OCCULT HBV INFECTION IN HIV-INFECTED LIBYAN PATIENTS WITH POSITIVE ANTI-HBC

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ABSTRACT

Occult hepatitis B infection (OBI) is common among human immunodeficiency virus (HIV) cases. Co-infection with HIV may worsen HBV infection. We aimed to determine the OBI prevalence in positive hepatitis B core antigen antibody (anti-HBc) HIV-infected patients. One hundred forty one hepatitis B surface antigen (HBsAg) negative Libyan HIV cases were tested for CD_4 T cell count and anti-HBc. Positive anti-HBc samples were evaluated for hepatitis B surface antigen antibody (anti-HBs) and HBV DNA by real-time polymerase chain reaction (PCR). Positive anti-HBc was detected in 82/141 (58.2%) of the studied cases. Isolated anti-HBc was found in 36/141 cases (25.5%) . OBI was detected in 9/82 (11%) cases out of anti-HBc positive cases. Five (55.6%) of them was associated with isolated anti-HBc. CD_4 T cell count was lower than 350 cells/mm^3 in all OBI cases (P = 0.001). We concluded that OBI is common in Libyan HIV patients with positive anti-HBc and a larger study to find the prevalence of OBI in HIV-infected cases would be of advantage.

Keywords: HBV, HIV, Isolated anti-HBc, OBI.

1. INTRODUCTION

Hepatitis B virus (HBV) which belongs to the Hepadnaviridae family, is an enveloped, partially double-stranded deoxyribonucleic acid (DNA) virus [1][2]. Globally, HBV causes chronic infection in approximately 370 million patients and the human immunodeficiency virus (HIV) causes around 40 million chronic infections. Nearly two to four million HIV patients are chronically co-infected with HBV [3]. Greater than two thirds of a million deaths occur yearly due to complications of chronic HBV infections, such as hepatoma and liver cirrhosis [4]. Co-infection of HIV cases with HBV has been observed to alter the prognosis of HBV infection,

increasing the mortality and morbidity rates. It is obvious that HBV and HIV share modes of transmission which makes infection with HBV is frequent among those infected with HIV [5]. While in several studies, the definition of occult HBV infection (OBI) is positivity of HBV DNA with negativity of hepatitis B surface antigen (HBsAg), with/without to HBV core antigen antibody (anti-HBc), in others the definition of OBI is viremia of HBV associated only with isolated anti-HBc positivity [6]. OBI is also defined by detection of less than 200 IU/mL HBV DNA in blood or liver with untraceable HBsAg , regardless of anti-HBc or hepatitis B surface antigen antibody (anti-HBs) status, taking in consideration the window period which should be excluded [2]. According to the serological profile of HBV, OBI cases can be divided into seropositive type with positivity of anti-HBs and/or anti-HBc, or seronegative type with negativity of both anti-HBs and anti-HBc [7].

The OBI is diagnosed by HBV DNA detection in the serum or in the liver of HBsAg negative cases. Detecting HBV DNA in hepatocyte DNA extracts is regarded as the gold standard. Though, detecting serum HBV DNA is a much easier procedure to be done and a largely more commonly performed diagnostic technique. In some circumstances, using anti-HBc positivity can be assumed as a substitute marker for detecting OBI [8]. Although the prevalence of OBI is higher among individuals infected with HIV than those who are not infected with HIV, the OBI natural history among HIV infected people is still unclear [9]. The growing evidence of clinical influences of OBI is the key point behind the rising interest in studying it. Really, the transmission of OBI may be occurred, chiefly via blood transfusion, causing hepatitis B; in immunosuppressed cases, it may result in viral reactivation;

it might have a damaging consequences on the progress of chronic liver disease; it plays a substantial role in developing hepatoma [8]. OBI diagnosis is with critical importance in immunosuppressed individuals and those on chemotherapy, as those people are at a higher risk of reactivation of HBV or even fulminant liver failure [10].

As the co-infection of HIV and HBV is frequent, the co-infection may greatly rise the disease evolution in comparison with HBV mono-infection. According to WHO recommendations; every HIV-infected individual should be routinely screened for HBV infection and antiretroviral treatment should be initiated immediately in HBV and HIV co-infected cases. In case of unavailability of molecular diagnosis, the anti-HBc positivity and the HBsAg negativity are used to predict OBI [7]. According to a serological survey run in Libya in 2003 [11]; the HBV prevalence was 2.2%. One study [3] showed that HCV, HIV, HBV and co-infection are quite frequent among Libyans. The HBsAg prevalence was 3.7%, anti-HIV was 0.15%, anti-HCV was 0.9%, while co-infection was 0.02%. So we should be aware of OBI in HIV-infected persons. We conducted this study in Tripoli, Libya, to determine the prevalence of OBI in anti-HBc positive Libyan HIV patients by evaluating HBV DNA.

2. METHODS

Our study is cross-sectional study was run in the Infectious Disease Department of (IDD) of Tripoli Medical Center (TMC) and in the National Center for Infectious Disease Prevention and Control (NCIDPC). In the period between January and May 2008. We decided to analyze these data which we collected a long time ago due to many reasons: limited resources, the importance of OBI among HIV patients and the fact that we

have not found any study regarding OBI in Libyan HIV patients. The cases were included if their diagnosis of HIV infection was confirmed by polymerase chain reaction (PCR) and they were HBsAg negative by enzyme-linked fluorescence immunoassay (ELFA). One hundred and forty one HIV-infected individuals with negative HBsAg were involved in this study. Informed consent was obtained and a sample of blood (5 ml) was collected under aseptic technique from every case. We divided every sample into 2 test tubes: one tube with EDTA for detecting CD_4 T helper cell count by flow cytometry. The other tube was centrifuged at 3000 rpm to separate the serum. After that samples with separated serum were stored at -20 °C till tested. The cases were divided into 5 age groups: 20-29, 30-39, 40-49, 50-59, older than 60 years. These negative HBsAg samples were further tested for anti-HBc. Samples with positive anti-HBc were then tested for anti-HBs and HBV DNA. Testing of HBsAg, anti-HBs and anti-Hbc IgG was done by ELFA, (Biomérieux, Marcy-l'Etoile, France) according to the guidelines of manufacturer. The research for HBV viral DNA was done by real-time PCR (HBV Real- TM Qual). iQ kit (NLM, Italy) that has a sensitivity of 105 IU/ml.

SPSS statistics software (version 26.0) was used to analyze data. While categorical variables were expressed in percentage, Fisher's exact test and chi-square test were used to identify the statistical significance of the difference between groups of categorical variables. The mean ± standard deviation was used to express continuous variables while Student's t-test analyzed the statistical significance of the difference between means of two groups of the continuous variables.

We considered P value ≤ 0.05 as statistically significant in our study.

3. RESULTS

In our study, 141 HBsAg-negative HIV-infected cases were studied for previous exposure to HBV infection by detecting anti-HBc. They aged between 20 years and 63 years with an age range of 43 years. The mean age was 35.77 ± 6.446 years. About 2/3 of cases aged between 30 and 39 (94, 66.7%) and 25 cases aged between 40 and 49 year (17.7 %). It was only one case older than 60 years (0.7%). The majority of the cases were males (118, 83.7%) while females were 23 cases (16.3%). Anti-HBc was positive in 82/141 (58.2%) cases which were further evaluated for anti-HBs, HBV DNA. Their ages were between 25 years and 48 years. Their mean age was 35.43 ± 5.092 years. More than 2/3 of them aged between 30 and 39 (57, 69.5%), 18 cases aged between 40 and 49 year (18.3 %) and 10 cases aged between 20 and 29 years. The majority of these cases were males (77, 93.9%) and there were only 5 female cases (6.1%). Forty six out of 141 cases (32.6%) were positive for both anti-HBc and anti-HBs. Thus in 36/141 cases (25.5%) the anti-HBc was the only positive serological marker.

HBV DNA was detected in 9 cases out of the 82 anti-HBc positive tested cases (11%); 5 (55.6%) of them was associated with isolated anti-HBc while the other 4 (44.4%) cases were positive for both anti-HBc and anti-HBs. CD_4 T cell count was lower than 350 cells/ mm^3 in all the nine cases with positive HBV DNA (P = 0.001). Otherwise, there was no any significant statistical association between positive HBV DNA and sex (P = 0.551), age (P = 0.415), isolated anti-HBc (P = 0.496) , age group (P = 0.3) (table 1).

TABLE 1. COMPARISON OF SOME DEMOGRAPHIC FACTORS, SEROLOGICAL PARAMETERS AND CD_4 T CELL COUNT BETWEEN HBV DNA POSITIVE AND HBV DNA NEGATIVE IN HBSAG NEGATIVE HIV-INFECTED CASES WITH POSITIVE ANTI-HBC.

Characteristics	HBV DNA (+)	HBV DNA (-)	*P* value	The involved test
Gender				
F	1	22	0.551	Fisher test
M	8	110		
Age (mean± stand deviation)	34.11 ± 4.137	35.59±5.198	0.415	t-test
CD_4 T cell				
< 350	9	31	0.001	Fisher test
> 350	0	42		
Isolated anti-HBc	5	31		Fisher test
Anti-HBc & anti-HBs	4	42	0.496	
Age groups				
20-29	1	9		
30-39	8	49		
40-49	0	15	0.3	Pearson
50-59	0	0		Chi-Square test
> 60	0	0		

4. DISCUSSION

OBI prevalence in HIV infected subjects is varied globally from zero to 90%, according to the location and risk of exposure to HBV [5]. According to some studies [12], the prevalence of OBI among HIV positive subjects was 19.1% in South Africa, 15% in Ivory Coast, 10–15% in Sudan and 6.9% in Cameroon. According to other studies, OBI prevalence in sub-Saha ranged between 15.1 and 24 % among HIV positive cases [9]. In India; Panigrahi *et al.* [13] found that 15.9% of studied HIV positive drug users

had OBI. A high rate of OBI (26.8%) among HIV infected cases was reported in Sudan [14]. Saha *et al.* [15] reported that the prevalence of OBI among Indian HIV positive cases was 6.3% and most of them were anti-HBc positive. Salyani [9] *et al.* detected OBI in 5.3% of HIV positive cases in Kenya. In Cameroon, Gachara *et al.* [4] reported OBI among 5.9% of HBsAg-negative HIV-infected cases. Several factors might be responsible for this variation in OBI prevalence including prevalence of HBV in these countries, hepatic diseases, screening tests used to detect HBV. Furthermore, the intermittent positive HBV DNA in some people might play a role in this variation [12].

In this study we evaluated HBV DNA in only HIV cases with HBsAg negative and anti-HBc positive due to a shortage of resources. We studied 141 HBsAg-negative HIV infected Libyan cases, of them 82 (58.2%) were anti-HBc positive. Honarmand *et al.* [16] found that 46.4% of HBsAg negative HIV Iranian cases were anti-HBc positive. In India, Panigrahi *et al.* [13] found that nearly 40% of HBsAg-negative HIV infected subjects were anti-HBc positive. Furthermore, Abdelaziz *et al*. [14] reported anti-HBc positivity in 41.62% of their 197 studied Egyptian cases. Also Opaleye *et al.* [5] in Nigerian study, found that 29.2% of HBsAg-negative HIV studied cases were anti-HBc positive. So it is obvious that these studies reported lower frequencies of anti-HBc positivity among HBsAg-negative HIV patients than what we did.

In our study, OBI was detected in 9 (11%) cases out of the 82 anti-HBc positive HBsAg-negative HIV patients; 5 (55.6%) of them was associated with isolated anti-HBc while the other 4 (44.4%) cases were positive for both anti-HBc and anti-HBs. Higher frequencies were detected in several

studies including Iranian study run by Honarmand *et al.*[16] who reported that OBI prevalence was 63% among HIV cases with positive anti-HBc. In addition; Panigrahi *et al.* [13] identified OBI in 31.5% of anti-HBc positive HIV infected cases in one study, and in their another study, they observed OBI in 21.3% of anti-HBc positive blood donors. Patel *et al.* [17] in Ethiopia, also described a high prevalence of OBI (19.1%) among anti-HBc positive HIV infected subjects. However; A lower prevalence was reported by Azmoudeh-Ardalan *et al.* [7] who detected OBI in 3.2% of anti-HBc-positive HIV infected cases in Iran. In our previous study [18], we found that 3% of 100 Libyan HBsAg-negative blood donors (HIV negative) had isolated anti-HBc and 11% were positive for both anti-HBc and anti-HBs without detecting any case with OBI among all positive anti-HBc blood donors. However; One study suggested [12] that in case of a shortage of PCR, anti-HBc can be used as an alternative indicator to predict seropositive OBI. Moreover, anti-HBc can be used as a screening test to detect individuals with previous HBV exposure whom are at high risk for HBV reactivation if their immunity status is disturbed.

We found isolated anti-HBc in 36 cases out of the 141 enrolled cases (25.5%). The prevalence of isolated anti-HBc among HIV patients varies from 17% to 40% [6].A higher prevalence than ours was detected in Mozambique which was 45.2% [19]. However; a lower frequency than ours was detected by Abdelaziz *et al.* [14] who found isolated anti-HBc in 17.77% of HIV Egyptian cases. Isolated anti-HBc pattern may be attributed to: i) waning immunity in resolved infection of HBV with undetectable levels of anti-HBs. ii) OBI. iii) the window phase of acute infection of HBV as the antigen antibody complexes precipitate producing an apparent

isolated anti-HBc pattern. iv) false positive results. The commonest cause of the isolated anti-HBc status is probably waning immunity [6].

We detected OBI in 5 cases (11.1%) of isolated anti-HBc HIV patients. Worldwide, The frequency of OBI among isolated anti-HBc HIV infected cases ranges from 0.6% to higher than 89.5% [6]. A lower frequency than ours was described in Mozambique by Carimo *et al.* [19] who found that OBI prevalence among isolated anti-HBc HIV positive cases was 8.3%, revealing a weak significance of isolated anti-HBc in predicting OBI. Moreover, In Ethiopia, Ayana *et al.* [12] reported a lower prevalence than we did, which was 6%. However, an amazing high OBI prevalence was declared by a Moroccan study [14] which revealed that 68.4% of isolated anti-HBc HIV infected cases had OBI.

We found that all cases with positive HBV DNA had CD_4 T cell count less than 350 cells/mm^3 with a significant statistical association (P = 0.001). In consistent with our finding, Six (66.7%) of the nine OBI cases detected by Gupta *et al.* [20] had CD_4 T cell count less than 200 cells/ mm^3. Furthermore, CD_4 T cell counts were lesser for OBI cases in comparison to both those who were co-infected with HBV/HIV and those who were mono-infected with HIV, as the CD_4 T cell counts in approximately 90% of OBI cases were lower than 250 cells/mm^3 [15]. In a study run in Netherlands [14] the CD_4 T cell levels of HIV positive cases with OBI were significantly lesser than that of HIV mono-infected cases. OBI was not detected in those with CD_4 T cell levels above 500 cells/mm^3. In contrast to our findings, Abdelaziz *et al.* [14] did not find any significant statistical association between positive HBV DNA and CD_4 T cell count, despite that most of the cases had CD_4 T cell count lower than 500 cells/ mm^3.

Improving public understanding of transmission ways of HBV and effective vaccination strategy can reduce HBV frequency [21]. World Health Organization recommends HBV vaccination for every HBV non-immune HIV-infected case. HBV vaccination is also recommended by the American Association for the Study of Liver Diseases particularly for HIV-infected patients with isolated anti-HBc [9].

5. CONCLUSION

We concluded that OBI is common in Libyan HIV patients with positive anti-HBc. A larger study is needed to evaluate the prevalence of OBI in Libyan HIV-infected cases, as a such study will help in making an appropriate strategy for management of both OBI and HIV.

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