



Frequency of Hepatitis Delta Virus in Hepatitis B Surface-antigen-positive Patients

Hepatit B Yüzey Antijeni-pozitif Hastalarda Hepatit Delta Virüsünün Sıklığı

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ABSTRACT

Objectives: The prevalence of hepatitis delta virus (HDV) worldwide shows geographical differences. Although there are several studies on anti-HDV seroprevalence rates in hepatitis B surface antigen (HBsAg)-positive patients in Turkey, studies on HDV-RNA prevalence in this patient population are limited. It was aimed to detect the frequency of anti-HDV antibodies and HDV-RNA in HBsAg-positive patients in this study.

Materials and Methods: This retrospective study included 2089 HBsAg-positive patients in whom anti-HDV was analyzed between April 2015 and March 2017. Anti-HDV test was performed in serum samples obtained from HBsAg-positive patients by enzyme-linked immunosorbent assay. In anti-HDV-positive patients, HDV-RNA was analyzed in serum samples by real-time polymerase chain reaction.

Results: The seroprevalence of anti-HDV was 4.1% (85/2089), while the rate of HDV-RNA positivity was 2.4% (51/2089). HDV-RNA was detected in 60% (51/85) of anti-HDV-positive patients. The frequency of anti-HDV and HDV-RNA was highest in the 50-59 age group.

Conclusion: The frequency of HDV in this study was found to be consistent with regional data. HDV viremia was detected in only 60% (51/85) of the anti-HDV-positive patients. Since anti-HDV antibodies may remain after recovery, it is important to investigate HDV-RNA to determine the true prevalence of HDV.

Keywords: Hepatitis delta virus, anti-HDV, HDV-RNA, HBV

ÖZ

Amaç: Dünya genelinde hepatit delta virüsünün (HDV) yaygınlığı coğrafi farklılıklar göstermektedir. Her ne kadar Türkiye’de hepatit B yüzey antijeni (HBsAg) pozitif olan hastalarda anti-HDV seroprevalans oranları konusunda birçok çalışma olmasına rağmen, bu hasta popülasyonunda HDV-RNA prevalansı ile ilgili çalışmalar sınırlıdır. Bu çalışmada HBsAg pozitif olan hastalarda anti-HDV antikorları ve HDV-RNA sıklığının saptanması amaçlandı.

Gereç ve Yöntemler: Bu retrospektif çalışma, Nisan 2015 ve Mart 2017 tarihleri arasında HBsAg-pozitif hastalarda anti-HDV testi çalışılan 2089 hastayı kapsadı. HBsAg-pozitif hastalardan alınan serum örneklerinde anti-HDV testi, enzim bağlı immünosorbent metod kullanılarak yapıldı. Anti-HDV-pozitif hastalarda, HDV-RNA, gerçek zamanlı polimeraz zincir reaksiyonu ile serum örneklerinde analiz edildi.

Bulgular: Anti-HDV seroprevalansı %4,1 (85/2089) iken HDV-RNA oranı %2,4 (51/2089) idi. HDV-RNA, anti-HDV pozitif olan hastaların %60’ında (51/85) tespit edildi. Anti-HDV ve HDV-RNA sıklığı 50-59 yaş grubunda en yüksekti.

Sonuç: Bu çalışmada HDV sıklığı bölgesel verilerle tutarlı bulundu. HDV viremi, anti-HDV pozitif olan hastaların sadece %60’ında (51/85) tespit edildi. Anti-HDV antikorları iyileşmeden sonra da pozitif kalabileceğinden dolayı, HDV’nin gerçek prevalansını belirlemek için HDV-RNA’nın araştırılması önemlidir.

Anahtar Kelimeler: Hepatit delta virüs, anti-HDV, HDV-RNA, HBV

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Introduction

Hepatitis delta virus (HDV), only affects patients with hepatitis B virus (HBV) infection, is a defective hepatotropic virus, as it requires hepatitis B surface antigen (HBsAg) to gain entry into the cell (1). HDV, first discovered in 1977 by Mario Rizzetto, is RNA virus with a negative polarity single-stranded circular genome (2). According to the International Committee on Taxonomy of Viruses, HDV is classified in the genus delta virus (3).

The routes of transmission of HDV are similar to those of HBV. It can be transmitted via blood transfusion, intravenous drug use, sexual contact, sharing personal care items, and nosocomial routes. It has been reported that this infection can be transmitted among family members in countries with a high viral prevalence. In addition, intravenous drug use is important in the transmission of HDV in Northern Europe and in countries where HDV prevalence is low (4,5). Globally, countries are classified for asymptomatic HBV carriers as very low endemicity areas for HDV rates of 0-2%, low for 3-9%, medium for 10-19%, and high endemic areas for >20%; while in chronic HBV-infected patients, it is classified as for <1%, 3-9%, 30-60%, and >60%, respectively. Turkey is considered a medium endemic area for HDV infection with regional differences (6,7).

HDV infection occurs always in association with hepatitis B infection because of HBV dependence of HDV. The clinical course of HDV infection varies from acute self-limiting infections to fulminant hepatitis. Chronic liver infection can cause end-stage liver disease-related complications such as rapid progression of fibrosis, hepatic decompensation, and hepatocellular carcinoma (1). Simultaneous occurrence of HBV and HDV infections is considered coinfection, while the subsequent HDV infection in a HBsAg-positive person is named as superinfection (8). Chronicity rate in coinfection and superinfection is 2-20% and 90%, respectively (9).

Detection of anti-HDV total, immunoglobulin (Ig) G or IgM antibodies in serum or plasma, HDV antigen in serum, liver biopsy and molecular methods are used for the diagnosis of HDV infection. Anti-HDV positivity does not always reflect the presence of active HDV infection. Detection of HDV-RNA is the most sensitive method for assessing active HDV infection (10).

Studies on HDV-RNA are limited, while there are many anti-HDV seroprevalence studies in HBsAg-positive patients in Turkey. Thus, it was aimed to detect the frequency of anti-HDV and HDV-RNA in HBsAg-positive patients in a reference university hospital in Istanbul, Turkey.

Materials and Methods

This retrospective study included 2089 HBsAg-positive patients who were analyzed for anti-HDV between April 2015 and March 2017 at the Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology, Division of Virology and Fundamental Immunology, Istanbul, Turkey. This study was approved by the Ethics Committee of Istanbul University, Istanbul Faculty of Medicine (approval number: 2017/651/11). This study was carried out in accordance with the principles of the Helsinki Declaration.

Anti-HDV and HBsAg tests were studied in serum samples using micro-ELISA kits (Dia. Pro, Diagnostic Bioprobes, Milano,

Italy) on a Triturus analyzer (Grifols, Parets del Valles, Spain). The positive and negative control samples were included in each run. Extraction of HDV-RNA was performed using a High Pure Viral Nucleic Acid Kit (Roche Applied Science, Basel, Switzerland) or EZ1 virus mini kit V2 (Qiagen, Germany). Extraction of HBV-DNA was performed using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's recommendations. HDV-RNA is firstly transcribed into cDNA using the transcriptor first strand cDNA synthesis V6 kit (Roche Diagnostics, Mannheim, Germany), and then cDNA was amplified on LightCycler 2.0 real-time polymerase chain reaction (PCR) (Roche Diagnostics GmbH, Switzerland) or Rotor-Gene Q (Qiagen, Germany). HBV-DNA was amplified by real-time PCR on Rotor-Gene Q (Qiagen, Germany).

Biopsy results of only 37 anti-HDV-positive patients could be included in this study between April 2015 and March 2017. The histological activity index and fibrosis were assessed by the modified Knodell scoring system.

Statistical Analysis

SPSS 21 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The suitability of the variables to the normal distribution was examined via visual methods (histogram and probability plots) and the Kolmogorov-Smirnov test. Difference in mean age between the genders was analyzed using the Mann-Whitney-U test, and the frequency of HDV-RNA positivity was compared between age groups and genders by the Pearson's chi-square test. A p value of less than 0.05 was considered statistically significant.

Results

Of the 2089 patients in this study, 1180 (56.5%) were male and 909 (43.5%) were female. The mean age of the patients was 46.3 ± 15.3 years (1-97). The mean age of male and female patients was 46.3 ± 15.3 and 46.4 ± 15.2 years, respectively. There was no statistically significant difference in mean age between genders ($p=0.79$). The frequency of anti-HDV positivity in all patients was 4.1% (85/2089). Of the anti-HDV positive patients, 42 (49%) were male and 43 (51%) were female and there was no difference in anti-HDV positivity between genders ($p=0.18$). The mean age of the anti-HDV-positive patients was 49.5 ± 11.1 years. When the distribution of anti-HDV-positive patients with respect to age groups was examined, a significant increase was found in the 50-59 years age group ($p=0.02$) (Figure 1).

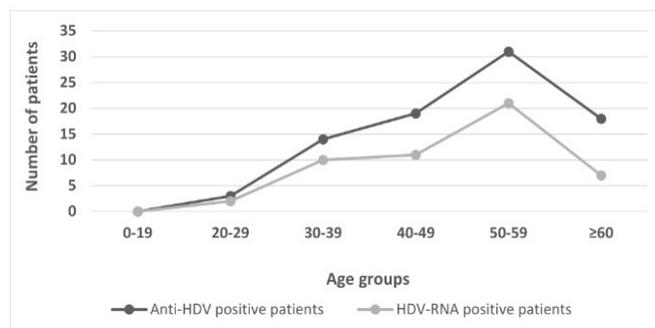


Figure 1. Distribution of anti-HDV and HDV-RNA positive patients according to age
HDV: Hepatitis delta virus

HDV-RNA was detected in 51 of 85 anti-HDV-positive patients. The frequency of HDV-RNA positivity was 2.4% (51/2089). The mean age of the HDV-RNA-positive patients was 48.7 ± 11.5 years. Of the HDV-RNA-positive patients, 27 (53%) were male and 24 (47%) were female. There was no difference in HDV-RNA positivity between the genders ($p=0.41$). When the distribution of the HDV-RNA-positive patients was examined according to age groups, it was observed that the number of patients was highest in the 50-59 age group, but the difference was not statistically significant ($p=0.23$). The demographic and laboratory characteristics of the HDV-RNA-positive patients were shown in Table 1. Median HBV-DNA viral load (20 IU/mL) in HDV-RNA-positive patients was lower than in HDV-RNA-negative patients (62 IU/mL), but no statistically significant difference was found ($p=0.09$).

Table 1. The demographic and laboratory characteristics of patients with hepatitis delta virus-RNA positive

		HDV-RNA positive patients (n=51)
Age (years) (mean \pm Standard deviation)		48.7 \pm 11.5
Gender n (%)	Female	24 (47%)
	Male	27 (53%)
HDV-RNA viral load (Copy/mL) (median)		39200 (IQR: 1250-356476)
HBV-DNA viral load (IU/mL) (median)		20 (IQR: 20-45)
HBV-DNA (n)	Positive	28
	Negative	23
Liver biopsy*	Fibrosis score (0-6) (median)	3
	Histological activity index (2-16) (median)	8

*The liver biopsy was evaluated by Modified Knodell scores
HDV: Hepatitis delta virus, IQR: Interquartile range, (range between the 25th to 75th percentiles), HBV: Hepatitis B virus

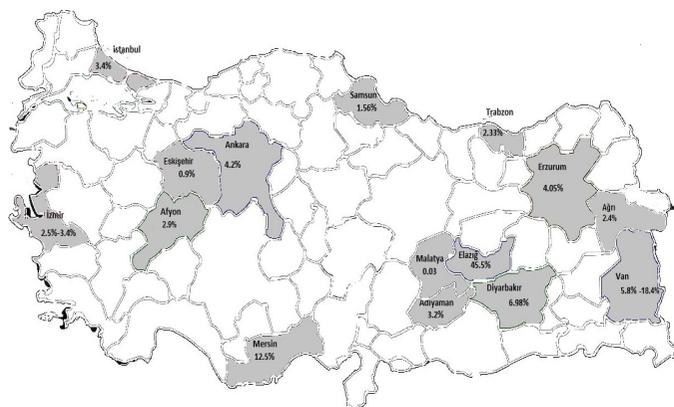


Figure 2. Anti-HDV seroprevalence in several areas of Turkey

Discussion

Despite the widespread application of HBV vaccine, HDV infection continues to be a global health problem, even in some developed countries, including some European countries, the US and Australia (1). The World Health Organization reported that there were globally 240 million people chronically infected with HBV, and about 15 million individuals chronically infected with both HDV and HBV. Globally, it is estimated that 5% of HBsAg-positive people are coinfecting with HDV (11). Epidemiological studies indicate that the rate of anti-HDV positivity is lower in the Far Eastern countries with high HBV endemicity and higher in the Mediterranean countries with moderate HBV endemicity (12). As in the world, HDV frequency also shows regional differences in Turkey (Figure 2) (12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28). Higher rates were reported in the Eastern and Southeastern Anatolian regions. Regional studies on seroprevalence of anti-HDV in Turkey showed a rate of 2.5% to 3.4% in the Western Anatolia region, 0.9% to 15% in the Central Anatolia region, 0.3% to 45.5% in the Eastern Anatolia region and 3.2% to 6.98% in the Southeastern Anatolia region. The reported rate of anti-HDV seroprevalence in studies performed in Turkey varies between 0.3% and 45.5% showing regional differences (21,28). In a study conducted in Istanbul, the frequency of anti-HDV positivity in patients with chronic HBV infections was 3.4% (46/1339) (13). There are limited studies on the prevalence of HDV in Istanbul. In this study, anti-HDV seroprevalence was found to be 4.1% (85/2089) and HDV-RNA positivity was 2.4% (51/2089) in 2089 HBsAg positive patients. The cause of detection of lower rates of HDV-RNA compared to anti-HDV may be related to the fact that anti-HDV antibodies may remain after recovery.

There are a limited number of HDV-RNA studies reflecting the true HDV prevalence in Turkey. The frequency rate of HDV-RNA positivity in 547 HBsAg-positive patients was found to be 0.9% in a study conducted in the Central Anatolia, Turkey (17). In the Western region of Turkey, the rate of HDV-RNA positivity in 88 patients was found to be 2.3% (13). In a study conducted in 180 patients in Southeastern Anatolia, HDV-RNA positivity was detected in only two patients (27). In their study including 282 patients performed in Eastern Anatolia, Bahcecioglu et al. (28) reported that 23.4% of the subjects were HDV-RNA-positive. In this study, the frequency of HDV-RNA positivity was 2.4% (51/2089). Immunization with hepatitis B vaccine and the exclusion of high-risk blood donors before blood donation may be associated with low prevalence rates. High prevalence rates may be related to the fact that the studies were conducted in regions where HBV is high endemic and in patients with chronic HBV infection.

In a study performed in Romania, anti-HDV positivity was found in 223 (20.4%) of 1094 patients with chronic HB infection. Hepatitis D viremia was detected in 67.7% of these patients (29). In another study conducted in Romania, anti-HDV IgG seroprevalence was found 23.1% in 2761 HBsAg-positive patients, whereas HDV-RNA was positive in 16.4% of these patients (30). The rate of anti-HDV seroprevalence was reported as 7.7% in 169 chronic HBV cases in Saudi Arabia, whereas only four patients were HDV-RNA-positive (31). HDV-RNA is an important parameter for determining the true HDV prevalence, because anti-HDV may remain positive for many years after infection, or antibodies against HDV infection may not

be detected in the window period. In this study, the anti-HDV positivity rate was 4.1% and the HDV-RNA positivity rate was 2.4% in 2089 HBsAg-positive patients. In Turkey, although the rates have decreased in the Western Anatolia region, the prevalence of HDV infection is still at high levels in the Eastern Anatolia region. However, the decline in these rates may be related to sociocultural and socio-economic improvements, good laboratory practices in the screening of blood and blood products.

Long-term studies have shown a decrease in HDV seroprevalence in some endemic regions. Infection rates have decreased especially in young patients. HDV infections in Italy have been reported to be limited to those infected around the 1980s. As well as improvements in socio-economic and hygiene conditions, media campaigns for mass vaccination programs are thought to play a role in this decline in incidence rates in Italy (32). It has been reported that there was also a decrease in prevalence rates in Spain, India, Taiwan and Turkey (33,34,35).

In the present study, the mean age of the anti-HDV-positive patients was 49.5±11.1 years, and a significant increase in anti-HDV positivity was detected in the 50-59 age group. Similar results were found in studies conducted in our country (14,36). Unlike this study, in a study conducted in the UK, the median age of 82 anti-HDV-positive patients was 36 years. The low median age was thought to be related to migration from endemic regions and intravenous drug use (37). In another study examining the anti-HDV seroprevalence in 362 patients aged between 4 and 70 in Pakistan, 212 patients (58.6%) with a mean age of 29.75±11.27 years were found to be anti-HDV-positive. The higher rates of anti-HDV positivity in young adults were thought to be associated with injectable drug abuse and the use contaminated needles for therapeutic injections in this age group (38). Sexual transmission may be another possible route that leads to these higher prevalence rates (38). In this study, high anti-HDV positivity rates in older ages may be related to the vaccination program implemented in Turkey for more than 20 years and the family screening programs for patients with hepatitis B.

This study has some limitations; due to its retrospective design, the clinical data of the patients and data regarding the clinical course of HBV infection could not be obtained.

Conclusion

The anti-HDV rates detected in this study were consistent with regional data in Turkey, but lower than in studies conducted in the Eastern region of the country. In addition, HDV viremia was detected in only 60% (51/85) of anti-HDV-positive patients. It is important to investigate HDV-RNA to determine the true prevalence of HDV because anti-HDV antibodies may remain after recovery.

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Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Istanbul University, Istanbul Faculty of Medicine (approval number: 2017/651/11).

Informed Consent: Retrospective study.

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Authorship Contributions

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

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