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ORIGINAL ARTICLE

Seroprevalence of Delta Hepatitis in Patients with Chronic Hepatitis B and its Clinical Impact in Khuzestan Province, Southwest Iran

Eskandar Hajiani *, Seyed Jalal Hashemi, Fariba Jalali

Division of Gastroenterology and Hepatology, Department of Internal Medicine, Emam Hospital,
Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background and Aims: **Hepatitis B virus (HBV) and hepatitis D virus (HDV) infections are major health problems in Iran. Preliminary reports indicate that HDV infection, a satellite virus of HBV, exists in this area. However, its prevalence in patients with different types of liver diseases has not been studied in detail. This study was carried out to determine the seroprevalence of HDV among individuals who tested positive for hepatitis B surface antigen (HBsAg) in a southwestern province of Iran.**

Methods: **In this cross-sectional study a total of 1,725 consecutive patients with HBV liver diseases attending the Ahvaz JundiShapur University Hospitals (AJSUH) and Hepatitis Clinics from 2002 to 2008 were included. We performed tests for HBV and HDV serum markers, using commercially available enzyme-linked immunosorbent assay kits. Patients were split into two groups according to their HDV antibody (anti-HDV) status (HDV positive or negative). The collected data were coded, and the statistical analyses were conducted.**

Results: **The mean age of the patients was 37 ± 13/8 years. There were 1,157 males and 568 females. Of the 1,725 patients with HBV liver disease, 150 were found to be reactive for anti-delta antibodies, yielding an overall HDV seroprevalence of 11.5%. Anti-HDV was found in 3.59% of patients with inactive chronic hepatitis, 45.5% of patients with chronic active hepatitis, and 43.2% of cirrhotic and hepatocellular carcinoma patients ($P < 0.001$). A higher proportion of individuals testing positive for antibodies to HDV were observed among males (72%) than among females (28%). The patients without HDV infection were younger than anti-HDV-positive/HBsAg-positive patients ($P < 0.01$).**

Conclusions: **HDV infection was common in patients with HBV in our community. All HBV patients should be screened for HDV infection. The results indicate that HDV co-infection was related to the severity of the liver disease. More studies designed to elucidate HDV's epidemiology are needed.**

Keywords: **HDV, HBV, Chronic Hepatitis, Iran, Khuzestan**

Introduction

Hepatitis B virus (HBV) infection is a global health problem, and more than 350 million people in the world are chronic carriers of the virus [1]. It is estimated that over 35% of Iranians have been exposed to HBV and about 3% are chronic carriers [2].

Among Iranian cirrhotics, 70-84% of patients have evidence of exposure to HBV infection [3]. In addition, Iranian patients with hepatocellular carcinoma (HCC) have been found to have a 72% exposure rate (as judged by a positive hepatitis B core

* ***Correspondence:***
Eskandar Hajiani, M.D.
Associate Professor of Gastroenterology and Hepatology,
Ahvaz Golestan Hospital, P.O. Box 89, Ahvaz, Iran.

Tel: *+98 611 553 0222*
Fax: *+98 611 334 0074*

E-mail: *ehajiani@ajums.ac.ir*

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antibody [HBcAb]) and a 46% carrier rate [4, 5].

Hepatitis D virus (HDV), a defective RNA virus that requires the presence of hepatitis B surface antigen (HBsAg) from HBV for packaging and transmission [6], plays an important role in fulminant hepatitis and the progression of chronic liver damage in patients with chronic hepatitis B [7]. HDV infection appears to have some geographical differences in prevalence rates in our country [8, 9].

However, in recent years it has been reported that the prevalence of HDV infection is decreasing in the country, along with the prevalence of HBV infection [10-12].

In recent studies, the prevalence rate of HDV infection has been reported to range from 2.4% in blood donors to 10% in chronic liver disease patients [13-16]. In a study by Malekzadeh *et al.* on asymptomatic hepatitis B carriers in Shiraz (in southern Iran), 13.9% were positive for HDV antibody (anti-HDV) [9]. In another study by Taghavi *et al.* (2008) on chronic hepatitis B patients in Shiraz, the rate of anti-HDV positivity was 9.7% [17]. Roshandel *et al.* (2007) reported that 5.8% of HBsAg-positive individuals had anti-HDV Ab in Golestan [18]. In another study Torabi reported a prevalence rate of 6.15% for anti-HDV Ab positivity in Tabriz (northwest Iran) [19].

However, information on the prevalence of HDV infection remains partial and scarce from the Khuzestan province in the southwest of Iran. No study has been done to evaluate the epidemiology of HDV in our area. In the present study, the prevalence of HDV with HBV infection was investigated in a cohort of HBsAg-positive carriers with special reference to the clinical profile and characteristics of those with HDV co-infection.

Materials and Methods

Study population

In this cross-sectional study a total of 1,725 consecutive patients with HBV liver diseases attending the Ahvaz JundiShapur University Hospitals (AJSUH) and Hepatitis Clinics from 2002 to 2008 were included.

On the basis of a specially designed protocol, standard, commercially available tests and physical examinations were performed. The analysis included data from the patient's medical history, a physical examination, and periodic clinical and serological evaluations.

All subjects were evaluated using a face-to-face interview using a questionnaire about demographic characteristics (gender and age), geographic origin, and socioeconomic background (education), parenteral exposure to blood or blood products, social and sexual behavior, occupational exposure, intravenous drug use, tattoos, acupuncture, surgery, previous hospitalizations, parenteral administration of drugs, and alcohol consumption. Data were also collected on each patient's history of jaundice or hepatitis, the date and mode of discovery of HBsAg positivity, the known duration of HBV infection when available, and the known duration of HBsAg since discovery of HBsAg positivity.

The requirement for inclusion in the study was performance of hepatitis B serology tests for at least 6 months. In either circumstance, the patients were cared for by physicians from the Hepatitis Clinic. All subjects were also evaluated for any signs and symptoms related to liver diseases.

Laboratory studies

A serum sample of each patient was checked for HBsAg, presence of hepatitis B e antigen (HBeAg) or antibodies to hepatitis e antigen (anti-HBe antibodies), HBcAb (commercially available enzyme-linked immunosorbent assay [ELISA] kit), and HBV-DNA (polymerase chain reaction [PCR] assay; commercially available kit [Roche Diagnostics, GmbH; UK]).

Patients were tested for HCV antibodies (anti-HCV) by an ELISA. Anti-HCV reactive samples were retested for confirmation by Abbott MATRIX Immunoblot assays and also for HCV-RNA by PCR.

The presence or absence of delta antibodies and co-infection with the human immunodeficiency virus (HIV) virus was also recorded. HDV was detected by serologic tests; both immunoglobulin M (IgM) and immunoglobulin G (IgG) anti-delta antibodies were tested for using Abbott Anti-Delta enzyme immunoassay (EIA) (Chicago, IL, USA). The serological tests were performed according to the instructions provided in the manufacturer's manual.

Sera from all patients were provided for the following liver function tests at the patients' first visit to our outpatient clinic: serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), g-glutamyl transpeptidase (G-GTP), total bilirubin (T Bil), total protein (TP), albumin (Alb), and G-globulin (g-glob). Ultrasonographic examination was performed in all patients to investigate hepatic shape and lesions occupying the hepatic space.

Patients were initially divided into two groups: HBsAg carriers (Group A), and patients with chronic active hepatitis, cirrhosis, or hepatocellular carcinoma (Group B).

Out of the 1,725 participants tested for HDV infection, 150 anti-HDV reactive patients were found.

The clinical records of this subset of patients were reviewed to determine the clinical impact and outcome of HDV infection; a number of clinical and laboratory parameters (inactive HBsAg carrier status; presence of chronic active hepatitis, cirrhosis, or hepatocellular carcinoma; age and gender) were compared with anti-HDV non-reactive/HBV-infected patients. The results of liver biopsy performed before any antiviral treatments were recorded.

Statistical analysis

The collected data were coded, analyzed and computed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). Chi-squared and unpaired Student's *t*-tests were used for the statistical analyses. Differences were considered significant when $P < 0.05$. Informed consent was obtained from all patients after the purpose and methods of the study were explained. The institutional Ethics Review Committee approved the study protocol.

Results

The mean age of the patients was 37 ± 13.8 years. There were 1,157 males and 568 females, with the male:female ratio being 2.03. Of the 1,725 patients with HBV liver diseases, 150 were found to be reactive for anti-delta antibodies, yielding an overall HDV seroprevalence of 11.5%. A higher proportion of individuals testing positive for antibodies to HDV was observed among males 108 (72%) than females 42 (28%). However, the HDV infection ratios did not differ significantly by sex in carriers, chronic hepatitis patients, or cirrhotic patients ($P > 0.05$).

The mean age of the individuals positive for HDV antibody was 42 years, and the mean age of non-reactive individuals was 37 ± 13/8 years, ($P < 0.01$). Thus, patients without HDV infection were younger than anti-HDV positive/HBsAg-positive patients. Anti-HDV was found in 62 of the 1,725 (3.59%) patients with inactive chronic hepatitis (Group A). Anti-HDV positivity was significantly more frequent in patients from Group B than Group A.

On the other hand, in Group B, 40 of the 88 (45.5%) patients with chronic active and sever hepatitis and 38 of the 88 (43.2%) cirrhotic and hepatocellular carcinoma patients were positive for anti-HDV, which is higher than the rate in patients of Group A ($P < 0.001$) (Fig. 1).

A comparative analysis of the HDV-positive group and the HDV-negative group was conducted. Statistical significance was detected in the serum glutamic pyruvic transaminase (SGPT) values, which were higher in the HDV-positive group, reflecting significantly higher inflammation (182 to -78; $P = 0.001$), whereas serum albumin levels were lower in the HDV-positive group (2.7 to 4.53; $P = 0.006$).

Anti-HCV antibodies were observed in 0.7% (12 / 1,725) of carriers, and anti-HIV antibodies were observed in 0.05% (1 / 1,725) of carriers (Table 1).

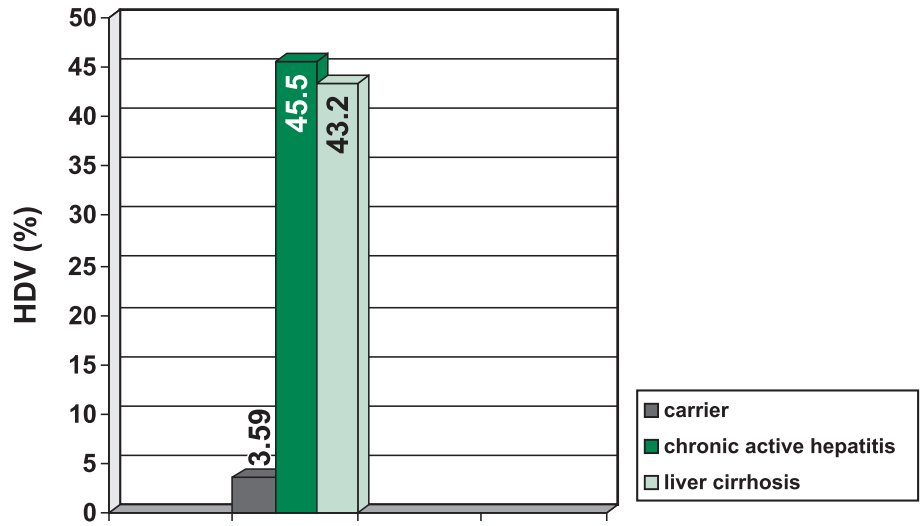

Figure 1. HDV infection in chronic liver disease patients.

Table 1. Comparative characteristics of HDV antibody in reactive and non-reactive patients.

Variable	Anti-HDV reactive (n = 150)	Anti-HDV non-reactive (n = 1565)	P-value
Mean age (years)	42	37±13.8	P < 0.01
Gender			
Male	108	1049	
Female	42	526	
Male:Female ratio	2.57	2.03	P<0.06
Group A	62/1725(3.59%)	1439/1725(83.4%)	P < 0.01
Group B	40/88(45.5%)	286/1725(16.6%)	P < 0.01
Serum albumin	2.7g/dl	4.53g/dl	P = 0.06
SGPT	182 IU	78 IU	P = 0.01
Overall prevalence	11.5%	88.5%	

Group A = inactive chronic hepatitis

Group B = patients with chronic active hepatitis, cirrhosis, or hepatocellular carcinoma

Out of these 13 cases, 6 (46.15%) also tested positive for HDV, whereas the remaining 7 (53.85%) tested negative for HDV. The difference in the frequency was not statistically significant (P = 0.25).

Discussion

HBV is one of the most common chronic pathogens in the world. About 5% of the world's population are chronic carriers (10). Chronic hepatitis B liver disease has become the major burden on the health delivery system in our country (Table 2). We are witnessing a decrease in the prevalence of HBV after the introduction of vaccinations against HBV and better treatment options [11, 20].

Table 2. Anti-HDV positivity in patients with chronic hepatitis B in several provinces of the country.

Region (References)	Year	Anti-HDV reactive (%)
Shiraz (9)	1989	13.9%
Hamadan (15)	1989	2.4%
Tehran (25)	1988	2.4%
Tehran (12)	2004	5.7%
Babol (26)	2002	2%
Golestan (18)	2007	5.8%
Kerman (14)	2003	20.7%
Tabriz (19)	2000	6.15%
Our study (Khuzestan)	2002-2008	11.5%

HDV is a defective RNA virus that requires HBV for its replication and infection. Therefore, the epidemiologic features of HDV infection are similar to those of HBV, although there are some differences. The clinical course of hepatitis D is variable. It may present with fulminant hepatitis or more commonly with chronic progressive liver disease [21]. Chronic HDV does progress to cirrhosis frequently but the pattern of disease progression appears to vary with geography, genotype, and mode of transmission [22].

The anti-HDV positivity in HBV patients in our study was a little higher (11.5%) compared to other Iranian studies [15-18], where it has been reported to range from 2.4% in blood donors to 10% in chronic carrier patients.

In Kerman (southern Iran), there is a relatively higher prevalence of hepatitis D infection (20.7%) compared to our study. The higher prevalence might be due to the presence of more intravenous drug abusers [14]. In two different studies by Malekzadeh *et al.* (1989) of asymptomatic hepatitis B carriers in Shiraz (southern Iran), 13.9% were positive for anti-HDV Ab. However, in another study by Taghavi *et al.* (2008), the anti-HDVAb positivity rate was 9.7%, which reveals a decrease in its prevalence and is somewhat similar to our positivity rate [9, 17].

Research on the prevalence of HDV infection in other regions of Asia, some of which are also endemic for HBV infection, is also insightful. Data from Pakistan have shown a high HDV prevalence of 16.6%, particularly among intravenous drug abusers [23]. In other studies in asymptomatic carriers of

HBsAg from Jordan, Kuwait, Saudi Arabia, and Turkey, the prevalence has been found to be 2%, 31%, 3.3%, and 5.2%, respectively [22].

These differences in HDV seroprevalence have been postulated to result from a variety of factors, such as active preventive measures directed against sexually transmitted diseases, close family contact, promotion of disposable needles, other risk factors, and better control of HBV infection itself. HDV has two predominant patterns of transmission. In some endemic areas transmission is thought to occur through person-to-person contact in the absence of overt percutaneous exposure. On the other hand, in Western Europe and the United States, frequent percutaneous exposure is the major route of transmission. There is little information available regarding the routes of HDV transmission in Iran. The predominant routes of transmission of HBV in Iran have been maternal, from infected mothers to infants, and horizontal during childhood [24, 25].

The epidemiology of hepatitis B has changed in Iran, and horizontal transmission in adults is increasing [16, 26, 27]. The risk factors for acquiring HDV infection in some studies in Iran include blood transfusion, surgery, family history, hejamat (traditional phlebotomy), tattooing, war injury, dentistry interventions, and endoscopy [16]. In our previous study, the most important risk factor for HBV and HDV infections was contact with an infected family member (17.8%), which is similar to other studies carried out in Iran and other countries in the region [28, 29].

In Iran, the hepatitis B vaccination has been applied to all children since 1993 (the Expanded Program on Immunization [EPI]), and now we can see the efficacy of EPI in our region, where hepatitis B has been frequently spreading via family contact [30]. This decreased prevalence of HBV infection leads to a decrease in the number of individuals who are susceptible to HDV infection, depriving this defective virus of the biological substrate necessary for its survival. Finally, the declining prevalence of both HBV and HDV infection may herald complete control of HDV infection in our area in the near future.

However, several other factors that might have contributed to the epidemiological differences of HDV in the present study need further evaluation. In our study, anti-HDV positivity was significantly more frequent in symptomatic patients (Group B) than in inactive chronic hepatitis patients (Group A) (3.59% versus 45.5%, respectively,

P = 0.001). In this study, we detected that 45.5% of chronic hepatitis B and 43.2 % of HBV cirrhosis and hepatocellular carcinoma patients tested positive for HDV infection in the Khuzestan region. The HDV carriage rate was 3.59% in asymptomatic HBV carriers.

HDV infection showed a severalfold increase in chronic hepatitis and cirrhosis patients ($P < 0.001$) compared to HBV carriers. In this regard, the higher prevalence of HDV infection in the severe form of HBV infection suggests that HDV infection increases the severity of HBV infection. Interestingly, we observed no significant difference in HDV infection in chronic hepatitis patients compared to HBV cirrhosis and hepatocellular carcinoma patients. The mean age of cirrhotic HDV patients was also younger than that of cirrhotic HBV patients without HDV. These findings reveal the higher severity of the prognosis of chronic HDV infection than of chronic HBV infection alone.

A final limitation of this study is that it is based on serological testing, and confirmation of ongoing HDV infection by PCR testing of HDV RNA is lacking. The impact of this lack of information is that patients with and without active delta infection cannot be differentiated.

In conclusion, this is the first study of HDV infection in our geographical region, and the findings showed that HDV infection is endemic in the Khuzestan province of Iran. It is important to determine whether delta hepatitis is present because chronic HDV infection often results in severe liver disease. Also, because the response to therapy is less satisfactory, the recommended treatment is different. The epidemiology of HDV is changing, with successful efforts to control HBV infection in our country. Education of the public, general practitioners, and other health care providers regarding dual HDV and HBV, as well as proper hepatitis B screening and vaccination, should be carried out in this province.

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