

Hepatitis D Virus Infection Among Hepatitis B Surface Antigen Carriers and in “Isolated anti-HBc” Antibodies Profile in Central Tunisia

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Abstract

Background: Hepatitis D Virus (HDV) causes accelerated liver diseases in patients with Hepatitis B Virus (HBV) infection. There is lack of data about its prevalence, related risk factors and interaction with HBV carriers in our country.

Objectives: The aim of this study was to estimate the prevalence of hepatitis delta and associated risk factors among Hepatitis B surface antigen (HBsAg) and “isolated anti-HBc” profile carriers in central Tunisia.

Patients and Methods: In this cross-sectional study, 540 patients with positive HBsAg and 109 “isolated anti-HBc” profile receiving care in a teaching hospital were tested for the presence of HDV serum-markers using commercially available enzyme immunoassay kit. HBV-DNA was detected by nested PCR in “isolated anti-HBc” profile group.

Results: Prevalence of HDV was 8.1% in HBsAg carriers group, but it was significantly higher in active than inactive hepatitis (30.2% and 4.5%, respectively, OR = 9, 95% CI: [4.48-18.58]). There was no significant association between studied risk factors and HDV infection. In the “isolated anti-HBc” profile group, prevalence of HDV was 4.6% and HBV-DNA had negative result in all patients with positive results for HDV.

Conclusions: Although HDV had low prevalence in our area, it is vital to plan preventive strategies for HDV spread as well as HBV prevention. It is particularly important to suspect HDV infection in active HBV carriers to manage a particularly severe dual infection. HDV infection should be suspected even in negative HBsAg patients having “isolated anti-HBc” profile.

Keywords: Hepatitis Delta Virus, Prevalence, Risk Factors, Asymptomatic Infection, Tunisia

1. Background

Hepatitis D is a major global health problem affecting 15 to 20 million individuals worldwide (1, 2). Hepatitis D virus (HDV) is a small defective RNA virus dependent on Hepatitis B virus (HBV) for its replication and expression (3). The global prevalence of HDV is poorly known due to lack of studies in several parts of the world (4, 5). It is estimated that 5-20% of Hepatitis B surface antigen (HBsAg) carriers are co-infected with HDV. HDV infection results in more severe chronic hepatitis B and occasionally fulminant form of acute hepatitis in co-infected patients (6). Dual HDV-HBV infection results in a 2-fold increase in mortality and a more rapid progression to cirrhosis and hepatocellular carcinoma compared with HBV infection without HDV (1, 4, 7).

Several cross-sectional studies showed that HDV infection is frequently associated with suppressed HBV replication, reducing the rate of HBV DNA and with fluctuation of HBsAg production (8, 9). Many Tunisian studies have been conducted to determine epidemiology and risk factors of HBV infection (10-12). HDV infection and its risk factors have been little studied in our country.

2. Objectives

The aim of this study was to estimate the prevalence and main related risk factors of HDV infection in the center of Tunisia in two groups of patients with different HBV serum markers.

3. Patients and Methods

3.1. Population

In this cross-sectional study, 649 patients attending Farhat HACHED hospital for known HBV infection or systematic screening were included. Patients' sera had reached the laboratory of virology between January 2011 and April 2012. There were two groups of patients; 540 HBsAg positive carriers and 109 “isolated anti-HBc” antibodies carriers with positive anti-HBc antibodies and negative HBsAg and anti-HBs antibodies (Table 1).

Informed consent was obtained from each patient and a questionnaire was filled including the following data: age, gender, history of blood transfusion or scarifications, intravenous drug abuse, “high-risk” sexual behavior and history of familial hepatitis, clinical symptoms and alanine aminotransferase (ALT) elevation level.

3.2. Serological Tests

Serum samples were already tested for markers of hepatitis B (HBsAg, HBeAg, anti-HBs, anti-HBc and anti-HBe antibodies) by automated microparticle enzyme immunoassay (AxSYM system / Abbott®, Germany) with sensitivity of 98-100% and specificity of 97-99.5%. Samples were then tested for anti-HDV antibodies (IgG) with third generation enzyme-linked immunosorbent assay (Globe Diagnostics®, Italy) with sensitivity and specificity of 99.8 and 100%.

3.3. Molecular Tests

Detection of HBV DNA by polymerase chain reaction (PCR) was performed in patients with an “isolated anti-HBc” profile and concerned two regions of the HBV genome: the pre-S and C regions with a global sensitivity of 10^3 copies/mL. After extraction of the DNA (QIAamp DNA Blood Mini kit; Qiagen®), amplification in the pre-S region was performed by a classical PCR according to the protocol of Lindh (13). Then, a nested PCR was performed in the C region according to a protocol described by Alhababi (14).

3.4. Statistical Analysis

Data analysis was performed by software Epi Info version 6.0, using Chi2 and Fisher’s exact tests. A $P < 0.05$ was considered statistically significant. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

4. Results

Overall, 649 patients were enrolled in this study. The

mean age was 36.01 ± 11.6 years (14 to 82 years) and male/female ratio was about 0.7. These patients were active carriers in 95 patients (14.6%). HBeAg had positive result in 34 patients and all of them belonged to the first group (Table 2). Among these positive HBeAg, there were 17 cases of chronic hepatitis B, including 14 active hepatitis and 17 cases discovered during a routine screening. In the “isolated anti-HBc” profile group, the average age was 31.3 ± 9.6 years, gender ratio was 0.6 and 17.4% of patients had at least one clinical symptom or biological disorders (Table 2).

Of 649 patients, 49 had positive result for HDV IgG antibodies, yielding an overall seroprevalence of 7.5%. There was no significant difference between HDV-positive and HDV-negative patients regarding sex (male/female ratio = 0.7) and mean age (32.2 years vs. 35 years, $P > 0.05$). HBeAg was absent in 84% of HDV positive sera.

In the first group, the prevalence of positive HDV antibodies was 8% (44/540 patients). No significant association was observed between hepatitis D and family history of jaundice or personal history of blood transfusion or having multiple sex partners (Table 3). However, there was a significant higher rate of HDV negative status in HBsAg asymptomatic (inactive) carriers ($P < 10^{-3}$, OR = 9, 95% CI: [4.48-18.58]). Among patients with active hepatitis and positive HDV status, most frequent symptoms were jaundice and elevated ALT ($P < 10^{-3}$) (Table 3).

In the second group, 5/109 patients had positive HDV antibodies (4.6%). These five patients had positive anti-HBe antibodies and all of them were asymptomatic; two candidates for medically assisted reproduction, two blood donors and one pregnant women. In this group, HBV DNA was detected in 4/109 sera (3.6%), but in none of the five HDV positive ones.

Table 1. General Characteristics of Studied Population^a

Variables	Group 1 Positive HBsAg	Group 2 “Isolated Anti-HBc” Profile	Total
Known chronic HBsAg carriers	335 (62)	0	335 (51.6)
Systematic screened patients for HBsAg			
Pregnant women	100 (18.5)	35 (32.1)	135 (21)
Candidates for medically assisted reproduction	64 (11.9)	44 (40.4)	108 (16.6)
Poly transfused	22 (4.1)	5 (4.6)	27 (4.1)
Health care workers	10 (1.8)	8 (7.3)	18 (2.7)
Blood donors	9 (1.7)	17 (15.6)	26 (4)
Total	540 (100)	109 (100)	649 (100)

^aValues are expressed as No. (%).

Table 2. Different Symptoms/Biological Disorders in the Two Studied Groups^a

Variables	Group 1 HBsAg Positive (n = 540)	Group 2 "Isolated Anti-HBc" Profile (n = 109)	Total
Asymptomatic patients	464 (86)	90 (82.5)	554 (85.4)
Symptomatic patients^b	76 (14)	19 (17.4)	95 (14.6)
Symptoms/biological disorders			
Jaundice	22 (4)	3 (2.7)	25 (3.8)
Elevated ALT > 40, UI/L ^c	55 (10)	12 (11)	67 (10.3)
Cirrhosis	8 (1.4)	4 (3.6)	12 (1.8)
Liver function failure	4 (0.7)	0	4 (0.6)
Positive HBeAg	34 (6.3)	0	34 (5.2)

^aValues are expressed as No. (%).^bOne patient may have more than one symptom or biological disorders.^cAlanine Aminotransferase.**Table 3.** Distribution of Positive HDV Antibodies According to Risk Factors and Clinical/Biological Condition Among 540 HBsAg Carriers

Variables	Prevalence of HDV Ab		Total	P Value	OR	CI 95%
	Positive (n = 44)	Negative (n = 496)				
Risk factors^a						
History of hepatitis B in the family	1 (3.5)	27 (5.4)	28	0.7	0.4	0.01 - 2.58
Surgery	0	20 (4)	20	0.3	NA	NA
Transfusions	0	24 (4.8)	24	0.2	NA	NA
Tattoo/scarifications	3 (12.5)	21 (4.2)	24	0.4	1.6	0.3 - 5.9
Multiple sex partners	0	7 (1.4)	7	> 0.99	NA	NA
Intravenous drug users	0	2 (0.4)	2	> 0.99	NA	NA
Clinical/biological condition						
Asymptomatic carriage of HBsAg	21 (4.5)	443 (89.3)	464	< 10 ⁻³	9	4.48 - 18.58
Symptomatic patients ^b	23 (30.2)	53 (10.7)	76			
NA Jaundice	6 (27.2)	16 (0.3)	22	< 10 ⁻³	4.7	1.42 - 13.63
NA Cirrhosis	1 (12.5)	7 (1.4)	8	0.49	1.6	0.03 - 13.11
NA liver function failure	1 (25)	3 (0.6)	4	0.28	3.8	0.07 - 48.5
NA Elevated ALT > 40, UI/L	10 (18.1)	45 (9)	55	< 10 ⁻³	2.9	1.21 - 6.59
Positive HBeAg	4 (11.7)	30 (6)	34	0.5	1.5	0.37 - 4.73

Abbreviations: Ab, antibodies; ALT, alanine aminotransferase.

^aOne patient may have more than one risk factor.^bOne patient may have more than one symptom or biological disorders.

5. Discussion

Prevalence and risk factors of HDV infection are closely related to those of HBV (15). Tunisia is an intermediate endemicity area for hepatitis B, but HDV prevalence is not well known in some parts of the country (11, 16). In our study, the prevalence of anti-HDV antibodies in inactive HBsAg carriers was 4%. When compared to previous Tunisian studies, our result confirms the declining trend of hepatitis D prevalence from 33% in 1990 (17) and 16.1% in 1997 (11) to 6.8% in 2009 (16). This decrease can be explained by improvements of socioeconomic conditions, awareness of transmitting viruses and above all, a better control of HBV infection and systematic vaccination since 1995. A decline

in HDV infection has also been reported since the 90th in others countries with low endemicity of HBV infection such as Italy, Spain, Turkey and Taiwan (18-20). However, in the last decade the prevalence of HDV remained constant in some countries such as England and France, due to migration of subjects from endemic areas (1). In contrast, the prevalence of HDV may exceed 60% in countries of high endemicity for HBV, such as India or center Africa (21, 22).

In our work, none of the studied risk factors was associated with HDV infection; although, the possibility of missing bias cannot be excluded. In Anglo-Saxon countries, the main risk factor for infection with HDV is intravenous

addiction (23, 24). In endemic areas, household transmission is the most common mode of transmission for HDV, but sexual and nosocomial risk were described (25). This intra-familial contamination is probably important in our country, as it is prominent in HBV transmission (11, 12, 26).

According to Tunisian and European data, our study showed no significant difference in HDV prevalence between the two genders (17, 27). Furthermore, more than 80% of HDV positive patients have negative result for HBeAg. Other studies showed similar results with up to 90% of HDV (+) patients having negative finding for HBeAg (28, 29). These findings may be due to high rate of precore region mutations, which can exceed 90% of Tunisian patients infected chronically with HBV (30-32). In addition, it has been described that HDV inhibits the expression of HBeAg through its ribozymes, which are enzymes able to destroy mRNAs that encode for Pre/C region (33).

Our data showed that patients with jaundice and elevated ALT were more likely to have HDV-positive status than inactive HBsAg carriers, which could be explained by a more severe infection in co-infected patients (HBV/HDV). As proposed in other countries such as the USA, HDV status is searched in all patients with active B hepatitis (30, 34, 35). In our study, there was no correlation between cirrhosis, liver failure and HDV-positive status, but these two pathologies were not enough represented. Indeed, other studies conducted on a larger population of positive HDV patients, have shown rapid and frequent progression to liver cirrhosis and liver failure (34).

In the "isolated anti-HBc" profile group, the prevalence of HDV was about 4.5%, which is almost similar to the level found in inactive HBsAg carrier's group. Positive HDV status may reflect either past healed co/super-infection or current co/super-infection with both viruses. HBsAg and HBV-DNA apparently fluctuate in longitudinally studied patients because of interaction and competition between HDV and HBV (1, 8, 9, 35). Thus, occult hepatitis B associated with HDV cannot be excluded and should be searched. In our study, none of the five positive-HDV patients had detectable HBV-DNA. The use of more sensitive technique with lower threshold may allow us not to miss occult hepatitis, since HBV-DNA is often low in double infection (8, 9, 36). Of these five patients, there was a pregnant woman and two candidates for assisted reproduction, exposed to a risk of mother to child viral transmission. Indeed, vertical transmission of HDV is possible and has been demonstrated by phylogenetic analysis of the strains of a mother and her child in Taiwan (37). Two other patients were also at high risk of HBV and HDV dissemination as they were blood donors (38). Systematic screening for anti-HBc or viral HBV-DNA in these populations could reduce the risk of spread of these two infections.

The prevalence of HDV infection in our region enhances other Tunisian data and introduces our country as a low endemicity area. It also confirms the downtrend in the prevalence of this infection in last decades. It would be interesting to complete this work by phylogenetic studies

clarifying the role of family transmission in HDV infection.

Regarding the severity of dual infection by HBV and HDV, we would propose that future Tunisian guidelines emphasize the relevance to test for HDV in all patients with active B hepatitis. Also not to miss HDV infection among patients with occult hepatitis B, especially in population of high risk of spread of both viruses.

Footnotes

Authors' Contribution: Study concept and design: Salma Mhalla and Naila Hannachi; acquisition of data: Salma Mhalla and Sana Alibi; analysis and interpretation of data: Salma Mhalla, Naila Hannachi and Yosr Kadri; rafting of the manuscript: Salma Mhalla; critical revision of the manuscript for important intellectual content: Naila Hannachi; statistical analysis: Salma Mhalla and Sana Alibi; administrative, technical and material support: Jalel Boukadida; study supervision: Jalel Boukadida and Amel Letaief.

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