

Evaluation of Patients with Hepatitis Delta Virus Infection at First Admission in Izmir, Turkey

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Abstract

Objective

To present the clinical and laboratory findings of patients with hepatitis D virus (HDV) at first admission to hospital.

Design

This retrospective study was conducted on patients with HDV infection at a State Hospital in Turkey.

Setting

Department of Infectious Diseases, Turkish Ministry of Health, Karsiyaka State Hospital, Izmir, Turkey.

Subjects

Some 35 982 samples were tested between 2004 and 2010; HBsAg-positive samples were subsequently tested for anti-HDV antibodies.

Main Outcome

Data of patients with anti-HDV seropositivity recorded at first admission.

Results

Among 1216 detected chronic HBV infections, a total of 26 (2.1%) cases of seropositive anti-HDV were diagnosed. Twelve of the study group were men, 14 were women. The mean age was 43.30 years (range, 24-79 years). Hepatitis B surface antigen (HBsAg) was positive in all patients, and hepatitis B envelope antigen (HBeAg) was negative in 19 (73.1%). HBV DNA levels were above 2000 IU/mL in six (23.1%) patients, whereas it was below 2000 IU/mL in the remainder. The mean alanine aminotransferase (ALT) and alpha-fetoprotein levels were 59.3 IU/mL (range, 21-152 IU/mL) and 1.9 IU/mL (range, 1.1-3.8 IU/mL), respectively. Liver biopsy was performed in 12 (46.2%) patients in the study group. The mean fibrosis score was 1.9 and the mean histologic activity index was 9.1.

Conclusion

Although HDV super infection has been associated with more severe liver disease and accelerated progression to cirrhosis, moderate fibrotic activity and histologic scores were seen at the first admission at our institution. Therefore, delta virus infection should be considered in patients with mild ALT elevation, even if HBV DNA levels are below 2000 IU/mL.

Keywords: Delta virus; HDV infection; HBV infection

Introduction

Hepatitis Delta Virus (HDV) infections always occur in association with hepatitis B virus (HBV) infections because of their dependence upon the HBV. The hepatitis delta virion is composed of an outer

lipoprotein envelope made of the surface antigen of HBV (HBsAg) and an inner ribonucleoprotein structure in which the HDV genome resides [1]. Due to the dependence of HDV on HBV, the presence of HBsAg is necessary for the diagnosis of HDV infection.

HDV causes both acute and chronic infections [1-3]. Asymptomatic and non-progressive illnesses are seen in a minority of cases, but a severe and progressive route to cirrhosis is present in most [4-6]. Available data suggest that approximately 5% of HBV carriers worldwide may be infected with HDV; it is estimated that there are 18-20 million people chronically infected with HDV worldwide [7]. However, the epidemiologic distribution of HDV infection does not parallel that of HBV, because areas endemic for HBV may be almost HDV free. The level of HDV endemicity is partly related to the route of transmission.

HDV infections are common in Southeast Asia, Eastern Europe, and South American countries; however, it is seldom found in the United States of America and parts of Europe [8]. Nevertheless, after a dramatic decrease in the seroprevalence of HDV infections in Europe, studies have shown that the seroprevalence of anti-HD in Italy among HBsAg carriers is again similar to that found in 1997 (9.7%) [9]. The virus has remained endemic in the Middle East, Central Africa, Mongolia, Tajikistan, and northern parts of South America, but data are lacking from many areas where hepatitis B is highly prevalent [1].

A previous comprehensive study of blood donors in Turkey reported an HBsAg carrier rate between 4.19% with significant differences in prevalence rates between the east and west of the country [10]. Moreover, Turkey's neighbouring countries in Eastern Europe and the Middle East also have high a prevalence of HDV. As with its neighbours, Turkey is in a high prevalence zone for both HBV and HDV infections. Accordingly, HDV is a problem in developing countries and throughout the world.

Although HDV is common in the Turkish population, there is a lack of comprehensive data related to prevalence. Most of the published data are in abstract form only in the English literature. Sufficient and comprehensive studies are still lacking. Furthermore, most studies have not included patients' clinical and laboratory findings. In this study, we investigated seroprevalence rates and clinical and laboratory findings of HDV infection among patients with HBV infection who were admitted to hospital for a first examination.

Subjects and Method

The Karsiyaka State Hospital, Izmir, Turkey, has a 250-bed capacity and its catchment area is the northern region of Izmir. With surgical, medical, and intensive care unit facilities, it serves more than 50 000 people who live in the Izmir region.

This retrospective study was conducted with patients with Hepatitis B virus infection from Izmir at the Department of Infectious Disease, Karsiyaka State Hospital, between 2004 and 2010. Only patients from the Izmir region were included in the study. All patients with chronic HBV infection were screened for HDV infection during the first examination and also at subsequent periods of elevated transaminase levels. Patients who were found to have HDV infection at the first examination were included in this study. All patients with HBV were carriers of inactive HBsAg or patients with chronic HBV infections. Patients with cirrhosis were excluded from this study. Patients with HBV were subdivided into two categories, patients with normal transaminase levels (group I), and those with permanently or intermittently increased transaminase levels (group II). HBV DNA levels were also recorded.

All patients were evaluated with physical examinations; laboratory biochemical and serologic tests including HBsAg, anti-HBs, anti-

HBcIgM, anti-HBcTotal, HBeAg, anti-HBe, anti-HDV antibodies; and ultrasonographic imaging. Liver biopsies were taken from patients with high aminotransferase levels or from those who were considered as having chronic hepatitis B infection. The first admission data of the patients with HDV infection were recorded. Anti-HDV antibodies (Ab) were tested using micro enzyme-linked immunosorbent assay (ELISA) (Murex anti-delta, Abbott, Portugal) for diagnosis of HDV. HDV RNA was determined only in patients with positive anti-HDV test results. Detection of HDV RNA was performed using qualitative reverse transcriptase polymerase chain reaction (PCR) amplification (ICycler IW Real-Time PCR; BioRad) using commercially available test kits (HDV Real-Time PCR, HDV QLP 1.0; Iontec, Istanbul, Turkey) in accordance with the manufacturers' instructions. The lower limit of detection of PCR was approximately 100 copies/mL. Patients who had not been screened for HDV were excluded from the study.

The clinical diagnosis was based on liver biopsy data if a biopsy had been performed. If such data were not available, the diagnosis was based on the presence of fluctuating or persistently high (>6 months) aminotransferase levels [upper limit of normal: 40 U/L for aspartate aminotransferase (AST) and 45 U/L for alanine aminotransferase (ALT)]. In the absence of clinical-, biochemical-, or ultrasound markers of cirrhosis, the diagnosis of HDV was based on the presence of serologic test results.

The Institutional review board (IRB) protocol and the consent form process were approved by the Institutional Review Board.

For the statistical analysis, SPSS 12.0 for Windows (SPSS for Windows, version 12.0; Statistical Package for Social Sciences Inc., Chicago, Illinois, USA) was used. Comparisons between the groups were performed with t-test, Mann-Whitney U test or Chi-square tests. The significant level was set at $P < 0.05$.

Results

Among the 1216 outpatients, 436 patients with HBV were included in this study. Of these, 191 (43.8%) were female and 245 (56.2%) were male. Two hundred thirty (52.8%) of those were in group I, and 206 (47.2%) were in group II, in whom transaminase levels were permanently high in 74, and ALT levels were intermittently high in 132.

Anti-HDV antibody was detected in 26 (6%) patients at their first presentation to hospital. According to the patient groups, anti-HDV seropositivity was found as 2% (5/230) in group I; 4% (3/74) in group II who had permanently increased ALT levels, and 14% (18/132) who had intermittently increased ALT levels ($p < 0.05$). The HBV DNA levels were more than 2000 IU/mL in all patients with HDV. Of all the patients with HBV infections, anti-HDV seropositivity was found as 6% (26/436) (Table 1). The mean HBV DNA level was higher in group I than in group II ($2 \times 10^5 \pm 4 \times 10^2$ vs $6 \times 10^3 \pm 3 \times 10^2$, $P = 0.01$, respectively). Conversely, the mean HDV levels were higher in group II compared with group I ($6 \times 10^3 \pm 80$ vs $3 \times 10^2 \pm 40$, $P = 0.06$, respectively).

Groups	Patients		Anti-Delta Positive	
	No	%	No	%
Inactive HBsAg Carriers	230	53	5	2
Chronic HBV	206	47	21	10
1st group*	74	17	3	4

2nd Group**	132	30	18	14
TOTAL	436	100	26	6
*: The patients who had normal transaminase levels .				
**: The patients who had continuously or intermittently increased transaminase levels, HBV: Hepatitis B Virus				

Table 1: Anti-HDV Seropositivity in HBV Infected Patients

Patients No	HBeAg	HBV DNA	HDV RNA	Fibrosis	HAI*	ALT	AFP
1	Positive	3x103	2x102	1	5	123	0,8
2	Positive	2x104	5x103	2	14	135	3,8
3	Positive	5x103	1x104	4	13	142	2,3
4	Positive	3x104	3x102	1	11	165	Unknown
5	Positive	1x105	3x103	3	14	76	1,6
6	Negative	2x104	6x102	1	9	60	3,3
7	Negative	2x105	Negative	3	14	117	3,6
8	Positive	6x103	5x102	3	14	217	3,4
9	Positive	8x103	4x104	1	9	122	Unknown
10	Positive	4x103	2x102	Cirrhosis		68	3,6
11	Positive	8x104	7x103	4	9	98	4,9

*HAI: Histologic Activity Index, ALT: Alanine aminotransferase, AFP: Alkaline phosphatase

Table 2: The findings of HDV patients who underwent liver biopsy

Discussion

Data on HDV epidemiology have mostly been sourced in chronic HBV carriers superinfected with HDV, in whom HDV infection had progressed to chronicity. Anti-HDV is present in high titers in these patients, and the prevalence of chronic HDV infection can be usually determined through screening. Mostly chronic HDV infection signs and symptoms are present when HDV infection is detected in HBV carriers. There is little information regarding these patients in the early period before the onset of signs and symptoms.

In our study, we aimed to determine HDV seroprevalence among HbsAg carriers admitted to hospital for the first time, and to reveal the clinical and laboratory findings in this patient population; infection of HDV in real-life situations.

In our study, moderate fibrotic and histologic scores were seen in the patients with HDV super infection at first admission; however, HDV super infection has been associated with more severe liver disease and accelerated progression to cirrhosis in most studies.

Several epidemiologic studies suggest that very high prevalence of HDV infection occurs in hyper-endemic regions of the world. The prevalence of HDV was reported 58.6% among 362 HbsAg-positive patients in Pakistan.[5] In a study from Iran, the seroprevalence of HDV was 9.3% among 847 patients with HBV, and as high as 12.7% in patients with chronic hepatitis B.[6] Similarly, the seroprevalence of HDV was about 10% in patients with increased ALT levels when they

Among the 26 patients with HDV, 12 (46%) were male and 14 (54%) were female. The mean age was 43.30 years (range, 24-79 years). HBsAg was positive in all patients, whereas HBeAg was negative in 19 (73%).

The mean alanine aminotransferase (ALT) and alpha-fetoprotein levels was 59.3 IU/mL (range, 21-152 IU/mL) and 1.9 IU/mL (range, 1.1-3.8 IU/mL), respectively. Among the study group, 11 (42%) patients underwent liver biopsy. The mean fibrosis score was 1.9 and the mean histologic activity index was 9.1 (Table 2).

first presented to the hospital. In concert with our results, there is a gradually decreasing trend in HDV seroprevalence rates in European countries. Study results from Germany, France, Spain, Greece, and Turkey have shown a reduction in the prevalence of patients with anti-HDV antibodies (anti-HD) among hepatitis B surface antigen (HBsAg) carriers.[11] The prevalence of HDV infection in Turkey has decreased in recent years as a result of more successful control of transfusion-transmitted infections, diagnostic developments, and increased levels of education. On the other hand, the effect of universal HBV vaccination, which started in Turkey after 1994, is expected to affect prevalence figures in years to come [11,12].

In several studies from Turkey, seropositivity of anti-HDV was reported between 0-11.2% in inactive HBsAg carriers, and 6.8-53.4% among patients with chronic HBV.[3] However, anti-HDV seropositivity was found as 15% in West Anatolia and 35% in East and Southeast Anatolia in another study.[4] It seems that our results were below the mean value of the general population. This may be related to the national HBV vaccination program, which gradually increased from 2000. The vaccination program, along with active blood donation and education of the population could be effective in decreasing seroprevalence. Another explanation could be related to the high sociocultural status and low endemicity of HBV infection in Izmir, a coastal region in the west of the Turkey, compared with other parts of the country. Perhaps more importantly, most of the patients in this study were inactive HBsAg carriers. When considered with previous

studies, the seropositivity rates of anti-HDV in our study are among the reported results in inactive HBsAg carriers.

We detected the prevalence of HDV in inactive HBsAg carriers as 2%. In a study by Degertekin et al [13], the mean anti-hepatitis D virus was reported as 4.0% in inactive HBsAg carriers in Turkey. The authors showed that the rate of inactive HBsAg carriers significantly decreased from 1980 to 2005 (4.1% and 2.9%, respectively). On the other hand, it is well known that HDV superinfection may progress silently because HDV can remain in inactive HBsAg carriers even if transaminase levels are low. This is particularly important among unidentified patients who are infected with HDV and have low transaminase levels.

Enzyme-linked immunosorbent assay is the most readily available assay for the diagnosis of HDV infection, and detection of total anti-HDV. In the acute form of hepatitis D, anti-HDV appears very late and may be missed if repeated testing is not performed. Thus, the real incidence of acute hepatitis D may be underestimated. This is especially true in immunodeficient patients (e.g., anti-HIV-positive), in whom a strong antibody response to HDV may be delayed or absent. Furthermore, after resolution of acute hepatitis D, anti-HDV may disappear with time. Thus, recognition of past HDV infection may be impossible. On the other hand, HDV superinfection of a chronic HBsAg carrier may present as typical severe acute hepatitis in a previously unrecognized HBV carrier, or as an exacerbation of preexisting HBV infection. Progression to chronic HDV infection occurs in almost all patients.[2] However, HBV replication is usually suppressed by HDV. In some circumstances, HDV superinfection is diagnosed in patients with chronic HBV who have moderately elevated transaminase levels,[13] which could explain the moderately elevated levels in most of our patients.

The fluctuating nature of HBV DNA levels in chronic HBV infection is common. During this fluctuation, transaminase levels may decrease and then increase. A recent study on HDV/HBV viremia also reported that patients with low HBV DNA and HDV RNA with delta hepatitis showed no major differences in biochemical activity and histologic grading and staging when compared with patients with high HDV and HBV or with high HBV and low HDV replication.[7] In our study, the prevalence rate of anti-HDV in inactive carriers was found as 2%. This finding suggests that HDV infection may be present in patients with HBV with low transaminase levels.

The disease is asymptomatic and not progressive in a minority of cases, but has a severe and rapidly progressive course to cirrhosis in most patients.[4-6] In our study, liver biopsy was performed in 11 patients and a high fibrotic score (4 and above) was found in two patients with high transaminase levels. HDV was detected in only one patient with cirrhosis. Unfortunately, we cannot comment further on this aspect because the number of patients who underwent liver biopsy in our study was too small.

There are some limitations in this study. First, this was a retrospective study and our results included available data on the sero-epidemiology of delta hepatitis in a small part of Turkey, Izmir. Hepatitis D is an important medical problem in Izmir and throughout Turkey. However, we believe that the results of Izmir provide a basis for Turkey's cosmopolitan society. The second limitation was the lack of

long-term data of the patients in this study. The scarcity of long-term follow-up for these patients may be because of migration or because patients chose another institution for their treatment. Another limitation was that liver biopsies were not performed in all patients with HDV; some patients may not have consented to biopsy because of its invasive nature.

Conclusion

The results of this study indicate that HDV continues to be a medical problem in Turkey. The key findings to arise from this study are that (i) the seroprevalence of HDV is not common in Izmir compared with previous studies conducted in other parts of Turkey; (ii) HDV is found in patients with chronic HBV infection and continues to be an important problem with inactive HBsAg carriers. For this reason, uninterrupted efforts need to be made to identify HDV infections among patients who are HBsAg positive to control transmission and to establish the most effective treatment.

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