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Prevalence of Hepatitis D Virus Infection in HBsAg Positive Subjects in Iran

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Abstract: This study was carried out to determine the seroprevalence of hepatitis D virus among HBsAg positive individuals in the northeast part of Iran. One hundred thirty nine 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 ELISA kits. Logistic regression was used to determine the relationship between independent variables and HDV seropositivity. Of 139 cases, 68(48.9%) were males and 71(51.1%) were females. The mean age was 41.89±11.30 years (25-64 years). Anti-HDV antibody was positive in 8 (5.8%) subjects with female predominance (9.9% versus 1.5%, $p = 0.06$; odds ratio = 7.32, 95%CI: 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. These findings showed that HDV infection was endemic in Golestan province (northeast) of Iran. Seroprevalence of Anti-HDV in the present study was higher than some previous studies from other parts of Iran. Our results suggest that the prevalence of HBV/HDV co-infection in Iran has increased during the last decade. Therefore, practitioners and all health care managers should be made aware of the risk of dual infection with HBV and HDV.

Key words: Hepatitis D, hepatitis B, Iran

INTRODUCTION

Hepatitis B Virus (HBV) infection is one of the most prevalent public health problems worldwide (especially in developing countries). It causes 1 million deaths annually (Zaki *et al.*, 2003). More than 3% of Iranian populations are involved with HBV infection (Massarat *et al.*, 2000; Hasanjani Roshan and Taheri, 2002).

Hepatitis D Virus (HDV) is a defective RNA virus dependent on hepatitis B Virus (HBV) infection for its replication and expression (Jacobson *et al.*, 1995; Kawal and Feinstone, 2000). 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, east of Europe and west of Asia is higher than the rest of the world (Gaeta *et al.*, 2000; Ivaniushina *et al.*, 1996). Its infection appears to be endemic in the Middle East (Tapalaga *et al.*, 1986). Infection with HDV can occur simultaneously with acute HBV infection or may be superimposed on chronic HBV infection (Chakraborty *et al.*, 2005). It is known that co-existent infection with HDV tends to accelerate the

progress of chronic HBV infection to chronic hepatitis, cirrhosis and hepatocellular carcinoma (Toukan and al-Khanderi, 1991; Dienstag and Isselbacher, 1998).

Fulminant hepatitis may develop in 20-30% of patients co-infected with both HBV and HDV but only 2% of patients infected with isolated HBV might experience such complication (Kawal and Feinstone, 2000).

This study carried out to determine the seroprevalence of hepatitis D virus among HBsAg positive individuals in the Golestan Province (northeast part) of Iran.

MATERIALS AND METHODS

A population based cross-sectional study had been conducted in Golestan province of Iran during 2004 to 2005. A total sample size of 1850 subjects had been enrolled using a single stage cluster sampling method. To achieving this sample, 92 clusters had been selected using a systemic random sampling according to last census tract in Golestan province of Iran (2004-2005). In each cluster, 20 subjects had been explored for hepatitis B virus surface

antigen using ELISA kit, Diasorin, Italy (Sensitivity: 100%; Specificity: 98.8%). 164 (8.9%) of 1850 subjects had been 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. Approximately 2 mL blood sample was collected from each subject. Separation of serum was done under complete aseptic conditions and then stored at -70°C until use. The serological test was performed using commercially available ELISA method according to the instructions provided in the manufacturers' manual. Anti-HDV antibody was detected using ELISA kit, Radim, England (Sensitivity: >98%; Specificity: >98%). All data were analyzed by SPSS (Chicago, IL) software, version 12 and STATA version 8. Proportions were compared by Chi-square and Fisher's exact tests. Logistic regression was used to determine the relationship between independent variables and HDV seropositivity. p-values of less than 0.05 were considered significant. Variables with p-values of less than 0.1 were analyzed by multivariable logistic regression. Model fitness was assessed by Hosmer-Lemeshow statistics.

RESULTS

Of the 139 cases, 68 (48.9%) were males and 71 (51.1%) were females. The mean age was 41.89±11.30 years (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 seroprevalence of 5.8% (95% CI: 2.5-11.0%). Anti-HDV antibody was positive in seven (9.9, 95 CI: 4.1-19.3%) of females and one (1.5, 95 CI: 0.04-7.9%) of males, but the difference was not statistically significant (p=0.06) (Table 2). The prevalence of HDV seropositivity in rural population was higher than urban population (7.5% versus 4.7%, respectively),

Table 1: Demographic characteristics of the studied subjects

Variables		No. of cases (%) ^a
Sex	Male	68 (48.9)
	Female	71 (51.1)
Age groups(years)	<30	32 (23)
	30-50	65 (46.8)
	>50	42 (30.2)
Marital status	Married	119 (85.6)
	Single	20 (14.4)
Body mass index (BMI) groups (kg m ⁻²)	<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	52 (38.10)

^aMissing data were deleted

although no significant relationship was seen (p = 0.48). Seroprevalence of hepatitis D virus did not significantly differ between age groups. Ten percent of singles and 5% of married subjects were positive for anti-HDV antibody, but the difference was not significant (p = 0.38) (Table 2). There was a significant relationship between HDV seropositivity and subjects' body mass index (BMI). That means, HDV infection was significantly higher in severe obese (BMI>35 kg m⁻²) than reference group (BMI<25 kg m⁻²). But when it was adjusted with sex by multivariate logistic regression, no significant relationship was found between BMI and HDV positivity (Table 2).

DISCUSSION

In present study, we conducted a seroepidemiological survey of co-infection with hepatitis D virus in 139 apparently healthy HBsAg positive individuals living in Golestan province (northeast part) of Iran. The seroprevalence of anti-HDV antibody in our study was 5.8% that indicates the endemicity of HDV infection in Golestan province (northeast) of Iran. It was considerably high, when compared with some previous similar Iranian studies (Hasanjani Roshan and Taheri, 2002; Rezvan *et al.*, 1990; Amini *et al.*, 1993). Rezvan *et al.* (1990) detected anti-HDV antibodies in 2.5% of asymptomatic HBsAg carriers. Amini *et al.* (1993) had reported the same prevalence (2.4%) of HDV infection in similar population from Hamadan. Hasanjani Roshan and Taheri (2002) from Babol, Iran had reported HDV positivity in 2% of HBV carriers. In contrast with these results, HDV seropositivity had been reported as 6% among HBsAg positive subjects from Tabriz (Torabi *et al.*, 2002). Recently, Alavian *et al.* (2005) have reported 5.7% of HDV seropositivity among HBV infected subjects in Iran (Alavian *et al.*, 2005). 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 (Dellic *et al.*, 1993), Spain (de Miguel *et al.*, 1994), Taiwan (Chen and Tseng, 1992), Mexico (Munoz Espinosa and Ibarra Salas, 1997), Pakistan (Mumtaz *et al.*, 2005) and Bangladesh (Zaki *et al.*, 2003), respectively. Analysis of sex-related seroprevalence of HDV antibody in our study showed that the females (9.9%) were more infected than males (1.5%). This finding was not in conformity with earlier reports from Babol, Iran (Hasanjani Roshan and Taheri, 2002) and Pakistan (Mumtaz *et al.*, 2005). In present study, subjects living in rural area showed a higher prevalence of HDV seropositivity than urban population. This is consistent

Table 2: Relationships between HDV seropositivity and socio-demographic factors in HBsAg subjects

Variables		Crude odds ratio (95% CI)	p-value	Adjusted odds ratio (95%CI) ^a	p-value
Sex	Male	1	-	-	-
	Female	7.32 (0.87-61.23)	0.06	6.24 (0.71-54.55)	0.09
Age groups (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 (BMI) groups (kg m ⁻²)	<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)	0.10
Place of residence	=35	8.5 (1.24-58.23)	0.03	5.52 (0.76-40.20)	0.09
	Urban area	1	-	-	-
	Rural area	1.67 (0.40-6.99)	0.48	-	-

^a Only variables with p-values <0.1 were analyzed by multivariable logistic regression

with findings of Mumtaz *et al.* (2005) from Pakistan. Present results showed that the prevalence of anti-HDV antibody was higher in young and single population. It was known that this is due to high-risk behaviors among them. Mumtaz *et al.* (2005) from Pakistan had reported similar findings.

We found that HDV infection was significantly higher in severe obese (BMI>35 kg m⁻²) than other individuals. We did not find this relationship in previous studies and no causal relationship had been proposed between HDV infection and patient's BMI. Therefore, we used multivariate logistic regression to detect possible confounder effects of other variables. For this reason, we adjusted BMI with sex (two variables with p-values of less than 0.1). After adjustment with multivariate logistic regression, no significant relationship was found between BMI and HDV positivity (Table 2). In fact, our data suggested that relationship between HDV infection and sex was more significant than that of BMI. However, because the power of our study was low (41%), we could not find statistically significant relationship between sex and HDV seropositivity. So more studies with larger sample sizes should be designed to determine this association.

In conclusion, present findings showed that HDV infection is endemic in Golestan province (northeast) of Iran. Seroprevalence of Anti-HDV in the present study was higher than some previous studies from other parts of Iran. Present results suggest that the prevalence of HBV/HDV co-infection in Iran has increased during the last decade. Therefore, practitioners and all health care managers should be made aware of the risk of dual infection with HBV and HDV.

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