

# Prevalence of hepatitis D virus infection in hepatitis B surface antigen-positive subjects in Golestan province, northeast Iran

Gholamreza Roshandel<sup>1</sup>, Shahryar Semnani<sup>1</sup>, Nafiseh Abdolahi<sup>1</sup>, Sima Besharat<sup>1</sup>, Abbas-Ali Keshtkar<sup>1</sup>, Hamidreza Jashaqani<sup>1</sup>, Abdolvahab Moradi<sup>1</sup>, Khodaberdi Kalavi<sup>2</sup>, Ali Jabbari<sup>1</sup>, Mohammad Javad Kabir<sup>3</sup>, Seyed Ahmad Hosseini<sup>3</sup>, Seyed Mehdi Sedaqat<sup>3</sup>, Ahmad Danesh<sup>3</sup>, Danyal Roshandel<sup>1</sup>, Seyed Mohammad Hedayat-Mofidi<sup>2</sup>

<sup>1</sup>Golestan Research Center of Gastroenterology and Hepatology, <sup>2</sup>Faculty of Paramedicine and Health Department, Golestan University of Medical Sciences, Gorgan, Iran

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**Background and Purpose:** Hepatitis D virus (HDV) is a defective RNA virus dependent on hepatitis B virus (HBV) infection for its replication and expression. It is known that coexistent infection with HDV tends to aggravate the course of HBV-associated liver disease. This study was carried out to determine the seroprevalence of HDV among hepatitis B surface antigen (HBsAg)-positive individuals in the northeast part of Iran.

**Methods:** 139 HBsAg-positive subjects detected from a population-based single stage cluster sampling in Golestan province of Iran were enrolled. All cases were evaluated for the presence of anti-HDV antibodies using commercially available enzyme-linked immunoabsorbent assay kits. Logistic regression was used to determine the relationship between independent variables and HDV seropositivity.

**Results:** Of 139 cases, 68 were males (48.9%) and 71 were females (51.1%). The mean age was  $41.9 \pm 11.3$  years (range, 25-64 years). Anti-HDV antibody was positive in 8 subjects (5.8%), with a female predominance (9.9% vs 1.5%,  $p=0.06$ ; odds ratio, 7.32; 95% confidence interval, 0.87-61.23). No significant relationship was seen between anti-HDV seropositivity and demographic factors such as age, place of residence and marital status.

**Conclusions:** These findings show that HDV infection is endemic in Golestan province (northeast) of Iran. Seroprevalence of anti-HDV in the present study was higher than in some previous studies from other parts of Iran. Our results suggest that the prevalence of HBV/HDV coinfection in Golestan province of Iran has increased during the last decade. Therefore, practitioners and health care managers should be made aware of the risk of dual infection with HBV and HDV.

**Key words:** Hepatitis B; Hepatitis D; Iran; Risk factors; Seroepidemiologic studies

## Introduction

Hepatitis B virus (HBV) infection is one of the most prevalent public health problems worldwide (especially in developing countries), causing 1 million deaths annually [1]. More than 3% of Iranian populations have HBV infection [2,3].

Hepatitis D virus (HDV) is a defective RNA virus dependent on HBV infection for its replication and

expression [4,5]. HDV is well known to induce a spectrum of acute and chronic liver diseases. More than 15 million patients are infected with HDV, and its prevalence in Italy, Eastern Europe and western regions of Asia is higher than in the rest of the world [6-8]. It appears to be endemic in the Middle East [9]. Infection with HDV can occur simultaneously with acute HBV infection or may be superimposed on chronic HBV infection [10]. It is known that coexistent infection with HDV tends to accelerate the progress of chronic HBV infection to chronic hepatitis, cirrhosis and hepatocellular carcinoma [6,11].

Fulminant hepatitis may develop in 20-30% of patients coinfecting with HBV and HDV, but only around

Corresponding author: Dr. Gholamreza Roshandel, Number 77, Qabooseieh Passage, Valiasr Street, Gorgan 49166-53588, Golestan Province, Iran.  
E-mail: roshandel\_md@yahoo.com

2% of patients infected with isolated HBV experience this complication [5].

This study was carried out to determine the seroprevalence of HDV virus among hepatitis B surface antigen (HBsAg)-positive individuals in the Golestan province, in northeast Iran.

## Methods

A population-based cross-sectional study was conducted in the Golestan province of Iran during 2004 to 2005. A total sample size of 1850 subjects was enrolled using a single-stage cluster sampling method. To achieving this sample, 92 clusters were selected using a systemic random sampling according to most recent census data in the Golestan province of Iran (2004-2005). In each cluster, 20 subjects were explored for HBsAg using an enzyme-linked immunoabsorbent assay (ELISA) kit (DiaSorin SpA, Saluggia, Italy) [sensitivity, 100.0%; specificity, 98.8%]. 164 of 1850 subjects (8.9%) were found to be positive for HBsAg. Unfortunately, the serum samples of 25 HBsAg-positive subjects were inadequate or had been lost during the sampling phase. Therefore, 139 of the HBsAg-positive subjects were enrolled in our study. An approximately 2-mL blood sample was collected from each subject. Serum was separated under complete aseptic conditions and then stored at  $-70^{\circ}\text{C}$  until use. Anti-HDV antibody was detected using an ELISA kit (Radim SpA, Pomezia, Italy) [sensitivity, >98%; specificity, >98%]. All data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 12.0; SPSS, Chicago, IL, USA) and STATA (Version 8; StataCorp LP, College Station, TX, USA) software packages. Proportions were compared by chi-squared and Fisher's exact tests. Logistic regression was used to determine the relationship between independent variables and HDV seropositivity. *p* Values of <0.05 were considered significant. Variables with *p* values of <0.1 were analyzed by multivariable logistic regression. Model fitness was assessed by Hosmer-Lemeshow statistics.

## Results

Of the 139 cases, 68 were males (48.9%) and 71 were females (51.1%). The mean age was  $41.89 \pm 11.30$  years (range, 25-64 years). Demographic characteristics of the subjects are summarized in Table 1. Of the 139 cases, eight were found to be reactive for anti-HDV antibodies, yielding an overall HDV

**Table 1.** Demographic characteristics of the study subjects

Variable	Number of cases (%) <sup>a</sup>
Gender	
Male	68 (48.9)
Female	71 (51.1)
Age (years)	
<30	32 (23.0)
30-50	65 (46.8)
>50	42 (30.2)
Marital status	
Married	119 (85.6)
Single	20 (14.4)
Body mass index (kg/m <sup>2</sup> )	
<25	53 (38.1)
25-29.9	49 (35.3)
30-34.9	22 (15.8)
≥35	15 (10.8)
Place of residence	
Urban area	86 (61.9)
Rural area	53 (38.1)

<sup>a</sup>Missing data were deleted.

seroprevalence of 5.8% (95% confidence interval (CI), 2.5-11.0%). Anti-HDV antibody was positive in seven (9.9%; 95% CI, 4.1-19.3%) females and one (1.5%; 95% CI, 0.04-7.9%) male, but the difference was not statistically significant ( $p=0.06$ ) [Table 2]. The prevalence of HDV seropositivity was higher in rural populations than in urban populations (7.5% vs 4.7% respectively), although no significant relationship was seen ( $p=0.48$ ). The seroprevalence of HDV did not significantly differ between age groups. Ten percent of single and 5% of married subjects were positive for anti-HDV antibody, but the difference was not significant ( $p=0.38$ ) [Table 2]. Crude data showed a significant relationship between HDV seropositivity and subjects' body mass index (BMI), HDV infection being significantly higher in severely obese subjects (BMI >35 kg/m<sup>2</sup>) than in the reference group (BMI <25 kg/m<sup>2</sup>). However, significance was not maintained after adjustment for gender on multivariate logistic regression analysis (Table 2).

## Discussion

We conducted a seroepidemiological survey of coinfection with HDV in 139 apparently healthy HBsAg-positive individuals living in the Golestan province (northeast part) of Iran. The seroprevalence of anti-HDV antibody was 5.8%, indicating the endemicity of HDV infection in Golestan province. This result was

**Table 2.** Relationships between hepatitis D virus seropositivity and sociodemographic factors in hepatitis B surface antigen subjects

Variable	Crude odds ratio (95% CI)	<i>p</i>	Adjusted odds ratio (95% CI) <sup>a</sup>	<i>p</i>
Gender				
Male	1	-	-	-
Female	7.32 (0.87-61.23)	0.06	6.24 (0.71-54.55)	0.09
Age (years)				
<30	1	-	-	-
30-50	0.63 (0.13-3.02)	0.57	-	-
>50	0.23 (0.2-2.38)	0.22	-	-
Marital status				
Married	1	-	-	-
Single	2.09 (0.39-11.18)	0.39	-	-
Body mass index (kg/m <sup>2</sup> )				
<25	1	-	-	-
25-29.9	1.11 (0.15-8.19)	0.92	0.83 (0.11-6.37)	0.86
30-34.9	1.11 (0.09-12.85)	0.93	1.00 (0.08-11.99)	0.1
≥35	8.5 (1.24-58.23)	0.03	5.52 (0.76-40.20)	0.09
Place of residence				
Urban area	1	-	-	-
Rural area	1.67 (0.40-6.99)	0.48	-	-

Abbreviation: CI = confidence interval

<sup>a</sup>Only variables with *p*<0.1 were analyzed by multivariable logistic regression.

substantially higher than recorded in some previous similar Iranian studies [3,12,13].

Rezvan et al in 1990 detected HDV antibodies in 2.5% of asymptomatic HsAg carriers [12]. Amini et al in 1993 reported the same prevalence (2.4%) of HDV infection in a similar population from Hamadan [13]. In 2000, Hassanjani-Roshan and Taheri from Babol reported HDV positivity in 2% of HBV carriers [3]. In contrast with these results, HDV seropositivity of 6% was reported among HBsAg-positive subjects from Tabriz [14]. Recently, Alavian et al have reported 5.7% HDV seropositivity among HBV-infected subjects in Iran [15]. These results suggest that the prevalence of HBV/HDV co-infection in Iran has increased during the last decade.

In other parts of the world, the seroprevalence of HDV among HBsAg-positive cases was 1.5%, 1.6%, 2.2%, 4%, 16.6% and 24.4% in Yugoslavia [16], Spain [17], Taiwan [18], Mexico [19], Pakistan [20] and Bangladesh [1], respectively. Analysis of the gender-related seroprevalence of HDV antibody in our study showed that females (9.9%) were more often infected than males (1.5%), in contrast to earlier reports from Babol, Iran [3] and Pakistan [20]. In the present study, subjects living in rural areas showed a higher prevalence of HDV seropositivity than the urban population, and the prevalence of HDV antibody was higher in young and single populations, known to result from

high-risk behaviors among these groups. The latter findings are consistent with those of Mumtaz et al [20] from Pakistan.

We found that HDV infection was significantly higher in the severely obese (BMI >35 kg/m<sup>2</sup>) than in other individuals. We did not find this relationship in previous studies and no causal relationship had been proposed between HDV infection and BMI. Therefore, we used multivariate logistic regression to detect possible confounder effects of other variables. After adjustment by multivariate logistic regression, no significant relationship was found between BMI and HDV positivity (Table 2). In fact, our data suggested that gender was more likely to be a correlate of HDV than BMI. However, because the power of our study was low (41%), we could not find a statistically significant relationship between gender and HDV seropositivity. Studies with larger sample sizes are needed to test for this association.

In conclusion, our findings show that HDV infection is endemic in the Golestan province of Iran. The seroprevalence of HDV antibodies in the present study was higher than in some previous studies from other parts of Iran. Our results suggest that the prevalence of HBV/HDV coinfection in Golestan province has increased during the last decade. Therefore, practitioners and health care managers should be made aware of the risk of dual infection with HBV and HDV.

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