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Original article

Prevalence and risk factors of hepatitis D virus infection in patients with chronic hepatitis B infection attending the three main tertiary hospitals in Libya

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ABSTRACT

Background and study aims: Globally, More than 350 million individuals are chronically infected with hepatitis B virus (HBV), and >20 million of them are co-infected with hepatitis D virus (HDV). The aim of this study was to determine the pattern of HDV infection in patients with chronic hepatitis B in three main tertiary hospitals in Tripoli and Benghazi, Libya.

Patients and methods: This cross sectional and descriptive study was conducted on 162 patients with chronic hepatitis B positive for more than six months) who were followed up at hepatitis clinics of the three main tertiary hospitals in Tripoli city (88 patients from Tripoli Medical Centre and Tripoli Central Hospital) and Benghazi city (74 patients from Aljomhoria Hospital) during the period from January 2010 to June 2012. HBV and HDV markers were detected by enzyme linked fluorescent assay (ELFA) or enzyme-linked immunosorbent assay and HBV-DNA was quantified by real-time PCR techniques.

Results: The mean age of patients was 36,92 ± 15,35. One hundred and three (63.6%) of them were males and 59 (36,4%) were females. Four patients (2,5%) were tested positive for anti-HD antibodies, all of them have had clinical and/or histological diagnosis of cirrhosis. In multivariable regression analysis, age ($p = .04$), elevation of serum ALT ($p = .03$), elevation of serum AST ($p = .04$), and presence of cirrhosis ($p = .003$) were significantly related to HDV seropositivity.

Conclusion: Although the study demonstrated that Libya has low to moderate prevalence of HDV (2,5%), it is important for policy makers and health care providers to continue the preventive measures for HDV spread, and HBV prevention program including utilization of HBV vaccine. Furthermore, it is imperative to screen chronic HBV patients for HDV for close observation for early diagnosis of subsequent development of liver cirrhosis. Moreover, further epidemiologic and genetic studies are needed to explore the trend for HDV infection in Libya.

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Introduction

Hepatitis D Virus (HDV) is a satellite virus, which was detected by Rizzetto in patients with severe hepatitis B virus (HBV) infection in 1977 [1]. It depends on HBV for the production of its envelope proteins [2]. The epidemiology of HDV infection is similar to HBV but with notable exceptions. HDV occurs worldwide but prevalence data are limited in many parts of the world due to inaccurate reporting and delayed detection. HBV/HDV co-infection is most commonly occurs in the Mediterranean area and parts of South America [3,4]. In Libya, a developing country of approximately 6 million people, hepatitis B remains an important communicable disease because of the inter-mediate prevalence of the carriers and the burden of acute and chronic disease, which is accompanied by an increasing rate of chronic liver disease and its complications including liver cirrhosis and hepatocellular carcinoma [5]. In a recent national and general population-based seroprevalence study included more than 65.000 individuals; the prevalence of HBsAg was reported to be 2.2% [6].

To the best of our knowledge, no data are available on epidemiologic status of HDV in Libya. The aim of present study was to determine the epidemiology of HDV infection among patients

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infected with chronic hepatitis B in the three main tertiary hospitals in Tripoli and Benghazi, Libya.

Patients and methods

Study population

This cross sectional and descriptive study was conducted on 162 consecutive patients with chronic hepatitis B (i.e., HBsAg positive for more than six months) who were followed up at hepatitis clinics in the three tertiary hospitals in Tripoli city (88 patients from Tripoli Medical Centre and Tripoli Central Hospital) and Benghazi city (74 patients from Aljomhoria Hospital) during the period January 2010 and June 2012.

Data collection

A pre-designed structured and anonymous data collection sheet was filled in each clinic for each patient. It included the following data: 1) demographic characteristics (i.e., sex, age, occupation, etc); 2) lifetime history of major invasive exposures, use of recreational intravenous drugs and multiple sexual partners; 3) vaccination history for HBV; and 4) clinical and biochemical laboratory investigations. The diagnosis of liver cirrhosis was established by a combination of physical, biochemical, radiological and endoscopic findings; or by liver biopsy.

Laboratory analysis

A 5–10 mL blood was collected in the clinic and transported within a few hours to the local laboratory in the hospital for separating serum aliquots and stored in a –20 °C deep freezer until testing at the laboratory of the National Center for Disease Control, Tripoli, Libya. HBV markers (i.e., HBeAg, anti-HBe antibodies and total anti-HBc antibodies) were detected using the enzyme linked fluorescent assay (ELFA) (mini Vidas analyzer-BioMerieux, France). Serum samples were tested for HBsAg (Diapro, Italy) and anti-HDV antibodies (DiaSorin, Italy) using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instruction. The sensitivity and specificity of HDV serology are 98%. Both HBsAg and Anti-HDV antibody results were considered positive (reactive) or negative (non-reactive) according to the interpretation parameters provided by the manufactures. A positive control and a negative control were integrated in each run to validate the assay. Extraction of DNA was carried out for all HBsAg reactive samples to by fully automated EZ1 advanced XL, using the extraction kit (EZ1 virus Mini kit v2.0), Germany. The detection of HBV viral load was performed by Real-time polymerase chain reaction (PCR) technique to quantify the levels of HBV-DNA viremia (Rotor-Gene) from Qiagen using detection kit "HBV RG PCR kit', Germany. The cut-off threshold of HBV-DNA by PCR is 3.8 IU/mL. Both HBV and HDV markers as well as PCR assays were performed at the National Centre for Disease Control of Libya, Tripoli. Liver function tests were assessed through auto-analyzer.

Ethical approval

The study was approved by the research ethical committee of Al-Arab Medical University, Benghazi, Libya. It was conducted in accordance with the Helsinki Declaration [7], and under the supervision of the National Center for Disease Control of Libya. All participants signed a consent form before collection of data and blood samples.

Statistical analysis

Clinical data and the test results were analysed using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL, version 19). Descriptive statistics in the form of mean, standard deviations and frequency with percentages were calculated for interval and categorical variables, respectively. Chi-square test between categorical variables and Student's *t*-test for interval variables was used as appropriate. Multivariate analysis was conducted using logistic regression, with HDV serologic results as the dependent variable. Results were considered as statistically significant if p-value (two-tailed) was less than .05.

Results

One hundred sixty-two patients with chronic hepatitis B infection were included in the study. They were diagnosed and followed up at the two tertiary hospitals in Tripoli and Benghazi during the study period. Four of them (2,5%, 95% confidence interval (CI) = 1, 3–3,5%) were tested positive for anti-HD antibodies. The main patient's characteristics according to status of Anti-HDV results are presented in Table 1. The mean age ± standard division (SD) of the total cohort of the patients was 36,92 ± 15,35 (ranged between 16 and 77) years. The mean age ± SD was significantly higher in the Anti-HDV positive patients 38.73 ± 13.48 than Anti-HDV negative patients (33.75 ± 11.73) (p = .040). There was no difference between the two groups in the distribution of gender, risk factors, and hepatitis B markers. In the total of hepatitis B patients, 19 (11,7%) had cirrhosis. The overall seroprevalence of HDV in cirrhotic HBV-infected patients was 21% (4/19). Furthermore, all HDV positive patients were negative for HBeAg.

All anti-HDV four positive patients (100%) had liver cirrhosis which was statistically significant (p = .003) Furthermore, all HDV positive patients were negative for HBeAg. In a multivariable analysis, age (p = .04), elevation of serum ALT (p = .03), serum AST (p = .04), and presence of cirrhosis (p = .003) were significantly related to HDV sero-positivity. Table 2 summarizes the demographic, risk factors, HBV markers and status of liver disease of the four Anti-D positive patients who co-infected with chronic hepatitis B infection.

Discussion

The present study was performed to investigate the seroprevalence of HDV infection and the associated risk factors in patients with chronic hepatitis B infection referred to the main three tertiary Medical Centers in Tripoli and Benghazi-Libya. The seroprevalence of HDV in Libyan patients with chronic hepatitis B was found to be 2.5%. To the best of our knowledge this study is the first report from Libya on HDV infection. This rate is comparable to some previous studies in the region which have reported the prevalence of HDV in regional hepatitis B infected patients [8]. However, a wide range of prevalence rates between 0.6% reported from Lebanon [9] and 23.5% from Egypt [10] observed in the regional studies. Our prevalence rate was similar [11,12] and lower than [13,14] studies reported from different global regions. This variation in HDV prevalence may be due to factors that influence the HDV transmission such as the generally lower socioeconomic status in some countries. As a country of Middle East and North Africa (MENA) region, Libya is located in endemic area of hepatitis B and D [3] and it may require more efficient concentrated screening, prevention, and public health education programs. Moreover, future studies are necessary to monitor the epidemiological trend of hepatitis D infection in Libya. The prevalence of HDV was reported less than 5% in majority of European

Table 1
Comparison between Anti-hepatitis D virus positive and negative groups for demographics, risk factors, hepatitis B markers, liver transaminases and status of liver disease.

Characteristics	Study patients			
	All patients (N = 162)	Anti-HDV positive (N = 4)	Anti-HDV negative (N = 158)	p-value
Age				
(Mean ± SD, years)	36.92 ± 15.35	38.73 ± 13.48	33.75 ± 11.73	.040
(range, years)	(16–77)	(22–47)	(16–77)	
Gender				
Male, N (%)	103 (63.6)	3 (75)	100 (63.3)	.544
Female, N (%)	59 (36.4)	1 (25)	58 (36.7)	
Risk factors N (%)*				
History of dental procedures, N (%)	87 (53.7)	2 (50)	85 (53.8)	.974
History of surgery, N (%)	47 (29%)	0 (0)	47 (29.7)	.422
History of hepatitis in the family, N (%)	39 (24.0)	0 (0)	39 (24.7)	.511
History of hepatitis, N (%)	23 (14.2)	1 (25)	22 (13.9)	.814
History of blood transfusion, N (%)	20 (12.3)	1 (25)	19 (12.0)	.731
History of tattoo, N (%)	7 (4.3%)	0 (0)	7 (4.4)	.899
History of Dialysis, N (%)	3 (1.8%)	0 (0)	3 (1.9)	.949
History of intra-venous drug abuse, N (%)	3 (1.8%)	0 (0)	3 (1.9)	.949
History extra-marital sexual activity N (%)	3 (1.8%)	0 (0)	3 (1.9)	.949
Unknown, N (%)	35 (21.6%)	2 (50)	33 (20.9)	.204
*Hepatitis B markers***				
HBeAg positive, No. (%)	32 (19.7)	0 (0)	32 (20.2)	.411
Anti-HBe antibody positive, No. (%)	136 (83.9)	4 (100)	132 (83.5)	.493
Anti-HBs antibody positive, No. (%)	11 (6.8)	1 (25)	10 (6.3)	.298
Anti-HBc antibody positive, No. (%)	160 (98.7)	4 (100)	155 (98.1)	.951
Hepatitis B level of viremia				
HBV-DNA levels, Mean ± SD (IU/mL) (u/mL)	5476 ± 1210	4751 ± 819	5125 ± 1023	.621
Elevated serum ALT, Mean ± SD	62 (38.3%)	125 ± 14.67	87 ± 10.53	.030
Elevated serum AST, Mean ± SD	55 (33.9%)	109 ± 12.56	79 ± 10.72	.040
Status of the disease:				
Inactive, N (%)	83 (51.2)	0 (0)	83 (52.5)	.836
Chronic hepatitis, N (%)	60 (37.0)	0 (0)	60 (37.9)	.849
Cirrhosis, N (%)	19 (11.7)	4 (100)	15 (9.5)	.003

*More than one risk factor could be present in one patient; ** More than one hepatitis B marker could be positive in one patient

Table 2
Summary of demographic and laboratory features of the four patients co-infected with hepatitis B and D.

Age/Sex	Occupation	Risk Factor	HBsAg	HBeAg	HBeAb	HBcAb	HBV-DNA* (Iu/mL)	Varity of liver disease	Serum ALT** (U/L)	Platelets†
47/Male	Employed	Unknown	(+)	(−)	(+)	(−)	Low (911)	Cirrhosis	149	66×10^9/L
40/Male	Policeman	Blood Transfusion & Dental Procedures	(+)	(−)	(+)	(−)	Undetectable	Cirrhosis	139	95×10^9/L
22/Female	Student	Unknown	(+)	(−)	(+)	(−)	High (5190)	Cirrhosis	19	287×10^9/L
26/Male	Employed	H/O hepatitis & Dental procedures	(+)	(−)	(+)	(−)	High (4520)	Cirrhosis	33	142×10^9/L

*Level of hepatitis B viremia (HBV-DNA, u/mL); **ALT, alanine aminotransferases (normal range 10–40 U/L); †Normal platelets count 150.000–400.000) $\times 10^9$/L; (+), positive; (−), negative.

countries [15]. Although, the rate of HDV infection in a study from Germany was 11%, only 20% of participants were originated in Germany and the others were immigrants from Eastern Europe and Turkey [15]. A similar rate of 8.5% for anti-HDV was reported from England and 85% of them were from Eastern and Southern Europe, sub-Saharan Africa, or Asia [15]. Furthermore, a study from California, USA reported a 6.3% prevalence of HDV [15].

In the present study, transmission of HBV infection was primarily through cutaneous routes. History of dental manipulations, blood transfusion, previous hepatitis, surgery and family history of hepatitis were most frequent risk factors regardless of presence or absence of HDV super- or co-infection. However, many of our patients did not have an identifiable risk factor (in 50% of HDV-positive and 20.9% in HDV-negative patients); probably unsafe injection in the past is responsible for their HBV infection in these patients, although the possibility of missing bias cannot be excluded. Based on these findings, HDV transmission and spread can be prevented by screening high-risk individuals and their families.

In the present study we found that HBV-DNA was lower in Anti-HDV negatives than Anti-HDV negatives. It is well established that hepatitis D is associated with reduced levels of HBV-DNA [16]. This suppression may be in support of the hypothesis that immune responses are likely to play a major role for HBV-DNA replication in hepatitis D infection.

In multivariate analysis, HDV infection remained associated with the older ages, high serum transaminases ALT and AST) and development of liver cirrhosis. In a recent longitudinal study of 237 patients with chronic hepatitis B followed up for an average of 10.6 years, older age, positive HDV, negative HBeAg, platelet count of $<150 \times 10^9$/L, and HBV-DNA level of $\geq$2000 IU/mL were identified as significant independent predictors of liver cirrhosis in multiple logistic analyses [17]. According to our findings, HDV positivity has been found to be the most significant risk factor for the incidence of cirrhosis in patients with chronic hepatitis B infection. Co-infection with HDV worsens the chronic hepatitis B patient's status. HDV-infected patients are more likely to present with serious complications compared to HBV patients without

HDV, and the relative risk of developing cirrhosis in HDV-infected patients is at least doubled [18,19]. The later development of liver cirrhosis in HDV-infected patients decreases the probability of survival to 49% and 40% at 5 and 10 years, respectively [20]. In fact all four HDV positive patients identified in the present study had liver cirrhosis and the overall seroprevalence of HDV in cirrhotic HBV-infected patients was 21%, this figure was unexpectedly high and was similar to the recently reported figures from Pakistan and Iran [12,21].

Even though HBV-DNA viral load was measured by PCR technique, this study has some limitations, such as the relatively small sample size and the method used for HDV detection. The method used for HDV detection was ELISA technique but the confirmation of ongoing HDV infection by PCR testing of HDV-RNA was not performed.

In conclusion, although the present study demonstrated that Libya has low to moderate prevalence of HDV (2.5%), it is vital for policy makers and health care providers to continue the preventive measures for HDV spread as well as HBV prevention program including routine utilization of HBV vaccine. Furthermore, it is important to screen chronic hepatitis B patients for HDV for close observation for the early diagnosis of subsequent development of liver cirrhosis. Moreover, further epidemiologic and genetic studies are needed to explore the trend for HDV infection in Libya.

Conflict of interest

The authors declare that there are no conflicts of interest.

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