral tedaviye cevap üzerinde anlamlı etkisi olmamıştır.

**Anahtar Kelimeler:** Kronik hepatit C, virolojik cevap, D vitamini

**Hussein HA, Mohamad HB, Mashaheet MA, Alamir MA, Ezzat GM. Does Vitamin D Level Affect the Response to Antiviral Treatment in Egyptian Patients with Chronic Hepatitis C? Viral Hepat J. 2017;23:50-54.**

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## Introduction

Vitamin D plays a central role in calcium and phosphate homeostasis and is essential for the proper development and maintenance of bone. It is also known to be involved in cell proliferation, differentiation, and immunomodulation (1). Vitamin D (D3 and D2) is hydroxylated in the liver to 25-hydroxyvitamin D [25(OH)D] and secreted in the circulation, again, mostly bound to vitamin D binding protein (DBP). DBP is synthesized in the liver. It represents a biomarker for severe liver disease (2).

An association between serum vitamin D level and chronic liver disease (CLD) has also been described (3,4). In their review, Stokes et al. (2) stated that the prevalence of vitamin D levels <20 ng/mL in CLD has been reported to range from 64 to 92% and was commonly inversely related to disease progression. Some studies showed no difference in vitamin D levels between patients with cirrhosis and patients without cirrhosis (5). Bitetto et al. (6) showed that a lower level of vitamin D is associated with a higher inflammatory grade and more advanced stage of fibrosis.

The aim of treatment is to cure hepatitis C virus (HCV) infection, to prevent the complications of chronic hepatitis C including liver cirrhosis, hepatocellular carcinoma, severe extra-hepatic complications and death. The goal of treatment is a sustained viral response (SVR), defined by undetectable HCV RNA in blood 12 weeks or 24 weeks (SVR24) after the end of treatment. The HCV genotype and genotype 1 subtype (1a or 1b) and baseline virological parameters that will be useful for tailoring therapy must be evaluated before starting treatment (7). In some studies, lower rates of SVR in HCV patients undergoing interferon-based therapy were detected to be associated with vitamin D deficiency (8,9). Other studies concluded that vitamin D replacement could improve the response to antiviral therapy in patients with chronic hepatitis C and increase the percentage of SVR (10,11).

Corey et al. (12) studied the effect of vitamin D levels on the progression of CLD through four years follow-up. They found no effect of vitamin D deficiency on disease progression. A recent Brazilian retrospective study documented that there was no relationship between vitamin D status, stage of liver fibrosis, and treatment response to interferon and ribavirin in patients with chronic hepatitis C (13). Accordingly, the relationship of serum vitamin D levels with both grade of liver inflammation and stage of fibrosis and virological response in patients with chronic hepatitis C remains controversial, and further research on Egyptian patients is required.

## Materials and Methods

All the study steps were explained to all the participants, and informed consent was obtained from all of them. The study was conducted after approval of the Ethics Committee of Faculty of Medicine Fayoum University. Our study was conducted on 110 Egyptian participants: 60 patients with chronic HCV infection who were scheduled for antiviral therapy (pegylated-interferon combined with ribavirin according to the official protocol of the Egyptian Ministry of Health irrespective of HCV genotype) for 48 weeks at the Hepatology Unit at Fayoum General Hospital, and 50 healthy age- and sex-matched individuals, non-reactive for HCV antibodies as controls.

Patients with malnutrition, malabsorption or renal failure and those on drugs affecting vitamin D levels, such as calcium or vitamin D supplementation, were excluded.

The results of polymerase chain reaction (PCR) for detecting HCV RNA before treatment and 24 weeks after completion of treatment to assess SVR (SVR24 means undetectable HCV-RNA at 24 weeks after the end of treatment) recorded from their files. Plasma HCV-RNA levels were measured using the COBAS TaqMan HCV assay, version 1.0 (Roche Molecular Systems), with a lower limit of quantification of 35-45 IU/mL and a lower limit of detection of 10 IU/mL. Therefore, the total duration of the study was about 1.5 years.

Pretreatment serum 25(OH)D levels were assessed in patients and controls. The DRG 25(OH)D Total enzyme-linked immunosorbent assay (ELISA) Kit is a solid phase ELISA based on the competitive principle for measurement of total 25(OH)D (vitamin D2 and vitamin D3). Accordingly, 25(OH)D levels were classified into three states: deficient - ≤10 ng/mL, insufficient -11-30 ng/mL, and optimal - >30 ng/mL.

The results of liver biopsy performed for histopathological analysis of fibrosis stage and degree of necroinflammatory activity and exclusion of other causes of CLD were recorded from their files. Biopsy specimen were evaluated for the grade of inflammation and stage of fibrosis using the Metavir system. Inflammatory activity was graded from A0 to A3 and the stage of liver fibrosis and architectural disturbances ranged from F0 to F4 (14).

SPSS software version 18 was used for statistical analysis. Simple descriptive qualitative data were expressed in the form of numbers and percentages. In quantitative analysis of data; we used standard deviation for dispersion, t-test to compare two independent groups and One-Way ANOVA test in comparing more than two independent groups. Comparison of two of more qualitative groups was assessed by chi-square test. A p level of ≤0.05 was considered statistically significant.

## Results

This study included 60 patients with chronic HCV infection (32 males and 28 females) with an age mean of 46±8 years. Fifty healthy individual with age mean of 43±10.3 years, non-reactive for HCV antibodies, constituted the control group (18 males and 32 females). Nine patients in the patients group had diabetes mellitus. Table 1 illustrates the characteristics of the studied patients before antiviral treatment. The mean alanine aminotransferase (ALT) level was 62.2±33.1 IU/L, aspartate aminotransferase (AST) level - 54.6±27.2 IU/L, PCR level - 576.039±1.025.556 IU/mL and the mean serum vitamin D level was 36.4±16.9 ng/mL. According to the results of the liver biopsy performed in 60 patients, 35 (58.4%) subjects had inflammatory activity grade A1, 23 (38.3%) - grade A2 and two (3.3%) had grade A0, while 23 (38.4%) had fibrosis stage F2, 20 (33.3%) - F1, and 17 (28.3%) patients had F3. Thirty-eight subjects (63.3%) had good SVR to interferon treatment and 22 (36.7%) had no response. There was no significant difference in vitamin D levels between patients and controls (36.4±16.9 and 34.9±16 ng/mL, respectively; p=0.3).

There was no significant correlation of serum vitamin D level with age, ALT, AST, and PCR (p>0.05) (Table 2).

Table 3 shows a comparison of serum levels of vitamin D according to liver biopsy activity and fibrosis scores. It showed a significant inverse correlation (p=0.05). The lowest mean vitamin D level (33.4 ng/mL) was detected in grade A2 activity and (31 ng/mL) and stage F3 fibrosis, followed by (40 ng/mL) grade A1 activity and (36 ng/mL) stage F2 fibrosis. The highest mean vitamin D level was detected in grade A0 activity (68 ng/mL) and stage F1 fibrosis (47 ng/mL).

The mean vitamin D level in responder and non-responder groups was 39.2±23.6 and 37.1±13.2, respectively and the difference was not statistically significant (p=0.7) (Table 4).

**Table 1.** Characteristics of the studied patients (number=60) at baseline

| Variable | Result |
|---|---|
| Age (years) | 46±8 |
| Gender | |
| Male | 32 (53%) |
| Female | 28 (47%) |
| Diabetes mellitus | 9 (15%) |
| AST (IU/L) mean | 54.6±27.2 |
| ALT (IU/L) mean | 62.2±33.1 |
| HCV RNA (IU/mL) mean | 576.039±1.025.556 |
| Liver biopsy activity | |
| A0 | 2 (3.3%) |
| A1 | 35 (58.4%) |
| A2 | 23 (38.3%) |
| Liver biopsy fibrosis | |
| F1 | 20 (33.3%) |
| F2 | 23 (38.4%) |
| F3 | 17 (28.3%) |
| Serum vitamin D level (ng/mL) mean | 36.4±16.9 (control 34.9±16) |
| Males versus females | 42.7±22.2, 32±13 (pv=0.002) |
| Virological response frequency | |
| Responders | 38 (63.3%) |
| Non-responders | 22 (36.7%) |

A0/A1/A2: Grades of activity, F0/F1/F2: Stages of fibrosis, pv: Probability value, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HCV: Hepatitis C virus

**Table 2.** Correlation between serum level of vitamin D and different study variables among cases of chronic hepatitis C virus infection

| Variables | Vitamin D level (ng/mL) | | |
|---|---|---|---|
| | R | p value | Sig. |
| Age (years) | 0.078 | 0.6 | NS |
| ALT | 0.043 | 0.7 | NS |
| AST | 0.07 | 0.6 | NS |
| HCV-PCR | -0.12 | 0.4 | NS |

Sig.: Significance, R: Linear correlation coefficient, NS: Non significant, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HCV: Hepatitis C virus, PCR: Polymerase chain reaction

**Table 3.** Comparisons of serum level of vitamin D in chronic hepatitis C patients subgroups according to liver biopsy

| Variables (n=60) | Level of vitamin D | p value | Sig. |
|---|---|---|---|
| | Mean ± SD | | |
| Liver biopsy activity | | | |
| A0 (n=2) | 68±36.8 | 0.05 | S |
| A1 (n=35) | 40.1±17.4 | | |
| A2 (n=23) | 33.4±21.7 | | |
| Liver biopsy fibrosis | | | |
| F1 (n=20) | 47.1±23.1 | 0.05 | S |
| F2 (n=23) | 36±10.1 | | |
| F3 (n=17) | 31.6±24.5 | | |

A0/A1/A2: Activity grades, F0/F1/F2: Fibrosis stages, Sig./S: Significant, SD: Standard deviation

**Table 4.** Comparisons of serum level of vitamin D in chronic hepatitis C patients subgroups according to response to interferon

| Response to interferon (n=60) | Level of vitamin D (ng/mL) | p value | Sig. |
|---|---|---|---|
| | Mean ± SD | | |
| Non-responders (n=22) | 37.1±13.2 | 0.7 | NS |
| Responders (n=38) | 39.2±3.6 | | |

Sig.: Significant, NS: Non significant

## Discussion

In the present study, the mean serum vitamin D level was 36.4±16.9 ng/mL in patients and 34.9±16 ng/mL in controls with no statistically significant difference. Miroliaee et al. (15) demonstrated that cirrhotic patients had lower vitamin D levels than non-cirrhotic patients. Lower vitamin D levels were detected in patients with Child-Pugh class B and C compared to those with class A (p<0.001) while the difference in levels of serum vitamin D between healthy controls and non-cirrhotic patients was not significant (95.28±29.41 and 81.37±30.44 nmol/L, respectively). Chen et al. (16) compared vitamin D levels between cirrhotic patients (various etiologies) and controls and found no significant difference in 25(OH)D levels between patients and controls, while there was a significant difference in 25(OH)D between patients with Child-Pugh class B and C and controls.

Conversely, Lange et al. (9) reported significantly lower levels of serum vitamin D in patients with chronic HCV infection than in those negative for HCV (p<0.00001). Petta et al. (8) showed that the prevalence of 25(OH)D <30 ng/mL among HCV genotype 1 Italian patients was 73% of while it was 6% in controls (p<0.001). A Spanish study (5) concluded that the prevalence of 25(OH)D <20 ng/mL was detected in 64% of CLD patients with cirrhosis when compared with controls. Therefore, in the current study, the non-significant difference in serum vitamin D levels between patients and healthy control can be explained by the absence of decompensated cirrhosis in our selected cases.

Regarding virological response to HCV antiviral therapy in the current study, 38 (63%) of cases had SVR24 to interferon combined with ribavirin therapy and 22 (36.7%) had no response. These results are in agreement with other studies (17,18,19) on

the treatment of patients with chronic HCV genotype 4 (pegylated-interferon and ribavirin) for 48 weeks. Correlation of serum levels of vitamin D grades of activity and stages of fibrosis in patients' subgroups according to liver biopsy showed significant difference. The lowest mean level of vitamin D, 33.4 ng/mL was among patients with grade A2 activity and 31 ng/mL in those with stage F3 fibrosis, followed by 40 ng/mL in subjects with grade A1 activity and 36 ng/mL among patients with stage F2 fibrosis, while the highest mean level of vitamin D, 68 ng/mL was among patients with grade A0 activity and 47 ng/mL among those with stage F1 fibrosis. Comparison of vitamin D categories (sufficient and insufficient) showed highly significant difference with higher percentage of patients with insufficient vitamin D level: 14 (66%) patients with grade A2 activity and 13 (61.9%) patients with stage F3 fibrosis followed by 7 (33.3%) patients with grade A1 activity and 6 (28%) patients with stage F2 fibrosis.

Similar results were showed by Bitetto et al. (6) who concluded that the grade of inflammation and stage of fibrosis were inversely correlated with serum vitamin D levels. Additionally, 25(OH)D serum concentrations were inversely correlated with stage of fibrosis in patients with genotype 1 chronic hepatitis C in another study done by Petta et al. (8). Other studies demonstrated the lower serum vitamin D levels, the higher the degree of inflammation and/or stage of fibrosis in chronic HCV patients (15,20,21,22). Arteh et al. (23) reported that vitamin D <32 ng/mL was detected in 92% of 118 patients with CLD. Another study (24) reported that 91% of patients with non-cholestatic CLD had inadequate 25(OH)D levels (<32 ng/mL), 68% of them had vitamin D-deficiency (<20 ng/mL).

These findings may be explained by the role of vitamin D in immune system. Deluca and Cantorna (25) demonstrated that vitamin D was important in immune system, either innate or T cell-mediated immunity. Vitamin D affects the adaptive immune response through regulation of T and B lymphocytes, cytokines release and production of immunoglobulin (26). El Husseiny et al. (27) assessed the relationship between vitamin D and markers of inflammation in Egyptian HCV-infected patients and found a negative correlation between vitamin D and interleukin (IL)-17, IL23 and macrophage chemoattractant protein 1.

In the present study, comparisons of serum level of vitamin D in chronic hepatitis C patients' subgroups according to response to interferon showed no significant difference between treatment responders and non-responders.

A recent Brazilian study (13) included 201 chronic HCV patients. 47% of patients had SVR. 69% of patients had low 25(OH)D levels (<30 ng/dL). The deficiency was not associated with stage of fibrosis or grade of inflammation. 49% of patients with vitamin D deficiency had SVR, while 40% of patients with adequate vitamin D had SVR with no statistically significance. Kitson et al. (22) reported that chronic HCV patients with high activity grade had lower vitamin D levels (21% vs. 11%; p=0.03). In their study the mean 25(OH)D level had no correlation with the score of fibrosis or activity. A meta-analysis in 2014 (28) included 11 related studies comprising 2605 patients. The analysis reported that there was no significant association between the pretreatment mean 25(OH)D level and SVR in different viral genotypes.

Conversely, other studies concluded significant correlation between serum vitamin D levels and virological response to HCV antiviral therapy (10,29,30). An Italian study retrospectively evaluated 206 HCV patients treated with pegylated-interferon and ribavirin. The authors reported that a baseline level of vitamin D >20 ng/mL strongly predicted the achievement of SVR (29). Another study (30) investigated 117 consecutive patients with chronic HCV patients genotype 1 evaluated by biopsy. Serum vitamin D levels and IL28B polymorphisms were evaluated. The patients received antiviral therapy with interferon and ribavirin. The authors reported that serum 25(OH)D levels and IL28B status were associated with higher percentages of both rapid and SVR. The results are still conflicting, may be due to different ethnicity and different disease course between populations.

### Study Limitations

Limitations of the current study include absence of virus genotype assessment and lack of seasonal consideration of vitamin D measurement. Vitamin D supplementation for insufficient group could have an impact on the results.

## Conclusion

In conclusion, the present study showed that vitamin D levels can affect chronic HCV progression in Egyptian patients but did not prove its association with patients' response to antiviral therapy.

### Ethics

**Ethics Committee Approval:** The study was conducted after approval of the Ethics Committee of Faculty of Medicine Fayoum University.

**Informed Consent:** It was taken.

**Peer-review:** Externally and internally peer-reviewed.

### Authorship Contribution

Surgical and Medical Practices: H.A.H., H.B.M., Concept: H.A.H., M.A.A., Data Collection or Processing: H.B.M., G.M.E., Analysis or Interpretation: G.M.E., M.A.M., H.B.M., H.A.H., Literature Search: M.A.A., M.A.M., H.A.H., Writing: H.A.H., H.B.M.

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## References

1. Gal-tanamy M, Bachmetov L, Ravid A, Koren R, Erman A, Tur-Kaspa R, Zemel R. Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. Hepatology. 2011;54:1570-1579.
2. Stokes CS, Volmer DA, Grunhage F, Lammert F. Vitamin D in chronic liver disease. Liver Int. 2013;33:338-352.
3. Schiefke I, Fach A, Wiedmann M, Aretin AV, Schenker E, Borte G, Wiese M, Moessner J. Reduced bone mineral density and altered bone turnover markers in patients with non-cirrhotic chronic hepatitis B or C infection. World J Gastroenterol. 2005;11:1843-1847.
4. Malham M, Jørgensen SP, Ott P, Agnholt J, Vilstrup H, Borre M, Dahlerup JF. Vitamin D deficiency in cirrhosis relates to liver dysfunction rather than aetiology. World J Gastroenterol. 2011;17:922-925.

5. Monegal A, Navasa M, Guañabens N, Peris P, Pons F, Martinez de Osaba MJ, Rimola A, Rodés J, Muñoz-Gómez J. Osteoporosis and bone mineral metabolism disorders in cirrhotic patients referred for orthotopic liver transplantation. Calcif Tissue Int. 1997;60:148-154.
6. Bitetto D, Fattovich G, Fabris C, Ceriani E, Falleti E, Fornasiere E, Pasino M, Ieluzzi D, Cussigh A, Cmet S, Pirisi M, Toniutto P. Complementary role of vitamin D deficiency and the interleukin-28B rs12979860 C/T polymorphism in predicting antiviral response in chronic hepatitis C. Hepatology. 2011;53:1118-1126.
7. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu. EASL Recommendations on Treatment of Hepatitis C 2016. J Hepatol. 2017;66:153-194.
8. Petta S, Cammà C, Scazzone C, Tripodo C, Di Marco V, Bono A, Cabibi D, Licata G, Porcasi R, Marchesini G, Craxí A. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology. 2010;51:1158-167.
9. Lange CM, Bojunga J, Ramos-Lopez E, von Wagner M, Hassler A, Vermehren J, Herrmann E, Badenhoop K, Zeuzem S, Sarrazin C. Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. J Hepatol. 2011;54:887-893.
10. Bitetto D, Fabris C, Fornasiere E, Pipan C, Fumolo E, Cussigh A, Bignulin S, Cmet S, Fontanini E, Falleti E, Martinella R, Pirisi M, Toniutto P. Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C. Transpl Int 2011;24:43-50.
11. Abu Mouch S, Fireman Z, Jarchovsky J, Assy N. Vitamin D supplement improves SVR in chronic hepatitis C (genotype 1) naive patients treated with PEG interferon and ribavirin. J Hepatol. 2010;52:23-41.
12. Corey KE, Zheng H, Mendez-Navarro J, Delgado-Borrego A, Dienstag JL, Chung RT; HALT-C Trial Group. Serum vitamin D levels are not predictive of the progression of chronic liver disease in hepatitis C patients with advanced fibrosis. PLoS ONE. 2012;7:e27144.
13. de Almeida Borges PS, Guimarães VM, de Farias JLR, Trindade LZ, El Bacha IAH, Carvalho-Filho RJ, Parise ER. Absence of Relationship between Serum Vitamin D Levels, Degree of Hepatic Fibrosis, and Virologic Response to Pegylated Interferon and Ribavirin Therapy in Patients with Chronic Hepatitis C. Journal of GHR. 2016;5:2015-2020.
14. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The Metavir Cooperative Study Group. Hepatology. 1996;24:289-293.
15. Miroliaee A, Nasiri-Toosi M, Khalilzadeh O, Esteghamati A, Abdollahi A, Mazloumi M. Disturbances of parathyroid hormone-vitamin D axis in noncholestatic chronic liver disease: a cross-sectional study. Hepatol Int. 2010;4:634-640.
16. Chen CC, Wang SS, Jeng FS, Lee SD. Metabolic bone disease of liver cirrhosis: Is it parallel to the clinical severity of cirrhosis? J Gastroenterol Hepatol. 1996;11:417-421.
17. Kamal S. Hepatitis C virus genotype 4 therapy: progress and challenges. Liver Int. 2011;31(Suppl 1):45-52.
18. Khattab MA, Eslam M, Shatat M, Abd-Aalhalim H, Mousa YI, Samir F, Aly H, Shaker O, Shaker Y. Changes in adipocytokines and insulin sensitivity during and after antiviral therapy for hepatitis C genotype 4. J gastrointestin Liver Dis. 2012;21:59-65.
19. Papastergiou V, Dimitroulopoulos D, Skorda L, Lisgos P, Ketikoglou I, Kostas N, Karatapanis S. Predictors of sustained virological response in Greek and Egyptian patients with hepatitis C genotype 4: does ethnicity matter? J Med Virol. 2012;84:1217-1223.
20. Baur K, Mertens JC, Schmitt J, Iwata R, Stieger B, Eloranta JJ, Frei P, Stickel F, Dill MT, Seifert B, Ferrari HA, von Eckardstein A, Bochud PY, Müllhaupt B, Geier A; Swiss Hepatitis C Cohort Study Group. Combined effect of 25-OH vitamin D plasma levels and genetic Vitamin D Receptor (NR 1I1) variants on fibrosis progression rate in HCV patients. Liver Int. 2012;32:635-643.
21. Putz-Bankuti C, Pilz S, Stojakovic T, Scharnagl H, Pieber TR, Trauner M, Obermayer-Pietsch B, Stauber RE. Association of 25-hydroxyvitamin D levels with liver dysfunction and mortality in chronic liver disease. Liver Int. 2012;32:845-851.
22. Kitson MT, Dore GJ, George J, Button P, McCaughan GW, Crawford DH, Sievert W, Weltman MD, Cheng WS, Roberts SK. Vitamin D status does not predict sustained virologic response or fibrosis stage in chronic hepatitis C genotype 1 infection. J Hepatol. 2013;58:467-472.
23. Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. Dig Dis Sci. 2010;55:2624-2628.
24. Fisher L, Fisher A. Vitamin D and parathyroid hormone in outpatients with noncholestatic chronic liver disease. Clin Gastroenterol Hepatol. 2007;5:513-520.
25. Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. FASEB J. 2001;15:2579-2585.
26. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25 dihdroxyvitamin D3 on human B cell differentiation. J Immunol. 2007;179:1634-1647.
27. El Husseiny NM, Fahmy HM, Mohamed WA, Amin HH, Relationship between vitamin D and IL-23, IL-17 and macrophage chemoattractant protein-1 as markers of fibrosis in hepatitis C virus Egyptians. World J Hepatol. 2012;4:242-247.
28. Kitson MT, Sarrazin C, Toniutto P, Eslick GD, Roberts SK. Vitamin D level and sustained virologic response to interferon-based antiviral therapy in chronic hepatitis C: a systematic review and meta-analysis. J Hepatol. 2014;61:1247-1252.
29. Falleti E, Bitetto D, Fabris C, Fattovich G, Cussigh A, Cmet S, Ceriani E, Fornasiere E, Pasino M, Ieluzzi D, Pirisi M, Toniutto P. Vitamin D Binding Protein Gene Polymorphisms and Baseline Vitamin D Levels as Predictors of Antiviral Response in Chronic Hepatitis C. Hepatology. 2012;56:1641-1650.
30. Petta S, Ferraro D, Cammà C, Cabibi D, Di Cristina A, Di Marco V, Di Stefano R, Grimaudo S, Mazzola A, Levrero M, Scazzone C. Vitamin D levels and IL28B polymorphisms are related to rapid virological response to standard of care in genotype 1 chronic hepatitis C. Antivir Ther. 2012;17:823-831.

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# *Can HBsAg Be Used as a Viral Replication Marker in Chronic Hepatitis B Patients?*

## Kronik Hepatit B Hastalarında HBsAg Viral Replikasyon Göstergesi Olarak Kullanılabilir mi?

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***ABSTRACT***

**Objective:** Monitoring hepatitis B virus (HBV) treatment responses and virus replication is performed with molecular tests. However, these tests are either expensive or invasive. A new and more practical marker is needed. We aimed to evaluate the correlation between serum hepatitis B surface antigen (HBsAg) and alanine aminotransferase (ALT) levels and HBV DNA level in hepatitis B e antigen (HBeAg) +/- patients and detect whether HBsAg can be used as a surrogate replication marker instead of HBV DNA.

**Material and Methods:** A retrospective study was conducted in 59 chronic hepatitis B patients. Serum ALT, HBsAg and HBeAg levels and HBV DNA levels were recorded. The results were analysed with the Mann-Whitney U test and Spearman correlation coefficient. A p value of ≤0.05 was considered statistically significant. Sequential results were compared using Blant-Alpman plot.

**Results:** The patients were grouped as HBeAg-positive (37.2%) and HBeAg-negative (62.8%). Serum ALT levels were elevated in 82% of HBeAg-positive and 70.2% of HBeAg-negative subjects. There was a statistically significant difference in HBsAg levels between the groups ($p<0.05$). However, there was no statistically significant difference in ALT and HBV DNA levels ($p>0.05$). A statistically significant negative correlation was detected between HBsAg and HBV DNA levels in HBeAg-positive patients. No correlation was found between HBsAg and HBV DNA levels in HBeAg-negative subjects ($p<0.05$). In both HBeAg-positive and -negative individuals, there was a positive correlation between serum ALT and HBV DNA levels ($p<0.05$). Blant-Alpman graph did not show an appropriate profile.

**Conclusion:** We found a negative correlation between HBsAg and HBV DNA levels in HBeAg-positive patients. However, this correlation is not practical in monitoring treatment response and replication.

**Keywords:** Hepatitis B surface antigen, hepatitis B virus DNA, hepatitis B e antigen

***ÖZ***

**Amaç:** Hepatit B virüsüne (HBV) olan tedavi cevabı ve virüs replikasyonun monitorizasyonu moleküler yöntemlerle yapılmaktadır. Ancak bu yöntemler ya pahalı ya da invazivdir. Bu konu da daha uygulanabilir bir belirtece ihtiyaç vardır. Bu çalışmada hepatit B e antijen (HBeAg) +/- hastalarda hepatit B yüzey antijeni (HBsAg), alanin aminotransferaz (ALT) ve HBV DNA düzeyleri arasındaki korelasyonu ve HBsAg'nin HBV DNA yerini alabilecek bir replikasyon göstergesi olarak kullanılıp kullanılamayacağını araştırmayı hedefledik.

**Gereç ve Yöntemler:** Çalışmamız 59 kronik hepatit B hastası ile retrospektif olarak yürütülmüştür. Hastaların serum ALT, HBsAg, HBeAg ve HBV DNA düzeyleri kayıt edilmiştir. Sonuçlar Mann-Whitney U ve Spearman korelasyon testleri aracılığıyla değerlendirilmiştir. P≤0,05 anlamlı kabul edilmiştir. Ardışık sonuçlar Blant-Alpman grafiği ile karşılaştırılmıştır.

**Bulgular:** Hastalar HBeAg pozitif (%37,2) and HBeAg negatif (%62,8) olarak gruplandırıldı. Serum ALT düzeyleri HBeAg pozitif hastalarda %82, HBeAg negatif hastalarda %70,2 oranında yüksekti. Gruplar arasında, HBsAg düzeyleri arasında istatistiksel olarak anlamlı fark varken ($p<0,05$), ALT ve HBV DNA düzeyleri arasında yoktu ($p>0,05$). HBeAg pozitif grupta HBsAg ve HBV DNA düzeyleri arasında, anlamlı negatif korelasyon saptanırken ($p<0,05$), HBeAg negatif olanlarda, HBsAg ve HBV DNA düzeyleri arasında korelasyon yoktu. Hem HBeAg pozitif hem de negatif grupta, serum ALT ve HBV DNA düzeyleri arasında pozitif bir korelasyon vardı ($p<0,05$). Blant-Alpman grafiği anlamlı bir profil göstermedi.

**Sonuç:** HBeAg pozitif hastalarda HBsAg ve HBV DNA düzeyleri arasında negatif bir korelasyon saptadık. Ancak bu korelasyon ne tedavinin monitorizasyonunda ne de replikasyonun takibinde kullanıma uygun değildir.

**Anahtar Kelimeler:** Hepatit B yüzey antijeni, hepatit B virüsü DNA, hepatit B e antijen

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## Introduction

Chronic hepatitis B (CHB) is a global health problem affecting 350 million patients and leading to 1 million deaths each year (1). Virological, serological, biochemical and histhopathological markers are used for monitoring treatment response (2). Quantification of intrahepatic hepatitis B virus (HBV) covalently closed circular DNA (ccc DNA) is recommended in evaluating efficacy of anti HBV therapy, however, it requires liver biopsy. Active virus replication can also be detected by quantifying total HBV DNA, however, these molecular-based assays are expensive and they are not available in many centers, especially in developing countries (3,4). Therefore, a surrogate marker which is cheap and more practical is required.

HBV has partially double-stranded and circular genome which encodes four major proteins, including S, P, C and X. The S [hepatitis B surface antigen (HBsAg)] protein that we detect by serologic tests and diagnose hepatitis B infection is the main protein of the viral envelope. *Hepadnaviridae* family members produce a large amount of viral envelope protein (HBsAg). The S protein is found both in intact viral particles and subviral particles which also contain M and L protein but not HBV DNA (5).These proteins are non-infectious but they are immunogenic (3). Both HBsAg and hepatitis B e antigen (HBeAg) represent viral replication and activation is suspected when an increase is detected in these markers. Although, there have been several studies declaring that there was a positive correlation between viral load and HBsAg, HBeAg and alanine aminotransferase (ALT) levels and these markers could be used in monitoring the treatment; this correlation may break down during HBV infection and antiviral therapy (6,7,8).

This study aimed to evaluate the correlation between serum HBsAg and ALT levels and serum HBV DNA level in HBeAg +/- patients and investigate if HBsAg can be used as a surrogate replication marker instead of HBV DNA in CHB patients.

## Materials and Methods

A retrospective study was conducted in 59 CHB patients who were followed up in our clinical microbiology and infectious diseases clinic and treated with nucleoside analogs between May 2012 and January 2017. The study was approved by Giresun University Prof. Dr. İlhan Özdemir Training and Research Hospital, Local Ethics Committee (approval no: 08/6, date: 15.11.2017). The patients had been HBsAg-positive for more than 6 months. Of the patients, 28 (47.5%) were male and 31 (52.50%) were female. The mean age was 42±11 years.

There was no patient co-infected with hepatitis A, hepatitis C, hepatitis E and hepatitis D viruses or human immunodeficiency virus. Patients with immune disorders, metabolic liver disease, hepatocellular carcinoma or end-stage liver disease were excluded. HBsAg, HBeAg, ALT and HBV DNA levels were recorded. Serum ALT levels were analysed with Cobas c 702 biochemical autoanalyser (Roche Diagnostics, Mannheim, Germany). Serum HBsAg and HBeAg were detected by electro-chemiluminescence assay with Cobas e 601 (Roche Diagnostics, Mannheim, Germany) wherein the semi quantitative test results was expressed in s/co (sample/cut off). HBV DNA was analysed by quantitative Montania 483 system (Anatolia Gene, Istanbul, Turkey) with Bosphore HBV Quantification kit. Linear limits were $1x101^{-1}x10^{9}$ IU/mL.

### Statistical Analysis

The patients were divided into two groups as HBeAg-positive and -negative. Mean and standard deviations of HBsAg, ALT and HBV DNA levels were calculated. Statistical difference in HBsAg, ALT and HBV DNA levels between HBeAg-positive and -negative groups were analysed with the Mann-Whitney U test. Spearman correlation coefficient was used to correlate serum levels of HBsAg, ALT and HBV DNA levels. SPSS 16 was used for statistical analysis. A p value of ≤0.05 was considered statistically significant. In order to determine whether HBsAg can be used for monitoring treatment, the sequential results of the patients were compared using Blant-Alpman plot.

## Results

The levels of HBsAg, ALT and HBV DNA in HBeAg-positive and -negative groups are shown in Table 1. Of the patients, 22 (37.2%) were HBeAg-positive and 37 (62.8%) were HBeAg-negative. In HBeAg-positive and -negative subjects, serum ALT levels were elevated in 18 (82%) and 26 (70.2%) patients, respectively.

Viral load was higher in HBeAg-positive patients than in HBeAg-negative individuals. On the other hand, HBsAg was higher in HBeAg-negative patients. The distribution of HBsAg levels are shown in Figure 1. Statistical analysis revealed that there was a statistically significant difference in HBsAg levels (p=0.011) between these two groups. However, the difference in ALT and HBV DNA levels was not statistically significant (p>0.05).

There was a statistically significant negative correlation between HBsAg and HBV DNA levels in HBeAg-positive patients. On the other hand, no correlation was found between HBsAg and HBV DNA levels in HBeAg-negative patients. In both HBeAg-positive and -negative patients, there was a statistically significant positive correlation between serum ALT and HBV DNA levels (p<0.05) (Table 2).

Blant-Alpman graph was drawn in order to analyze whether HBsAg can be used instead of HBV DNA for monitoring response to treatment but the data did not constitute an appropriate profile (Data not shown).

**Table 1.** Median (maximum-minimum) of hepatitis B surface antigen, alanine aminotransferase and hepatitis B virus DNA levels for both hepatitis B e antigen positive and negative patients

| | HBeAg positive (n=22) median (Q1-Q3) | HBeAg negative (n=37) median (Q1-Q3) | p value |
|---|---|---|---|
| HBsAg | 2235.5 (611.275-4544.75) | 4096 (2187-5681) | p<0.05 |
| ALT | 43.5 (29.5-59.25) | 43 (28-49.5) | p>0.05 |
| HBV DNA | 192800 (2254-648325000) | 16680 (2234-755300) | p>0.05 |

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

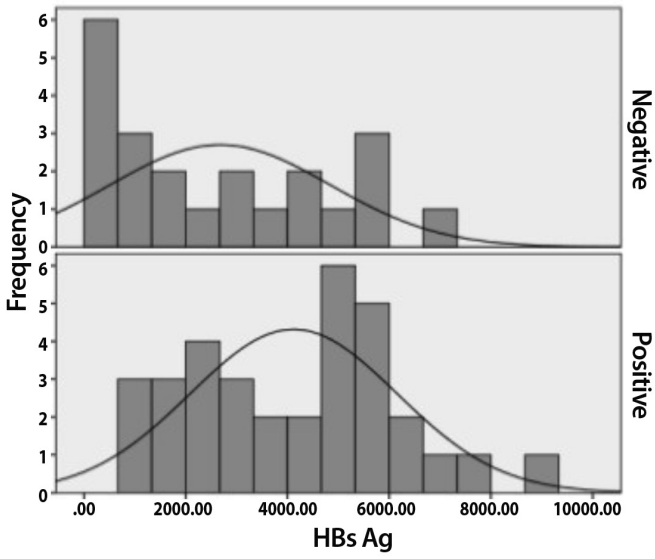


**Figure 1.** The distribution of serum hepatitis B surface antigen levels in hepatitis B e antigen positive and negative groups
*HBsAg: Hepatitis B surface antigen*

**Table 2.** Spearman's correlation coefficients showing the correlation between hepatitis B surface antigen, alanine aminotransferase and hepatitis B virus DNA levels in hepatitis B e antigen positive and negative patients

| | HBeAg positive Spearman RHO | HBeAg negative Spearman RHO |
|---|---|---|
| HBsAg-HBV DNA | -0.699* | 0.021 |
| ALT-HBV DNA | 0.531* | 0.511* |

*Statistically significance at 5% level
HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B virüs, HBeAg: Hepatitis B e antigen, ALT: Alanine aminotransferase, RHO: Speraman's Rank order correlation

## Discussion

In our study, the difference and correlation between HBsAg and ALT levels and HBV DNA level were investigated in HBeAg-positive and -negative patients. We hypothesized that HBsAg can be used during monitoring response to treatment in CHB patients. However, in our study, when an increase was detected in HBV DNA levels, a decrease was observed in HBsAg levels or vice versa in HBeAg-positive patients. There was no correlation between HBsAg and HBV DNA levels in HBeAg-negative patients. On the other, hand serum ALT levels were increasing with increasing levels of HBV DNA in both groups.

Several studies investigating the correlation between HBsAg and ALT levels and HBV DNA level in CHB patients have revealed conflicting results (5,6,7,9,10). To our knowledge, researchers detected a correlation between HBsAg and HBV DNA only in HBeAg-positive patients (3,9,10). In a cross-sectional study from Iran, the majority of the cases (87%) were HBeAg-negative and no correlation was detected between HBsAg and HBV DNA levels (3). Similar results were also demonstrated by Zeng et al. (8) in a Chinese cohort, by Jaroszewicz et al. (11) in a European cohort, by Nguyen et al. (12) in an Asian cohort, and by Ramachandran et al. (13) in an Indian cohort. In our study, 62.8% of the cases were HBeAg-negative and we also could not find any correlation between HBsAg and HBV DNA in HBeAg-negative patients. Our results revealed that using HBsAg instead of HBV DNA for monitoring does not seem possible in HBeAg-negative cases.

In HBeAg-positive cases, HBV DNA, which demonstrates viral load, was higher when compared to HBeAg-negative cases but, in contrast, HBsAg was lower. Therefore, there was a negative correlation between HBsAg and HBV DNA levels in HBeAg-positive patients. HBsAg and HBeAg are both accepted as the indicators of viral replication and a positive correlation is expected in fact. In the literature, there are studies that revealed positive correlation as well as many studies demonstrating negative correlation (5,6,8,14). We conclude that a negative correlation was detected in our study due to several different scenarios.

In our laboratory, we use the HBsAg II kit (Roche Diagnostics, Mannheim, Germany) which produces semi-quantitative results wherein the results are expressed in s/co and we do not dilute the samples. However, there is a type of interference which is also called hook effect especially in immunoassays. This effect may cause misdetection of HBsAg due to a very high analyte concentration (15). In order to obtain accurate results, during quantification experiments performed with HBsAg II quantification kits (Roche Diagnostics, Mannheim, Germany), 1/400 times dilution of the samples is suggested by the manufacturer (16,17). On the other hand, Zhang et al. (14) carried out a study with a quantitative kit (Abbott Diagnostics, Germany) and they have diluted the samples to 1:500 or 1:1000 if they were greater than 250 IU/mL according to the manufacturer's instructions. They have subdivided the patients into three groups as immune tolerant (IT), immune clearance (IC) and acute or chronic liver failure (ACLF) considering the phase of the disease. They found a weak correlation between HBsAg and HBV DNA levels in IT and ACLF groups and a modest correlation in IC group. They explained this difference with the degree of immune responses in different stages (14). In a cohort study from China, the researchers also worked with a quantitative kit of Abbott Diagnostics and they found the strongest correlation between HBsAg and HBV in IC group and the poorest correlation in low replicate and liver cirrhosis groups (8).

HBsAg clearance is a complex phenomenon. In IT phase, the host immune response is not triggered against HBV infected hepatocytes. However, when IC phase begins, depending on the degree of host immune response, the clearance of the virus begins and the level of HBsAg declines. ACLF patients show a dramatic immune response compared with IC patients and HBsAg levels are significantly lower in ACLF patients. However, these hepatocytes still synthesize $10^2$-$10^5$ HBsAg, which is in number much more than required for formation of complete virus particle and these non infective, filamentous or sphenoid S antigens are detected in the serum. Even novel diagnostic techniques cannot differ the complete virion from these particles and detect total HBsAg levels (14,8). Tuaillon et al. (18) quantified serum HBsAg levels by using four different immunoassay methods and investigated the relationship between HBsAg and HBV DNA levels. They found

the highest correlation in the early phase of the infection. They declared that in the latest phase of the infection, the correlation between these two parameters was weakest and this was not related with the test used.

Mutation is another important factor that may also affect the efficiency of diagnostic immunoassays and the correlation between the quantitative tests. Mutations in HBsAg cause false-negative results in diagnostic tests (19). Külah et al. (20) found this mutation rate as 12% in their study. In addition, HBeAg production is interrupted secondary to the mutations in pre-core region and it is possible to detect HBV DNA in these cases because of continuing virus replication (21). Therefore, novel diagnostic tools are urgently needed for the antigenicity and immunogenicity analyses of these mutant cases.

ALT levels showed a moderate correlation with HBV DNA results. As the cases were chronic hepatitis patients, this was not a surprising finding. Serial values of the patients were also analysed in order to define whether HBsAg can be used instead of HBV DNA in monitoring the treatment. However, Bland-Alpman plot, which is used to evaluate whether one parameter can be used instead of another, revealed that HBsAg does not seem to be appropriate for being used in monitoring the treatment instead of HBV DNA.

### Study Limitations

There were several limitations of our study. The semiquantitative method we used, the escape HBV mutants that we could not detect, the phase of the disease and/or degree of immune response of the host can be considered as the factors that might affect the serum HBsAg levels.

## Conclusion

In conclusion, serum HBsAg levels were negatively correlated with HBV DNA levels in HBsAg-positive patients, however, this correlation was not strong enough to use HBsAg instead of HBV DNA in monitoring treatment. Another quantification study is planned by our group with quantification kits and patients in different stages of the disease. Furthermore, new test methods which detect both infectious and non infectious virus particles containing S proteins might be beneficial.

### Ethics

**Ethics Committee Approval:** The study was approved by Giresun University Prof. Dr. İlhan Özdemir Training and Research Hospital, Local Ethics Committee (approval no: 08/6, date: 15.11.2017).

**Informed Consent:** Retrospective study.

**Peer-review:** Internally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: E.U., A.M.Ş., H.K., G.G., Concept: E.U., A.M.Ş., H.K., Design E.U., A.M.Ş., H.K., Data Collection or Processing: E.U., G.G., Analysis or Interpretation: E.U., E.A., G.G., Literature Search: E.U., A.M.Ş., H.K., E.A., G.G., Writing: E.U., A.M.Ş., H.K., E.A., G.G.

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## References

1. Wright TL. Introduction to chronic hepatitis B infection. Am J Gastroenterol. 2006;101(Suppl 1):1-6.
2. European Association For The Study Of The Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol. 2009;50:227-242.
3. Ganji A, Esmaeilzadeh A, Ghafarzadegan K, Helalat H, Rafatpanah H, Mokhtarifar A. Correlation between HBsAg quantitative assay results and HBV DNA levels in chronic HBV. Hepat Mon. 2011;11:342-345.
4. Li W, Zhao J, Zou Z, Liu Y, Li B, Sun Y, Li X, Liu S, Cai S, Yao W, Xin S, Lu F, Xu D. Analysis of hepatitis B virus intrahepatic covalently closed circular DNA and serum viral markers in treatment-naive patients with acute and chronic HBV infection. PLoS One. 2014;9:e89046.
5. Ozdil B, Cosar AM, Akkiz H, Sandikci MU, Kece C. Negative correlation between viral load and HBsAg levels in chronic HBV-infected patients. Arch Virol. 2009;154:1451-1455.
6. Saglik I, Mutlu D,Ongut G, Guvenc H.I,Akbas H, Ogunc D, Colak D. Comparison of HBsAg and HBeAg levels with HBV DNA and ALT levels in patients with chronic hepatitis B infections. Viral Hepat J. 2013;19;119-122.
7. Sener AG, Kirdar S, Serter M, Afsar I, Demir EM, Ceylan C, Aydın N. Investigation of the relationship between serum nitric oxide levels, HBV-DNA and ALT levels in chronic hepatitis B patients. Mikrobiyol Bul. 2009;43:83-89.
8. Zeng LY, Lian JS, Chen JY, Jia HY, Zhang YM, Xiang DR, Yu L, Hu JH, Lu YF, Zheng L, Li LJ, Yang YD. Hepatitis B surface antigen levels during natural history of chronic hepatitis B: a Chinese perspective study. World J Gastroenterol. 2014;20:9178-9184.
9. Martinot-Peignoux M, Carvalho-Filho R, Lapalus M, Netto-Cardosso AC, Lada O, Batrla R, Krause F, Asselah T, Marcellin P. Hepatitis B surface antigen serum level is associated with fibrosis severity in treatment-naive, . e antigen-positive patients. J Hepatol. 2013;58:1089-1095.
10. Seto WK, Wong DK, Fung J, Ip PPC, Yuen JC, Hung IF, Lai CH, Yuen MF. High hepatitis B surface antigen levels predict insignificant fibrosis in hepatitis B e antigen positive chronic hepatitis B. PLoS One. 2012;7:e43087.
11. Jaroszewicz J, Calle Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R,Flisiak R, Bock CT, Manns MP, Wedemeyer H, Cornberg M. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus(HBV)-infection: a European perspective. J Hepatol. 2010;52:514-522.
12. Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, Levy M, Locarnini SA. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. J Hepatol. 2010;52:508-513.
13. Ramachandran J, Ismail AM, Chawla G, Fletcher GJ, Goel A, Eapen CE, Abraham P. Serum HBsAg quantification in treatment-naive Indian patients with chronic hepatitis B. Indian J Gastroenterol. 2014;33:131-135.
14. Zhang YM, Yang YD, Jia HY, Zeng LY, Wei Y, Ning Z, Li LJ. HBsAg levels in HBeAg-positive chronic hepatitis B patients with different immune conditions. World J Gastroenterol. 2014;20:4407-4413.
15. Akhtar K, Sherwani RK, Sofi LA, Sharma A, Singh P. Hook's effect – a rare presentation in HBsAg screening. Indian J Hematol Blood Transfus. 2009;25:27-29.
16. Maylin S, Boyd A, Delaugerre C, Zoulim F, Lavocat F, Simon F, Girard PM, Lacombe K. Comparison between Elecsys HBsAg II and Architect HBsAg QT assays for quantification of Hepatitis B Surface Antigen among Patients Coinfected with HIV and Hepatitis B Virus. Clin Vaccine Immunol. 2012;19:242-248.

17. Gupta E, Pandey P, Kumar A, Sharma MK, Sarin SK. Correlation between two chemiluminescence based assays for quantification of hepatitis B surface antigen in patients with chronic hepatitis B infection. Indian J Med Microbiol. 2015;33:96-100.
18. Tuaillon E, Mondain AM, Nagot N, Ottomani L, Kania D, Nogue E, Rubbo PA, Pageaux GP, Van de Perre P, Ducos J. Comparison of serum HBsAg quantitation by four immunoassays, and relationships of HBsAg level with HBV replication and HBV genotypes. PLoS One. 2012;7:e32143.
19. Hossain MG, Ueda K. Investigation of a Novel Hepatitis B Virus Surface Antigen (HBsAg) Escape Mutant Affecting Immunogenicity. PLoS One. 2017;12:e0167871.
20. Külah C, Cömert F, Özlü N, Eroğlu O, Tekin İÖ. Hepatit B Virus (HBV) İnfeksiyonunda Serolojik Belirteçler, Transaminaz Düzeyleri Ve HBV DNA'nın Birlikte Değerlendirilmesi. Viral Hepat J. 2007;12: 111-115.
21. Saab S, Martin P. Tests for acute and chronic viral hepatitis. Finding your way through the alphabet soup of infection and superinfection. Postgrad Med. 2000;107:123-126.

Research Article

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# *Investigation of Mean Platelet Volume, Platelet Distribution Width and Erythrocyte Distribution Width in Patients with Hepatitis B Virus Infection*

## Hepatit B Virüs Enfeksiyonu Bulunan Kişilerde Ortalama Trombosit Hacmi, Trombosit Dağılım Genişliğinin ve Eritrosit Dağılım Genişliğinin Araştırılması

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***ABSTRACT***

**Objective:** Hepatitis B virus (HBV) infection is an important public health issue all over the world, and it has a high morbidity and mortality rates caused by chronic liver disease. Liver biopsy is the primary procedure for evaluating the fibrosis grade. Recently, non-invasive methods are used to predict liver histology. Complete blood count (CBC) is one of the most needed and used laboratory tests in clinics. CBC parameters have been used in various studies to estimate the severity of the disease and the risk of mortality. In the present study, we aimed to determine the relationship of HBV infection with mean platelet volume (MPV), platelet distribution width (PDW) and red cell distribution width (RDW).

**Materials and Methods:** Two hundred fifty-nine hepatitis B surface antigen (HBsAg)-positive patients, who attended the Infectious Diseases outpatient Clinic at Van Military Hospital between October 2013 and December 2014, were included in the study group. A total of 245 food handlers with similar socio-demographic characteristics with the study group, who applied at the same period, formed the control group. HBsAg-positive patients were studied in two groups as chronic active hepatitis and inactive carriers according to their follow-up. CBC results of the patients and the healthy controls were screened from the hospital information system and they were evaluated retrospectively.

**Results:** The average platelet count in HBsAg-positive patients and controls was 262.59±62.13x103/mm$^3$ and 245.28±60.78x103/mm$^3$, respectively and the difference between the groups was statistically significant (p=0.002). There was also statistically significant difference in RDW values between the two groups. The average RDW was 12.14±1.05 in HBV group, while it was 12.49±1.28 in control group (p=0.001). On the other hand, no significant difference was observed in PDW and MPV between the groups.

***ÖZ***

**Amaç:** Hepatit B virüs (HBV) enfeksiyonu tüm dünyada önemli bir halk sağlığı sorunudur ve kronik karaciğer hastalığına bağlı yüksek bir morbidite ve mortalite oranına sahiptir. Karaciğer biyopsisi, fibrozisin derecesini belirlemek için primer yöntemdir. Son zamanlarda, karaciğer histolojisini öngörmek için non-invaziv yöntemler kullanılmaktadır. Tam kan sayımı, kliniklerde en çok ihtiyaç duyulan ve kullanılan laboratuvar testlerinden biridir. Tam kan sayımı parametreleri, hastalığın ciddiyetini ve mortalite riskini belirlemek için çeşitli çalışmalarda kullanılmıştır. Bu çalışmada HBV enfeksiyonu ile ortalama trombosit hacmi (MPV), trombosit dağılım genişliği (PDW) ve eritrosit dağılım genişliği (RDW) parametreleri arasındaki ilişkiyi saptamayı amaçladık.

**Gereç ve Yöntemler:** Ekim 2013-Aralık 2014 tarihleri arasında Van Asker Hastanesi Enfeksiyon Hastalıkları Polikliniği'ne, hepatit B yüzey antijeni (HBsAg) pozitifliği nedeni ile başvuran 259 hasta, araştırmamızda çalışma grubunu oluşturdu. Aynı tarihlerde polikliniğine başvuran, çalışma grubu ile benzer sosyo-demografik özelliklere sahip 245 gıda elleyicisi de kontrol grubunu oluşturdu. HBsAg pozitif hastalar, izlemlerine göre kronik hepatit ve inaktif taşıyıcılar olmak üzere iki grupta incelendi. Hastaların ve sağlıklı grubun tam kan sayımı sonuçları, hastane bilgi sisteminden tarandı ve retrospektif olarak değerlendirildi.

**Bulgular:** HBsAg pozitif hastalarda trombosit ortalaması 245,28±60,78x103/mm$^3$, kontrol grubunda 262,59±62,13x103/mm$^3$'tü ve bu gruplar arasındaki fark istatistiksel olarak anlamlıydı (p=0,002). İki grup arasındaki RDW değerleri de istatistiksel olarak anlamlı bir farklılık gösterdi. HBV grubunda RDW ortalaması 12,14±1,05, kontrol grubunda ise 12,49±1,28'di (p=0,001). Öte yandan, PDW ve MPV ile ilgili gruplar arasında anlamlı bir fark gözlenmedi.

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***ABSTRACT***

**Conclusion:** It is thought that simple, inexpensive and routinely used platelet and erythrocyte parameters in combination with other inflammatory parameters may help predict liver inflammation. While platelets and RDW are decreased in people with HBV infection, MPV and PDW are not changed. We assume that platelets and RDW should be evaluated together in HBsAg-positive patients, and further studies with larger samples should be performed.
**Keywords:** Hepatitis B, chronic liver disease, mean platelet volume, erythrocyte distribution width

***ÖZ***

**Sonuç:** Basit, ucuz ve rutinde sıklıkla kullanılan trombosit ve eritrosit parametrelerinin, diğer enflamatuvar parametreler ile birlikte karaciğer enflamasyonunu öngörmede yardımcı olabileceği düşünülmektedir. HBV enfeksiyonu olan kişilerde trombosit ve RDW azalırken, MPV'nin ve PDW'nin değişmediği gözlendi. HBsAg pozitif hastalarda trombosit ve RDW'nin birlikte değerlendirilmesini, ayrıca bu konu ile ilgili çalışmaların daha geniş gruplarda devam etmesi gerektiğini düşünmekteyiz.
**Anahtar Kelimeler:** Hepatit B, kronik karaciğer hastalığı, ortalama trombosit hacmi, eritrosit dağılım genişliği

**Kıratlı K, Çevik E. Investigation of Mean Platelet Volume, Platelet Distribution Width and Erythrocyte Distribution Width in Patients with Hepatitis B Virus Infection. Viral Hepat J. 2017;23:60-63.**

## Introduction

Hepatitis B virus (HBV) infection is an important public health issue all over the world, and it has a high morbidity and mortality rates caused by chronic liver disease (1). Countries with a HBV carrier rate of less than 2% of are considered low endemic, those with between 2% and 10%, moderate, and those with >10% are considered high endemic regions. Although there are regional differences in Turkey, the seroprevalence of hepatitis B surface antigen (HBsAg) is between 3.9% and 12.5% and we are considered to be in the moderate endemic region (2).

Liver biopsy is the primary procedure for evaluating the fibrosis stage (3). However, the method is expensive and its invasive nature limits its use in some cases due to complications and contraindications (4). For this reason, non-invasive parameters are used to predict liver histology (5,6,7,8).

Inactive HBV carriers constitute the majority of chronic hepatitis B cases and they have a low risk of hepatocellular carcinoma (HCC) or cirrhosis (9). Therefore, close follow-up for inactive carriers is important in terms of development of liver cirrhosis and HCC. The relatively low risk of HCC limits the usage of invasive methods such as biopsy. Consequently, the use of non-invasive methods is important to determine the level of inflammation and chronicity in inactive HBV carriers (10).

Mean platelet volume (MPV) and platelet distribution width (PDW) are some parameters in complete blood count (CBC), which define thrombocyte size and the extent of diversity in thrombocyte size (4). MPV is also a marker of inflammation and its severity (11).

Red cell distribution width (RDW) is an automatic measurement of the variability of the red blood cell size. It is used in predicting the cause of anemia (12). New studies have declared that elevation of RDW values is associated with high mortality risk in different patient groups (13).

CBC is one of the most needed and used laboratory tests in clinics. CBC parameters have been used in various studies to estimate the severity of the disease and the risk of mortality. In the present study, we aimed to determine the relationship between HBV infection and MPV, PDW and RDW parameters.

## Materials and Methods

In our study, 259 HBsAg positive patients, who attended the infectious diseases outpatient clinics at Van Military Hospital between October 2013 and December 2014, were included in the study. A total of 245 food handlers with similar socio-demographic characteristics with the study group, who applied at the same period formed the control group. Serum HBsAg and anti-HBc IgG titres were analysed using the enzyme-linked immunosorbent assay (ELISA) method by Beckman Coulter Access 2 (Beckman Coulter, FL, USA). CBC (platelet and erythrocyte indices) was performed by LH780 Beckman Coulter (Beckman Coulter, FL, USA).

The HBsAg positive patients were studied in two groups as chronic hepatitis and inactive carriers according to their follow-up. CBC results of the patients and the healthy controls were screened from the hospital information system and they were evaluated retrospectively. There was no additional disease in HBsAg positive patients (e.g. diabetes mellitus, ischemic heart disease, thyroid disease, etc.).

We used SPSS version 16.0 to perform statistical procedures. All continuous variables were expressed as mean ± standard deviation. Student's t-test was used for comparison of independent quantitative data showing normal distribution. One-Way analysis of variance (ANOVA) was used for comparison between independent subgroups. Categorical data were evaluated by the chi-square test. Data were evaluated at 95% confidence interval and a p value of less than 0.05 was considered statistically significant. This research was approved by the Gülhane Military Medical Faculty's Ethics Committee (12.01.2015/50687469-1491-28-14/1648.4-66).

## Results

The average platelet count in HBsAg-positive patients and controls was $245.28\pm6078x10^3/mm^3$ (79-444) and $262.59\pm62.13x10^3/mm^3$, respectively (85-595), and the difference between the groups was statistically significant (p=0.002). There was a statistically significant difference also in RDW values between the two groups. The average RDW in HBV group and

control group was 12.14±1.05 and 12.49±1.28, respectively (p=0.001). However, no significant difference was observed in PDW and MPV between the groups (Table 1).

When we divided HBsAg positive subjects into chronic infection and inactive carriers groups, there was no significant difference between the groups, but we found a significant difference in platelet count between chronic infection group and the healthy group (p=0.009). In addition, RDW values showed significant difference between the inactive carrier group and the healthy group (p=0.003) (Table 2). On the other hand, there was no significant difference in MPV and PDW values between the groups.

## Discussion

HBV infection is a common disease worldwide, with a prevalence of over 8% in some regions, especially in Southeast Asia, Sub-Saharan Africa and Central America. It is estimated that 2 billion people worldwide are suffering from this infection and 350 million of them are carriers or chronically infected (14). HBV infection may appear in a wide range of spectrum between asymptomatic carriage and fulminant hepatitis requiring liver transplantation as well. Liver cirrhosis and HCC may develop later in patients who are followed up for chronic hepatitis in this process (15).

Platelets have an important role in the pathogenesis of local and systemic inflammation-related disorders. Thrombotic and inflammatory agents released from platelets can trigger disease-specific complications. Platelets are also closely associated with hemostasis, inflammation, immune cell activation, tissue regeneration and other physiological and pathological processes (16). It has been reported that MPV increased in patients with essential hypertension, hypothyroidism, obesity, coronary heart disease, and diabetes and in smokers (1).

RDW is an index showing the distribution of the sizes of circulating erythrocytes in CBC. RDW is used in the diagnosis of anemia and its elevation is observed in hemolysis and erythrocyte production disorders. RDW does not only increase in hematologic diseases, but also increases in pulmonary embolism, acute renal failure, pulmonary arterial hypertension, peripheral arterial disease and stroke. Since RDW has been shown to be correlated with inflammatory markers such as CRP, it is now accepted as a marker of inflammation (17).

Turhan et al. (1) reported statistically higher MPV values in 260 inactive hepatitis B patients compared to controls in their study. In another study of 59 patients with chronic hepatitis B, increased MPV was found to be associated with advanced fibrosis. It has been suggested that MPV might be useful in assessing fibrosis in patients with chronic hepatitis B (18).

In a study by Karabulut and Namlı (19), significant high values were found in the PDW parameter in HBsAg positive subjects, but no significant difference was found in MPV between HBsAg positive patients and controls. However, Ceylan et al. (4) reported that low MPV values and high PDW values were associated with severe liver fibrosis in patients with hepatitis B. Uluca et al. (10) found no difference in MPV values between inactive HBV carriers and controls.

In a study by Demircan et al. (20), significantly elevated MPV levels were detected in patients with HCV infection which was thought to be due to the effect of the virus leading to platelet dysfunction. Hakyemez et al. (21) reported no association between PDW value and severe cirrhosis. However, the association of advanced liver fibrosis with RDW, PLT and MPV was statistically significant in chronic hepatitis B cases. They declared that platelet-derived indices may play a critical role in monitoring liver fibrosis and cirrhosis progression. Karagoz et al. (13) found significantly high MPV and RDW values in HBV infected patients, and they declared that these parameters could be used in determining liver damage. In our study, there was no difference between the groups in terms of MPV and PDW values.

In a study by Lou et al. (12), high RDW values were reported to be associated with disease severity in hepatitis B patients. Therefore, this difference is an important factor affecting the progression of the disease and is considered an important marker in patients with HBV infection. In a recent study, RDW was found to be higher in HBV related cirrhotic patients than in chronic hepatitis B cases and healthy subjects (22).

In the present study, the average platelet count in HBsAg-positive patients and controls was 245.28±60.78x$10^3$/$mm^3$ and 262.59±62.13x$10^3$/$mm^3$, respectively and there was a statistically significant difference between the groups as expected. The average RDW was 12.14±1.05 in HBV infected group, while it was 12.49±1.28 in control group. This difference was observed between the HBV carriers and the controls. In contrast to many studies, lower RDW values were detected in hepatitis B cases.

**Table 1.** Relationship between hepatitis B surface antigen positive patients and healthy group

| Parameter | HBsAg (+) patients | Healthy group | p |
|---|---|---|---|
| Platelet x 1000 (K/uL) | 245.28±60.78 | 262.59±62.13 | 0.002 |
| Mean platelet volume (fL) | 8.64±4.29 | 8.54±1.03 | 0.724 |
| Platelet distribution width (%) | 12.14±2.7 | 12.02±4.06 | 0.704 |
| Erythrocyte distribution width (%) | 12.14±1.05 | 12.49±1.28 | 0.001 |

HBsAg: Hepatitis B surface antigen

**Table 2**. Relationship between inactive carrier, chronic infection and healthy group

| Parameter | Inactive carrier | Chronic infection | Healthy group | p |
|---|---|---|---|---|
| Platelet x 1000 (K/uL) | 247.97±55.30 | 241.75±67.40* | 262.59±62.13* | 0.009* |
| Erythrocyte distribution width (%) | 12.08±1.0** | 12.23±1.11 | 12.49±1.28** | 0.003** |

## Conclusion

It is envisaged that simple, inexpensive and also routinely used platelet and erythrocyte parameters in combination with other inflammatory parameters may help predict liver inflammation. While platelet count and RDW decreased in people with HBV infection, MPV and PDW did not change. We assume that platelets and RDW should be evaluated together in HBsAg positive patients, and further studies on this subject should be continued in larger groups.

### Ethics

**Ethics Committee Approval:** This research was approved by the Gülhane Military Medical Faculty's Ethics Committee (12.01.2015/50687469-1491-28-14/1648.4-66).

**Informed Consent:** The Declaration of Helsinki and Good Clinical Practice Guidelines were respected during the entire process of enrolling the patients in the study and collecting/analyzing/reporting the data.

**Peer-review:** Internally peer-reviewed.

### Authorship Contributions

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

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

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## References

1. Turhan O, Coban E, Inan D, Yalcin AN. Increased mean platelet volume in chronic hepatitis B patients with inactive disease. Med Sci Monit. 2010;16:202-205.
2. Bilgiç A, Özacar T. Hepatit B Virüsü. İçinde: Topçu AW, Söyletir G, Doğanay M. İnfeksiyon Hastalıkları ve Mikrobiyolojisi, 2. Baskı, İstanbul: Nobel Tıp Kitapevleri; 2002; s. 1350-1370.
3. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Chronic Hepatitis B Guideline Working Party of the Asian-Pacific Association for the Study of the Liver. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int. 2008;2:263-283.
4. Ceylan B, Mete B, Fincanci M, Aslan T, Akkoyunlu Y, Ozguneş N, Colak O, Gunduz A, Senates E, Ozaras R, Inci A, Tabak F. A new model using platelet indices to predict liver fibrosis in patients with chronic hepatitis B infection. Wien Klin Wochenschr. 2013;125:453-460.
5. Koda M, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. Fibroindex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. Hepatology. 2007;45:297-306.
6. Rossi E, Adams L, Prins A, Bulsara M, de Boer B, Garas G, MacQuillan G, Speers D, Jeffrey G. Validation of the fibrotest biochemical markers score in assessing liver fibrosis in hepatitis C patients. Clin Chem. 2003;49:450-454.
7. Adams LA, Bulsara M, Rossi E, DeBoer B, Speers D, George J, Kench J, Farrell G, McCaughan GW, Jeffrey GP. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clin Chem. 2005;51:1867-1873.
8. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518-526.
9. Yilmaz B, Aydin H, Can G, Şentürk Z, Üstüner B, Yilmaz H, Öztürkler M, Roach EC, Korkmaz U, Kurt M, Çelebi A, Şentürk Ö, Hülagü S. The relationship between fibrosis level and blood neutrophil to lymphocyte ratio in inactive hepatitis B carriers. Eur J Gastroenterol Hepatol. 2014;26:1325-1328.
10. Uluca U, Sen V, Gunes A, Tan I, Aktar F, Cubuk E, Sabaz MN. İnaktif Hepatit B Taşıyıcılarında Nötrofil Lenfosit Oranı ve Ortalama Trombosit Hacminin Değerlendirilmesi, Mustafa Kemal Üniversitesi Tıp Dergisi. 2015;6:8-13.
11. Gasparyan AY, Ayvazyan L, Mikhailidis DP, Kitas GD. Mean platelet volume: a link between thrombosis and inflammation? Curr Pharm Des. 2011;17:47-58.
12. Lou Y, Wang M, Mao W. Clinical usefulness of measuring red blood cell distribution width in patients with hepatitis B. PLoS One. 2012;7:e37644.
13. Karagoz E, Ulcay A, Tanoglu A, Kara M, Turhan V, Erdem H, Oncul O, Gorenek L. Clinical usefulness of mean platelet volume and red blood cell distribution width to platelet ratio for predicting the severity of hepatic fibrosis in chronic hepatitis B virus patients. Eur J Gastroenterol Hepatol. 2014;26:1320-1324.
14. Ağca H. Hepatit B Serolojisi. Altındiş M, Tabak F. Hepatit Mikrobiyolojisi,1.Baskı, İstanbul: İstanbul Medikal Yayıncılık; 2015; s. 76-82.
15. De Paschale M, Ceriani C, Cerulli T, Caqnin D, Cavallari S, Ndayake J, Zaongo D, Priuli G, Vigano P, Clerici P. Prevalence of HBV, HDV, HCV, and HIV Infection During Pregnancy in Northern Benin. J Med Virol. 2014;86:1281-1287.
16. Tanju C, Ekrem G, Berksoy Emel A, Nur A. Mean platelet volume as a negative marker of inflammation in children with rotavirus gastroenteritis. Iran J Pediatr. 2014;24:617-622.
17. Karakaş MF, Büyükkaya E, Kurt M, Büyükkaya Ş, Karakaş E, Akçay AB, Şen N. Kardiyak Sendrom X'de Eritrosit Dağılım Genişliği (RDW) ile Hs-CRP Seviyelerinin İncelenmesi. Abant Med J. 2013;2:17-22.
18. Ekiz F, Yüksel O, Koçak E, Yılmaz B, Altınbaş A, Çoban S, Yüksel I, Üsküdar O, Köklü S. Mean platelet volume as a fibrosis marker in patients with chronic hepatitis B. J Clin Lab Anal. 2011;25:162-165.
19. Karabulut N, Namlı MN. HBsAg pozitif hastaların trombosit indekslerinin değerlendirilmesi. ANKEM Derg. 2015;29:73-78.
20. Demircan F, Kılınç F, Gözel N, Şenateş BE, Şenateş E. The Evaluation of Mean Platelet Volume in Hepatitis C Infection, Viral Hepat J. 2014;20:11-14.
21. Hakyemez İN, Bolukçu S, Durdu B, Aslan T. Red Cell Volume Distribution Width to Platelet Ratio is an Important Predictor of Liver Fibrosis and Cirrhosis in Chronic Hepatitis B. Viral Hepat J. 2016;22:52-57.

# *Erratum*

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

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

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

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

**The corrected version**

**Results:** Among HCV RNA-positive 554 patients, 312 (56.3%) patients had genotype 1b, 127 (22.9%) - genotype 1a, and 94 (16.9%) patients had genotype 3a. A total of 10 samples were identified to be mixed genotype; 3 (0.5%) - genotype 4c/d, 3 (0.5%) - genotype 2a/c, 3 (0.5%) - genotype 1a/1b, and 1 (0.1%) - genotype 1b/4.

**Bulgular:** HCV RNA pozitif 554 hastada HCV genotiplerinin prevalansı genotip 1b: 312 (%56,3), genotip 1a: 127 (%22,9), genotip 3a: 94 (%16,9) ve genotip 4c/d: 3(%0,5), genotip 2a/c: 3 (%0,5), genotip 1a/1b: 3 (%0,5), genotip 1b/4: 1 (%0,1) olmak üzere 10 hastada (%1) mix tip olarak tespit edildi.