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ORIGINAL ARTICLE

Hepatitis D in Chronic Active Hepatitis B: Prevalence, Liver Enzyme levels and Histopathology-an Epidemiological Study in Shiraz, Southern Iran, 2003-2004

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Background and Aims: **At least 5% of hepatitis B carriers worldwide are infected with Hepatitis D virus (HDV). This study aims to determine the prevalence, transaminase levels and histopathological findings of HDV among patients with chronic active hepatitis B in southern Iran.**

Methods: **During 2003-2004, 93 patients ≥15 years with chronic active hepatitis B were enrolled from referrals to Shiraz University of Medical Sciences in southern Iran.**

Results: **Nine (9.7%) patients were seropositive for the anti HDV antibody. 76.3% of patients were male and among the HDV positive group, all subjects were male too. A significantly higher AST and more advanced grade and stage of liver disease were observed in the HDV positive group. The most common mode of transmission in the positive group was intravenous drug use.**

Conclusions: **The risk of liver disease progression in chronic hepatitis B appears to be higher in HDV infected patients. Intravenous drug abuse is an important risk factor for acquiring HDV infection. Checking anti-HDV is suggested in any patient with positive HBsAg, especially in males or those with history of intravenous drug abuse.**

Keywords: **Hepatitis D, Hepatitis B, Liver Enzymes, Histopathology**

Introduction

Six main viruses have been recognized, as agents responsible for viral hepatitis, including hepatitis A, B, C, D, E and G viruses [1]. Delta virus or hepatitis D virus (HDV) is a small RNA-containing virus requiring concomitant presence of Hepatitis B Virus (HBV) for its survival and pathogenicity [2]. It was first described in 1997 in HBsAg positive patients. Within a few years of its discovery, it was linked to cases of progressive chronic hepatitis B and fulminant hepatitis [3, 4]. It is important to know that HDV infection is present in a patient with HBV infection because firstly, it allows a more accurate determination of prognosis and secondly, the response of HDV patients to antiviral therapy and needed dosage therapeutic regimens are significantly different from those with chronic hepatitis B alone [5].

HDV is distributed worldwide with the highest endemicity in South America. It is believed that at least 5% of hepatitis B carriers worldwide are infected with HDV. A considerable regional variation exists, with some countries reporting a much higher co-infection rate. This is particularly true in Mediterranean countries, North Africa, and the Middle East. Based on blood donor screening, infection with HDV was much more prevalent among intravenous drug abusers (20-53%) and hemophiliacs (48-80%) [6]. HDV infection is transmissible only if the recipient is a carrier of HBV. In cases of acute co-primary infection, HBV infection needs to be established in the host before HDV infection can occur. In a host with a

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previously established HBV infection, superinfection with HDV is much more common than co-infection. Co-primary and superinfections occur because the mode of transmission of HDV is similar to that of HBV.

The epidemiologic pattern of HDV infection varies throughout the world and in countries such as Iran, it differs greatly from regions like the USA [7]. In Iran, previous studies report a 2.4 to 14% rate of HDV infection in otherwise healthy HBsAg carriers and 50% in HBV induced cirrhotic cases [8-10]. Among Iranian hemodialysis HBsAg positive patients, 44.5% were reported to be infected with HDV [11]. The most useful markers of HDV infection include HDV antigen, anti HDV antibody, HDV RNA and immunohistochemistry of the liver. Detection of HDV-RNA by reverse transcriptase PCR amplification is the most reliable technique, with nearly 100% sensitivity [12].

This study aimed to determine the prevalence of HDV infection among chronic active hepatitis B patients in Fars Province and its possible effects in histopathology of the liver.

Materials and Methods

In a cross-sectional study, 93 patients ≥15 years who were HBsAg positive and with chronic active hepatitis (CAH) B were selected consecutively over 14 months (April 2003-June 2004) from referrals to Hepatology Clinic of Motahari Center affiliated to Shiraz University of Medical Sciences in Shiraz, southern Iran. The clinic represents the only referral center for hepatic disease and transplantation in Fars province. The inclusion criteria were positive HBsAg, age above 15 and baseline liver enzymes of ≥1.5 times the normal range on at least two occasions. A written consent form was provided for each patient and the study approved by the ethics committee of the university. Patients were classified into two groups of positive and negative for total anti-HDV (IgG +IgM) measured by a one step competitive enzyme-linked immunosorbent assay (ELISA) method [DIA. PRO, Italy; Cut-off= (NC+PC)/5; OD=450 nm; Positivity of <1.0].

The level of liver enzymes (aminotransferases) and liver histopathology (grade and stage of liver disease) were compared between the two groups. The sex, age (between 15 and 75 years), method of transmission and habitat of patients were recorded. Most patients received treatment (lamivudine or interferon), and liver enzymes had decreased or even normalized after treatment, so baseline levels, prior to starting treatment, were included. The histopathologic severity rated from I to VIII for inflammation (grade) and from I to IV for stage according to Scheuer classification [13]. No cases of hepatocellular carcinoma were present in the study group. Modes of transmission were classified as follows: 1. Unknown (detected randomly from routine blood donation or detected without any known risk factors), 2. Positive family history of hepatitis B, 3. Intravenous drug abuse, 4. History of transfusion of blood or blood products and 5. Hemodialysis. Data were analyzed using t, Fisher Exact and Non-Parametric tests.

Results

Frequency of seropositivity for HDV was 9.7%; the rest of the patients (84, 90.3%) were seronegative. Among all patients, 71 were male (76.3%) and 22 female (23.7%). In the positive group, all nine patients were male (100%). In the negative group with 84 patients, 62 (73.8%) were male and 22 (26.2%) female.

Mean AST was higher in the positive group (94.7±50.1) compared to the negative group (64.5±62.8) (P=0.01). Mean ALT in the positive and negative groups were 86.1±34.8 and 77.8±58.6, respectively (P=0.13). The mean grade of inflammation was 3.33±0.86 and 2.08±1.21 in the positive and negative groups, respectively (P=0.004). The mean stage (fibrosis) was 3.11±0.92 and 1.84±1.42 in the positive and negative groups, respectively (P=0.009) (Table 1). It should be mentioned that for five patients in the HDV-negative group, no liver biopsy was available (four refused the procedure and for one with hemophilia, liver biopsy was avoided because of associated high risk). None of these were included in the statistical analysis.

In the positive group, the most common route of transmission was intravenous drug abuse (44.4%) followed by unknown routes (33.3%), positive family history (11.1%) and history of receiving

Table 1. Gender, liver enzymes and histology in HDV-positive and negative groups.

		HDV-positive	HDV-negative	P-value
Sex	Male	9 (100.0%)	62 (73.8%)	0.109
	Female	0 (0.00%)	22 (26.2%)	
Enzymes	Mean AST (mean±SD)	94.7±50.1	64.5±62.8	0.01
	Mean ALT (mean±SD)	86.1±34.8	77.8±58.6	0.13
Histology	Grade (mean±SD)	3.33±0.86	2.08±1.21	0.004
	Stage (mean±SD)	3.11±0.92	1.84±1.42	0.009

blood or blood products (11.1%). In the negative group, the most frequent route of transmission was unknown (41.7%), followed by positive family history (28.6%), history of receiving blood or blood products (16.7%), intravenous drug abuse (11.9%) and hemodialysis (1.2%) (Table 2). Patients were also tested for HIV and HCV and all were negative.

Table 2. Routes of transmission among HDV-positive and negative groups.

	HDV-positive	HDV-negative	P-value
Unknown	3 (33.35)	35 (41.7%)	0.733
Positive family history	1 (11.1%)	24 (28.6%)	0.436
IV drug abuse	4 (44.4%)	10 (11.9%)	0.027
History of blood /blood product transmission	1 (11.1%)	14 (16.7%)	0.988
Hemodialysis	0 (0.00%)	1 (1.2%)	0.995

Discussion

There are several reports on HDV prevalence in Iran. According to Alavian *et al.*, 5.7% of HBV patients in Tehran were also infected with HDV [7]. Gholamreza *et al.* reported a prevalence of 5.8% for anti-HDV in HBsAg positive cases in northeastern Iran [14]. In Hamadan Province, west of Iran, anti-HDV was present in 2.4% of HBsAg carriers [8]. Anti-HDV was found in 8.8% of HBsAg-positive patients in Tehran, central Iran [15]. In a study by Rezvan *et al.*, anti-HDV positivity was 2.5% in asymptomatic chronic HBsAg carriers, but as high as 49.2% in HBsAg positive patients with chronic active hepatitis and cirrhosis [9].

Our results showed a prevalence of 9.3% for hepatitis D infection among our patients with chronic active hepatitis B. The difference at least in part may be due to different inclusion criteria. In previous studies, a significant proportion of the patients were asymptomatic HBV carriers with normal transaminases contrary to our study in which only those with elevated transaminases were included. Since HDV infection is presumed to be associated with an increase in histological activity and inflammation, a higher rate of HDV infection is to be expected in those with elevated liver enzymes.

Among all previous reports in Iran, only in Rezvan *et al.* study in 1991, the study group comprised of HBsAg positive patients with chronic active hepatitis [9], which was similar to our study group. However, there is a striking difference between their result and ours. It may be explained by the changing epidemiology of hepatitis B and D in the last two decades or may be due to regional differences.

The overall incidence of HBV in this province is also the lowest in the country, which may indicate less high-risk behavior in this region. The possible effect of regional differences on HDV prevalence may also be demonstrated by comparing our study to that of Alavian *et al.* Although gender distribution was the same in both studies (23-26% of participants were females), HDV infection was more common among HBV patients in our study. It should be noted, however, that we only included chronic active HBV patients whereas Alavian *et al.* studied the inactive carriers as well as chronic active HBV and cirrhotic patients. This, as previously mentioned, could account for some of the difference between the findings of these two studies.

Studies in other countries have reported a wide range of prevalences. According to Hsieh *et al.*, approximately 5% of the global HBV carriers were co-infected with HDV [16]. This was about 4.8% in northern Poland [17], 13.6% in Mongolia [18] and 15.3% in Taiwan [19]. The anti-HDV was positive in 20% of patients with chronic hepatitis B [20] and in 27.5% of patients with chronic active hepatitis B [21] in Turkey. Among HBsAg positive patients seeking treatment, 26.8% had the anti-HDV in India [22] while the prevalence of HDV-virus was 37.9% in HBsAg-positive chronic hepatitis patients in Romania [23].

All chronic active hepatitis B subjects in our study infected with HDV, were male. This finding is also reported by Veta *et al.* in Romania who investigated hepatitis B patients with positive HDV serology and reported that most patients were male [24]. This clear male predominance could be due to the higher rate of high-risk behavior including IV drug abuse. On the other hand, there was no significant relation between gender and HDV seropositivity in northern Iran [14].

We found that in the HDV-positive group, the AST level was significantly higher and the histological liver lesions (fibrosis, inflammation) were significantly more than the HDV-negative group. Similarly, a study in China on hepatitis B patients showed that the incidence and mortality of serious chronic hepatitis, severe hepatitis, and liver cirrhosis were all higher in the hepatitis B patients with positive HDV compared to those with negative serology. Level of ALT was also higher in the positive group [25]. These findings are consistent with the general belief that hepatitis D infection in patients with chronic hepatitis B may aggravate the clinical course with a rapid progression of liver disease documented by a higher histological activity and

liver enzymes. It should be noted that opposite results are also reported in literature like Zuberi *et al.*, which reported no significant difference between frequency and severity (stage) of fibrosis between the two groups, although mean ALT was significantly different in the same study [22].

Among the HDV-positive group in our study, the most frequent route of transmission was IV drug abuse, which is quite common in Iran. This finding is supported by Sheng *et al.* who compared HDV-infected case patients with HDV-uninfected matched controls and found that case patients had a higher rate of injection drug use [26]. According to Davaalkham *et al.*, however, parenteral exposures in healthcare settings and household transmission were the main routes of transmission in Mongolian children [18].

Conclusions

The risk of progression of liver disease in chronic hepatitis B appears to be higher in HDV infected patients, although this requires confirmation with longitudinal follow up studies. Intravenous drug abuse is an important risk factor for acquiring HDV infection, being the most frequent mode of transmission in patients with positive anti-HDV. Based on our findings, we suggest that the HDV status should be checked in any patient with positive HBsAg.

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