



The Prevalence of Hepatitis Delta Virus in Patients with Chronic Hepatitis B and Its Association with Risk Factors

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Abstract

Background: Hepatitis B infection is a serious health problem and two billion people worldwide are infected with the virus. The hepatitis delta virus (HDV) is a satellite virus. Hepatitis D virus infection in HBsAg carriers can be present as a simultaneous and acute infection.

Objectives: The aim of this study was to evaluate the frequency of HDV in patients with chronic hepatitis B and its association with risk factors.

Methods: In this descriptive study, 74 patients with chronic HBV infection were selected from patients that had referred to the Clinical Lab of Blood Transfusion Organization. All patients were positive for HBsAg for more than six months and anti-HBc. All samples were negative for HIV and HCV. An anti-HDV test was performed on HBsAg-positive specimens by the enzyme linked immunosorbent assay (ELISA) method. Also, HBV-real-time polymerase chain reaction (PCR) testing was done to determine the viral load.

Results: In this study, 74 HBsAg positive patients with a mean age of 50.22 ± 15.09 years were studied. Five (6.8%) patients had anti-HDV antibodies. Furthermore, 60% of the patients with HDV had risk factors, such as addiction, family history of hepatitis B, and a history of surgery. Maximal viral load in plasma samples of patients with anti-HDV antibodies, 531 IU/mL, was determined.

Conclusions: For prevention of HDV transmission, all patients of chronic hepatitis B with low-level viral load should be evaluated for hepatitis D infection. Also, for determining the relationship between HDV infections with its risk factors, another study with a larger sample size should be performed.

Keywords: Hepatitis B, Hepatitis Delta Virus, Prevalence

1. Background

Hepatitis B virus (HBV) B is a DNA virus belonging to the Hepadnaviridae family. Hepatitis B infection is a serious health problem and two billion people worldwide are infected with this virus and 350 million people are infected with the chronic infection (1). Hepatitis delta virus (HDV) is a satellite virus and a single-stranded RNA virus that belongs to the delta viride family. Delta antigen was identified by Rizzetto et al. in patients with hepatitis B during year 1977 (2). Hepatitis delta virus requires the surface antigen of hepatitis B virus to replicate and transmit. Hepatitis D virus infection in HBsAg carriers can be found as a simultaneous and acute infection. This infection simultaneously leads to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (3). Currently, eight genotypes have been described for HDV, HDV-1 to HDV-8, with the exception of HDV-1; all genotypes are found in distinct and different geographic regions. Most studies in Iran have reported on

the HDV-1 genotype (4, 5).

Hepatitis delta virus is mainly transmitted through injections. The highest transmission rate is found among injecting drug users and those exposed to contaminated blood or its products. In addition, intra-familial transmission is further described in high endemic areas of sexual transmission. Acute hepatitis is a result of co-infection of HBV and HDV, which is usually associated with recovery from both HBV and HDV infections. However, infection with HDV in patients with HBV (super-infection) usually causes chronic hepatitis due to both viruses. In comparison with patients with chronic hepatitis B, patients with chronic hepatitis B and D are significantly more likely to have cirrhosis and hepatocellular carcinoma. More than 350 million people are infected with chronic hepatitis and 15 to 20 million people are infected with HDV. Furthermore, HDV is endemic in Mediterranean countries, the Middle East, Central Africa, and the northern part of South America (1).

Patients that had both chronic HBV and HDV infections developed two- and three-fold higher cirrhosis and hepatocellular complications, respectively, and showed more than two folds increase in mortality compared to those with HBV only. The severity of liver disease caused by combined infection with HBV/HDV is often heavier than the HBV infection alone and is linked to HDV genotype and viral load (6-9). Vaccination against hepatitis B was started in 1989 in Zanjan and Semnan provinces of Iran. National vaccination began for all infants and high-risk groups since 1993 in Iran. Studies have shown that the prevalence of hepatitis B virus in 2010 declined dramatically throughout Iran (10). Thus, the reduction in the prevalence of hepatitis B virus is expected to have an effect on reducing the prevalence of HDV.

2. Objectives

The aim of this study was to evaluate the frequency of HDV in patients with chronic hepatitis B and its association with risk factors.

3. Methods

In this cross-sectional study, from individuals that had referred to the Clinical Lab of the Tehran Blood Transfusion Organization, 74 patients with chronic HBV infection having positive HBsAg test results for more than six months and positive anti-HBc test by ELISA (Siemens ELISA Marburg/Germany), were included in the study. Also, all patients that had HCV, HIV-1+, and HTLV-1+ results were excluded. All participants completed a questionnaire including age, gender, marital status, occupation, education, or other risk factors. All participants completed the consent form. This study was approved by the Medical Ethics Committee of the Semnan University of Medical Sciences under the following code IR.TMI.1394.6.1.

About 6 mL whole blood samples were collected in tubes containing potassium ethylene diamine tetra-acetic acid (EDTA) anticoagulant. The samples were centrifuged at 4°C for 10 minutes at 2700 rpm and the plasma was isolated and used to extract genomic DNA. An anti-HDV test was performed on HBsAg positive specimens by ELISA (DIA.PRO). Positive cases were detected according to the kit's instructions ($\text{cut-off} = (\text{NC} + \text{PC}) / 5$; OD = 450 nm; positivity of > 1.1). To determine the viral load, after DNA extraction by QIAamp DNA mini kit (Qiagen, Hilden, Germany), real-time PCR was performed using HBV Real Star kit (Altona Diagnostics, Germany). The test was performed according to the kit's instructions, and the viral load was calculated based on IU/mL. Statistical analysis was performed

using SPSS software version 23 and chi-square test for data analysis.

4. Results

In this study, from 74 patients, 48 (64.9%) were males and 26 (35.1%) females with an age range of 23 to 86 years and mean age of 50.22 ± 15.09 years. Five patients, about 6.8% of patients, had anti-HDV antibodies. The frequency of anti-HDV was four times greater in males than females and all patients with HDV were over 40 years of age. About 60% of patients with HDV had risk factors, including addiction, familial hepatitis B infection, and history of surgery. The maximal viral load was determined in plasma samples of patients with anti-HDV antibodies, 531 IU/mL.

The five patients with anti-HDV had risk factors, such as a family history of HBV, a history of injecting drug use, a history of surgery and hospitalization. The relationship between anti-HDV antibody and age, gender, job, injecting drug addiction, HBV familial history, suspected sexual relationship, surgery, etc. were statistically analyzed. There was no significant relationship between anti-HDV and risk factors (Table 1).

5. Discussion

The epidemiology of HDV is associated with the frequency of HBV infection due to the dependence of hepatitis D virus on the hepatitis B virus. Hepatitis delta virus is an important issue for general health in endemic areas, including Iran. The prevalence of HDV in carriers of HBsAg has been reported around the world at 5% (11). Different reports have been made on the prevalence of HDV in HBsAg positive patients in different regions of Iran. High prevalence of HDV in some regions of Iran, such as Hamedan (17.3%) and in Khuzestan (11.5%), and low HDV prevalence in areas, such as Babol (2%) and Isfahan (2.9%), have been reported (12-15).

In a study on HBsAg carriers in 2014 and HBsAg positive blood donors, the prevalence of HDV was 2.2% and 2.5%, respectively. In another study in 2014, an anti-HDV outbreak was reported as 2% on HBsAg positive blood donors. In a meta-analysis study in 2013, Amini et al. reported that the prevalence of anti-HDV was 7.8% in HBsAg positive patients in Iran (16).

Amini et al. (17) also reported that the prevalence of HDV in patients with HBsAg positivity in Iran was 6.6% in a meta-analysis study during year 2011. In the present study, the prevalence of anti-HDV in HBsAg positive patients was 6.8%, which is similar to the above-mentioned study. The prevalence of anti-HDV in patients with HBsAg positivity

Table 1. The Frequency of Anti-Hepatitis Delta Virus Positivity in Patients with Chronic Hepatitis B

	Chronic HBV Group	Anti-HDV Positive Group
Gender		
Male	48 (64.9)	4 (5.4)
Female	26 (35.1)	1 (1.4)
Age, mean \pm SD	15.09 \pm 50.22	3.14 \pm 48.6
Marital status		
Single	36 (48.6)	0 (0)
Married	34 (51.4)	5 (6.8)
Risk factors		
Family history of HB	9 (12.2)	1 (1.36)
Surgical history	22 (29.7)	1 (1.36)
Suspicious sexual contact history	3 (4.1)	0 (0)
Addiction history	3 (4.1)	1 (1.36)
Tattoo history	1 (1.4)	0 (0)
History of dentistry	2 (2.8)	0 (0)
No risk factors	31 (41.9)	2 (2.72)

^a Values are expressed as No. (%) unless otherwise indicated.

was 16.75% in Kerman during years 2012 to 2013 and the cause of the high prevalence of anti-HDV in Kerman was reported due to the phenomenon of foreigners' migration from Afghanistan and Pakistan (18). In the present study, the frequency of anti-HDV was higher in males, which was consistent with studies in Zahedan (19) and Pakistan (20), which could be due to high-risk behaviors in males. The frequency of HDV antibodies in patients with chronic hepatitis B in different cities of Iran is presented in Table 2.

In this study all patients with anti-HDV were older than 40 years of age, indicating that delta infection was common in the fourth decade of life. Therefore, this does not fully reflect the current serologic anti-HDV status and may have occurred before the beginning of the HBV vaccination program. Also, this study showed the presence of low viral load of HBV in HDV patients, which confirms previous studies that HDV can reduce the proliferation of HBV. Studies have shown that the level of HBV viral load is low or undetectable in HBV and HDV co-infections, which suggests that HDV replication is associated with suppression of HBV replication. The prevalence of HDV in the current study (6.8%) was lower than in endemic regions of Italy (8.3%) and Turkey (27.1%). The prevalence of anti-HDV in 258 cases of HBV-positive cases in Lebanon and Greece was 1.2% and 0.23% respectively. The prevalence of anti-HDV in HBsAg positive patients has been reported in Burkina Faso as 3.4%, in Libya as 2.5%, in California as 3.6%, and in Cameroon as 10.5% (28-31). In general, studies have shown

that the prevalence of HDV is declining around the world and this decline can be the result of global HBV vaccination, increased awareness, strategies for the importance of prevention and socioeconomic conditions (16). The epidemiological pattern of HDV is different in endemic areas compared with non-endemic areas. In countries where HDV infection is endemic in people with hepatitis B, such as in the Mediterranean, transmission is mainly through familial and sexual contact. Hepatitis D Virus infection in nonendemic areas, such as the United States and Northern Europe, is confined to blood products, addicts, and people with hemophilia.

In the present study, there was no significant relationship between the risk factors of injecting drug use, familial history of HBV, and the frequency of anti-HDV antibodies in patients with chronic hepatitis B, which can be due to the limited number of samples.

5.1. Conclusions

For prevention of HDV transmission, all patients of chronic hepatitis B with low-level viral load should be evaluated for hepatitis D infection. Also, the most important limitation of the study was the collection of HDV + samples, therefore, it is recommended to achieve more significant results regarding the risk factors of HDV infection through another study with a larger sample size.

Table 2. Frequency of Anti-Hepatitis Delta Virus in Patients with Chronic Hepatitis B in Different Cities of Iran

City	HBsAg+	Anti-HDV ^a	RNA-HDV ^a	Genotype	Ref Number
Kerman	400	67 (16.75)	7 (1.75)	I	(18)
Tehran	280	16 (5.7)	ND	ND	(21)
Tabriz	355	22 (6.01)	ND	ND	(22)
Hamedan	81	ND (17.3)	ND	ND	(12)
Khuzestan	1725	ND (11.5)	ND	ND	(13)
Babol	546	ND (2)	ND	ND	(14)
Isfahan	346	ND (2.9)	ND	ND	(15)
Tehran	509	39 (7.7)	ND	ND	(23)
Tehran	854	18 (2)	0.6	I	(5)
Birjand	413	ND	13 (3.1)	I - II	(24)
Kermanshah	1749	30 (1.7)	ND	ND	(25)
Tehran	1038	23 (2.2)	14 (1.37)	ND	(26)
Mashhad	25	ND	12 (48)	I - II	(27)

Abbreviations: anti-HDV, HDV antibody; HBsAg, hepatitis B surface antigen; HDV, hepatitis D virus; ND, no data.

^a Values are expressed as No. (%).

Footnotes

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

Ethical Considerations: All participants filled the consent form. This study was approved by the Medical Ethics Committee under the following code: IR.TMI.1394.6.1.

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