Sero-prevalence of hepatitis D virus in patients with hepatitis B virus in North-Western Libya

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Abstract

Several reports indicated a declining trend in the occurrence of hepatitis D virus (HDV) infection in some geographical areas. However, no study has been published in Libya to determine the sero-prevalence of HDV in this part of the world.

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The present study was undertaken to evaluate the sero-prevalence of HDV in patients with hepatitis B virus (HBV) attending Saint James hospital in Tripoli. A total of 346 patients with HBV were evaluated for the presence of anti HDV antibodies and delta antigen using commercially available ELISA kits. HDV co-infection was detected in 6 patients (anti HDV antibodies in 3 patients and the delta antigen in another 3 patients) (1.734%). Our results suggest that delta infection may not be very common in

1 Introduction

Hepatitis delta virus (HDV), which was first defined by Rizetto in 1977 in Italy, is a defective RNA virus that lacks pathogenicity on its own. It requires the presence of hepatitis B virus surface antigen (HBsAg) for replication (1). Following its replication in the nucleus of the hepatocyte, it is transferred to cytoplasm, where it is enveloped by HBsAg and secreted into the circulation (2). Hepatitis delta virus is a member of the viral sub-family referred to as satellites and has co-infected more than 10 million people who are infected by hepatitis B virus (HBV). Clinically delta infection manifests itself as a co-infection or a super-infection. In co-infection, both HBV and HDV are responsible for the acute infection (3, 4). This form of the disease spontaneously resolves in the majority of the cases, with 5% developing chronic disease and the reported mortality is between 2% and 20%. In super-infection, additional HDV infection develops in an HBsAg carrier. In this form, the risk of developing chronic disease is high (50-70%) with accompanying risks of chronic active hepatitis and cirrhosis. Hepatitis delta virus accounts for 3-25% of fulminant hepatitis cases. The most important difference between super-infection and co-infection is the higher rate of chronicity and cirrhosis following the acute infection in the former.(5) In

contrast to sufficient amounts of data regarding HBV infections for Libya, data regarding HDV infection is scarce and, to our knowledge; there has been no report on HDV infection in Libya. Therefore, we assessed the anti-HDV prevalence in HBsAg carriers.

METHODS:

Between January 2006 and February 2007 HBV DNA, Anti-HDV and Delta Ag screening was performed at Division of Molecular Diagnostics, Saint James Medical Laboratory, Saint James Hospital, Tripoli, Libya in a total of 346 patients with hepatitis B infection. The diagnosis of hepatitis B infection was based on HBV DNA positivity (Molecular diagnosis), so HBV DNA diagnosis was performed using real time polymerase chain reaction (RT PCR), that by using HBV quantification kit (RoboGene, Germany). However, we used RT PCR to evaluated levels of HBV DNA in serum and then assessed the relationship between these levels and the extent of HDV infection HDV detection was done by serology, anti-HDV antibodies and HD-Ag was detected using third generation ELISA kits (Adaltis, Italy).

The molecular and serological tests were performed using commercially available PCR and ELISA kits according to the instructions provided in the manufacturer’s manual.

The association between Hepatitis Delta infection and hepatitis B infection status, patient's age and gender were explored. Statistical analyses were performed with manual Statistical formulas.

Results:

In the total of 346 patients with Hepatitis B infection (HBsAg and HBV DNA positive), 211 patients

(61%) were males and 135 (39 %) were females. The patient's age was ranging from 11 – 70 years and the mean ages of 346 HBV patients were 31.82 (Table 1, 2).

Table 1: Hepatitis B, Delta infection percentages.

	SEX		Age groups			Patients total n= 346
	Male n= 211	Female 135 =n	Y 25-10 n= 114	Y 40-26 n= 163	Y n= 69 41≤	
DNA -HBV	(%61) 211	(%39) 135	(%33) 114	(%47) 163	(%20) 69	346
HDV-Anti	(%0.9) 2	(%0.7) 1	(%0.87) 1	(%1.2) 2	(%0) 0	(%0.86) 3
Ag-HD	(%1.4) 3	(%0) 0	(%0.87) 1	(%0.6) 1	(%1.4) 1	(%0.86) 3
HDV positively	(%2.4) 5	(%0.7) 1	(%1.7) 2	(%1.8) 3	(%1.4) 1	(%1.73) 6

Table 2: Analysis of patients' age

Statistical of HBV patients age	
Mean	31.82
Std. Error of mean	0.63
Mode	29
Std. Deviation	11.75
Variance	137.95
Range	59
Minimum	11
Maximum	70
Patients Total	346

HDV infection was detected in 1.73% (6/346) of these cases (Table 3). The prevalence of anti-HDV positivity among the HDV positive cases were 50% (3/6), and the prevalence of Delta Ag positivity among the HDV patients were 50% (3/6) (Table 4).

Table 3: HDV Prevalence in 346 patients with HBV infection

HDV	Count	Percentage	Mean of ages	Std. Deviation
NEG	340	%98.27	31.59	11.78
POS	6	%1.73	33.50	10.03
Total	346	100	31.82	11.75

Table 4: HDV infection in the positive cases

HDV	Count	Percentages	Mean of ages	Std. Deviation
Ag	3	%50	36.00	13.45
Abs	3	%50	31.00	7.21
Total	6	100	33.50	10.03

The sero-prevalence of Hepatitis Delta infection among 211 males was 2.37% (5/211), and the sero-prevalence of Anti-Delta-Abs and Delta Ag among 135 females was 0.74% (1/135) (Table 5).

Table 5 :Analysis of patients' gender

Gender	HDV		Total	Mean	.Dev .Std	SE Mean
	NEG	POS				
M	206	5	211	32.2	12.1	0.83
F	134	1	135	31.4	11.8	1.00
Total	340	6	346	31.8	11.7	0.63

Data from this experiment was prepared and tabulated in Excel. The mean age for male and female were calculated. The significance of the difference between mean ages was tested using a Two-sample t – test at a confidence interval for differences in means of 95 %, i.e. A P value < 0.05 was considered to be statistically significant. All statistical analysis was carried out using Mini Tap 14.

A one-way analysis of variance was carried out to evaluate the difference in the effect of age on HBV.

DNA (IU/ml). When necessary, the significantly different pairs of means were identified using Fisher's test (the student t test). Simple regression analysis was carried out to investigate relationship between age for a total of 346 HBV patients, Optical Density (OD) at 450 nm and cut-off for Hepatitis Delta Antigen (HDV Ag) and Anti Hepatitis Delta Antibodies (Anti HDV Abs) positive samples, and a corresponding HBV DNA (IU/ml). Statistically, the difference in mean age for male and female was not significant. Also, the analysis indicated that the HDV prevalence and HBV DNA (IU/ml) significantly were not affected by age. No statistically significant relationship was found between patients' age, gender and a corresponding HBV DNA (IU/ml).

DISCUSSION:-

The reported incidence of anti-HDV and Delta Ag sero-positivity among HBsAg carriers shows a great variation at different regions of the world, so Delta hepatitis is common in some areas of the world with a high prevalence of HBV infection, particularly the Mediterranean region, parts of Eastern Europe, the Middle East, Africa and South America (6). However, there were many reports indicated a declining trend in the occurrence of HDV infection in some geographical areas (7,8,9). For example, while HDV was responsible for a high proportion of cases of acute and chronic liver disease in Southern Europe during the 1970s, its sero-prevalence was reported to have declined substantially in 1997 (10, 11).

In our country, there were no reported incidences of HDV infection among healthy carriers and in patients with Hepatitis B infection. Considerable epidemiological data exist regarding HBV and HDV in other regions of North Africa and Middle East, while information about HDV carriage is lacking in Libya.

In the present study 6 (1.734%) of the 346 patients with Hepatitis B infection were anti-HDV and Delta Ag positive. So sero-prevalence of HD-Ag was 0.867% (3/346), and sero-prevalence of Anti-HDV Abs was 0.867% (3/346), our results suggest that delta infection may not be very common in Libyan patients with HBV infection. However, at the nearly geographical areas such as Tunisia and Egypt there were several reports incidence of Delta Ag and Anti-HDV sero-prevalence. So a study in Tunisia tested serum samples from 33363 healthy people for serological markers of hepatitis B, C and Delta viruses, concluded that overall sero-prevalence for HDV was 17.7%. In this study HDV super-infection occurred later than HBV infection and increased with age in parallel with HBV. In addition, HDV super-infection was also found to be common in Tunisia and occurred in almost 44% of individuals infected with HBV (12). And another study indicated that Hepatitis Delta virus may be endemic in Tunisia and does not inhibit HBV replication (13). While in our study the frequency of Anti-HDV and HD-Ag was significantly lower in patients with Hepatitis B infection, and presence of HDV infection does not inhibit HBV replication.

In a study from Egypt 45 Egyptian children, 24 of them have liver cirrhosis and 21 have chronic hepatitis were examined against Anti-HDV and Delta-Ag, concluded that, the prevalence of HDV infection was 8.9% of Egyptian children with chronic liver disease, and HDV infection in children was associated with advanced chronic liver disease (14). In our study we didn't have any positive cases of a total of seven children screened against HDV infection.

In Nigeria, HDV antigen (HD-Ag) was found in the sera of two of 31 (6.5%) patients. Among the blood donors and university freshmen HD-Ag was present in none. In addition, liver biopsies of 28 other patients were stained for HD-Ag, none of these was positive. The study

concluded that HDV prevalence was low in our community, and suggest that the virus might play only a minor role in the pathogenesis of HBsAg associated chronic liver disease (15). While in our study the prevalence of HD-Ag was 3/346 (0.86%).

A study from Saudi Arabia evaluated the prevalence of Anti-HDV in a total of 81 HBsAg carries (75 were intravenous drug users and six were non-IDUs). The overall prevalence of anti-HDV among HBsAg positive patients was 13.6%. Among HBsAg positive IDUs, it was 14.7% and it was 0.0% for HBsAg positive non-IDUs (16). While in our study we didn't have patients with IDUs, but the prevalence of Anti-HDV-Abs in HBsAg carries was 3/346 (0.86%).

In Switzerland, 640 HBV infected women, were screened for HBsAg and Anti-HDV. Of these 640 patients, 61 (9.5%) were positive for HBsAg, and only five women were positive for Anti-HDV-Abs (17). While in our study, a total of 135 HBV infected female were screened for Anti-HDV-Abs, so the prevalence of Anti-HDV was 0.7% (1/135). In conclusion, in this study from Tripoli, Libya, where a moderate prevalence of HBV infection exists, the frequency of anti-HDV and Delta-Ag positivity was significantly lower compared to that at nearly geographical areas, and Hepatitis Delta virus may be dose not endemic in north-western Libya and does not inhibit HBV replication.

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