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Original Article

Occult Hepatitis B Virus Infection in Egyptian HIV-Infected Patients with Isolated Anti-HBc

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Abstract

Background : Occult hepatitis B infection (OBI), defined as the presence of hepatitis B virus (HBV) DNA in liver or serum despite the absence of detectable hepatitis B surface antigen (HBsAg) is a frequent contaminant with human immunodeficiency virus (HIV). HIV has a negative effect on HBV disease accelerating its natural course.

Objective: This study aimed to estimate the occurrence of OBI in Egyptian HIV-infected patients with isolated anti-HBc.

Methods: This cross-sectional study was conducted on 197 HIV infected patients. They were tested for HBsAg, Antibody to hepatitis B surface antigen (anti-HBs), antibody to HBV core antigen (anti-HBc), and CD4 count. Patients with sole anti-HBc were screened for HBV DNA by polymerase chain reaction (PCR).

Results: Among those patients, 13 (6.60%) were positive for HBsAg, 82 (41.62%) for anti-HBc and 70 (35.53%) for anti-HBs. Their corresponding median CD4 count was 310.00 cells/mm^3, 497.50 cells/mm^3 and 525.50 cells/mm^3, respectively. Anti-HBc was the sole marker in 35 (17.77%) patients of whom 7 (20%) were HBV DNA positive indicating OBI. Most OBI patients were non-vaccinated against HBV. There was no significant statistical relationship between the presence of OBI and CD4 count, although most of them had CD4 count less than 500 cells/mm^3.

Conclusion: The present study underscores the importance of OBI screening among HIV patients with isolated anti-HBc.

Keywords: Occult hepatitis B infection (OBI), Hepatitis B virus DNA (HBV DNA), Human immunodeficiency virus (HIV), CD4, Sole antibody to HBV core antigen (anti-HBc)

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INTRODUCTION

Hepatitis B virus (HBV) infection remains a major public health problem, where out of the 2 billion people previously infected with the virus, 240 million suffer from chronicity and its complications.(1) Serological markers of HBV are traditionally used for its diagnosis notably the most important one, hepatitis B surface antigen (HBsAg). However, advances in molecular techniques have offered additional means for HBV diagnosis.(2) Using such techniques showed that individuals without detectable HBsAg may still harbor HBV-DNA in their liver and/or serum with or without antibody to HBV core antigen (anti-HBc) and antibody to hepatitis B surface antigen (anti-HBs). This situation has been termed occult hepatitis B infection (OBI).(3) OBI patients are classified into seropositive (positive for anti-HBc and/or anti-HBs) and seronegative (negative for anti-HBc and anti-HBs) groups. Approximately, 20% of OBI patients belong to the latter group showing no HBV markers in their serum. Reduced HBsAg production or expression as well as suppressed replication rate of the virus have been proposed to explain the absence of HBsAg in blood. These may have an impact on HBsAg detection by conventional assays.(4)

Similar transmission routes and risk factors for both HBV and human immunodeficiency virus (HIV) increase the frequency of HIV-HBV co-infection. It is known that infection with HIV causes disruption of the whole immune system not merely CD4 reduction. This severe damage to the immune system contributes to the development of OBI.(5) Although HBV has no remarkable effect on the HIV progression, HIV has a significant negative effect on HBV natural course. This effect appears as accelerating its progression to cirrhosis and hepatocellular carcinoma (HCC), in addition to the higher rate of reactivation of inactive HBV carriers.(6) HIV patients' survival has considerably improved owing to implementation of the

highly active anti-retroviral therapy (HAART), however, viral hepatitis has resulted in substantial morbidity and mortality in these patients. The attention was raised towards OBI diagnosis by the need for nucleotide treatment to prevent recurrence of HBV in HIV infected patients.[7]

HIV positive patients with chronic hepatitis C virus (HCV) co-infection may present with an OBI as HCV infection has an inhibitory effect on the replication of HBV. Different studies have shown the variation in OBI rate in different countries. This could be attributed to different prevalence rate of HBV, HCV and HIV as well as the difference in the sensitivity of the assays used for HBV DNA detection and absence of standardization of diagnostic methods.[4,5,8]

A prevalence of 3.6 to 45% HBV-HIV co-infection has been reported depending on HBsAg positivity. It has been stated that the diagnostic algorithm for persons with HIV who are HBsAg-negative include anti-HBc detection and, if positive, follow-up with molecular diagnosis to detect OBI. Such a diagnostic strategy could help reduce sequelae and HBV transmission in this population, thus improving their quality of life.[9]

Egypt is an intermediate endemic area for HBV infection. However, there are only a few reports of OBI in HIV patients. In this study, we attempted to estimate the burden of OBI in HIV-positive patients by detecting HBV-DNA among patients with sole anti-HBc.

METHODS

Patients

This cross-sectional study was conducted during the period from March through December 2017. Using Epi Info 6, the minimal required sample size was calculated to be 197 assuming the prevalence of OBI among HIV patients is 15%,[10] 95% confidence and precision 5%.[11] All HIV patients diagnosed and treated at Alexandria Fever Hospital were enrolled consecutively in the study until the required sample size was reached.

Sample collection and processing

Six ml blood samples were collected from each HIV positive patient. Each sample was divided into two tubes: The first was placed onto EDTA to be used for CD4 count by flow cytometry. [12] The second was centrifuged and serum separated. The serum was stored at (-80°C) till being used.

Serological testing

A) Detection of HBV markers by ELISA

Initially, all samples were screened by enzyme linked immunosorbent assay (ELISA) for HBsAg, HBsAb (using commercially available one step sandwich ELISA kit from Dialab, Wiener Neudorf, Austria) and total anti-HBc (using competitive ELISA kit from Dialab, Wiener Neudorf, Austria).Samples positive for anti-HBc alone were subjected to HBV DNA testing using polymerase chain reaction (PCR).

B) Detection of HBV DNA by semi-nested PCR technique[13]

HBV DNA was extracted from serum samples positive for anti-HBc alone using PREP-NA total DNA extraction kit (Portvino, Moscow region, Russia) according to the manufacturer instructions. The extracted DNA was subjected to semi-nested PCR. The lyophilized primers (Biosearch Tech, Petaluma, California, United States) were reconstituted by the addition of sterile water to a final concentration of 100 Pico mol/µl. Primers of the first round are SI 5 -ATGGAGAACATCACATCAGGA-3 and SIR 5_-TTAGGGTTTAAATGTATACCC-3. Primers of the second round are SI 5 and P29 5_-ATACCCAAAGACAAAAGAAAA-3.

DNA amplification was done using Dream Taq Green PCR Master mix (Thermo Scientific, Waltham, United States). To each tube a total volume of 50µl was reached by adding 25µl Master mix, 1µlSI primer, 1µlSIR primer, 10µl DNA template (sample) and 13µl nuclease free water. For negative control 10µl of nuclease free water were used instead of the sample. For positive control 10µ1 of known HBV DNA positive sample were used. The tubes were transferred to the thermal cycler (BOECO, Hamburg, Germany) where they were subjected to initial denaturation at 94°C for 3 min then forty cycles of denaturation (94° C for 45 sec), annealing(53° C for 60 sec) and extension (72° C for 90 sec).This was followed by final extension (72° C for 1 min).

Reamplification of the resulted amplicons was done using SI and P29 primers with 10µ1 of the amplicon product from the first round of PCR as a template with the same amplification protocol to yield a final amplicon of 672 base pairs (bp). PCR products were loaded on 2% agarose in Tris Borate EDTA (TBE) containing 0.5µg of ethidium bromide per ml. After electrophoresis, the amplicon was visualized on agarose gel using UV transilluminator.

CD_4 Estimation by flow cytometry was performed using CD_4 easy count kit (Partec, Germany). All laboratory work was carried out at the Microbiology Lab at the High Institute of Public Health except for the CD4 count which was conducted at the Virology Lab of Alexandria Fever Hospital.

Statistical analysis

Data were collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis (version 21). Data were entered as numerical or categorical, as appropriate. Kolmogorov-Smirnov test of normality revealed significance in the distribution of some variables, so the non-parametric statistics were adopted. Data were described using minimum, maximum, median and inter-quartile range. Categorical variables were described using frequency and percentage. Comparisons were carried out between two studied independent not-normally distributed subgroups using Mann-Whitney U test. Chi-square test was used to test association between qualitative variables. Fisher Exact

test was carried out when indicated (expected cells less than 5). Agreement was analyzed using Kappa and weighted Kappa test. Intraclass correlation was used to quantify the degree to agreement (reliability). An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%.[14]

Ethical considerations

The study was approved by the Ethics Committee of the High Institute of Public Health as well as by the Ethics Committee of the Ministry of Health and Population. After obtaining an informed consent from HIV-infected patients, the following data were collected from the patients' records: patient number (anonymity was a must), age, sex, alcohol intake, marital status, occupation and history of HBV vaccination.

RESULTS

Among the 197 HIV patients tested, 13 (6.60%) were positive for HBsAg, 82 (41.62%) for anti-HBc and 70 (35.53%) for anti-HBs. Their corresponding median CD4 count was 310.00 cells/mm^3, 497.50 cells/mm^3 and 525.50 cells/mm^3, respectively. In Table 1, among the 197 HIV patients screened for HBV markers, HBsAg, anti-HBs and anti-HBc were sole in 5 (2.54%), 31 (15.74%), and 35 (17.77%), respectively. Among the 31 sole anti-HBs, 17 (54.84%) gave a history of vaccination, while 14 (45.16%) did not give such a history. Thirty nine patients (19.80%) were positive for both anti-HBc and anti-HBs, while 8 (4.06%) had dual anti-HBc and HBsAg. Seven patients (3.55%) had sole anti-HBc with HBV DNA. No HBV markers were detected in 79 (40.10%) of the HIV studied population. It is worth mentioning that all HBsAg positive patients were negative for anti-HBs.

In Table 2, five (38.46%) HBsAg positive patients were negative for anti-HBc marker, while 8 (61.54%) were positive for it. These results show that not all HBsAg positive patients are anti-HBc positive. However, these results were not statistically significant.

Table 3 shows that among the 35 patients with sole anti-HBc, 7 (20%) were positive for HBV-DNA and identified as OBI. Most of patients with OBI were males, non-vaccinated and non-alcoholic with a percent of 85.71% in each of those parameters. These results were not statistically significant. The median of CD4 count was lower among DNA positive patients than among DNA negative patients (247.00 vs., 506.50, respectively). Most of OBI patients (71.43%) had CD4 count less than 500 cells/mm3. These results were not statistically significant.

Table 1: HBV markers among HIV patients in relation to vaccination

Viral marker	Total (n=197)		History of vaccination			
			Vaccinated		Non-vaccinated	
	n	%**	n	%*	n	%*
HBsAg alone	5	2.54	1	20	4	80
Anti-HBs alone	31	15.74	17	54.84	14	45.16
Anti-HBc alone	35	17.77	5	14.29	30	85.71
Anti-HBc + anti-HBs	39	19.80	10	25.64	29	74.36
Anti-HBc + HBsAg	8	4.06	2	25	6	75
Sole anti-HBc with DNA	7	3.55	1	14.29	6	85.71
Sole anti-HBc without DNA	28	14.21	4	14.29	24	85.71

%*: percent calculated from row total
%**: percent calculated from column total

Table 2: Relation between HBsAg and anti-HBc among HIV patients

Anti HBc	HBsAg		Total (n=197)
	Negative	Positive	
Negative	110 (59.78%)	5 (38.46%)	115 (58.38%)
Positive	74 (40.22%)	8 (61.54%)	82 (41.62%)
Total	184(93.40%)	13(6.60%)	197(100%)
Pearson Chi-Square	χ^2=2.272, *p*= 0.132		
Kappa	0.125		
p value	0.006*		
Weighted kappa	0.125		
95% CI	-0.0187-0.0635		
Correlation coefficient	-0.161		
95% CI	-0.294 – -0.022		
p value	0.988		

Table 3: HBV-DNA among HIV patients with sole anti-HBc in relation to demographic data, vaccination, alcohol intake and CD4 count

Variable	HIV patients with sole anti HBc (n=35)				Significance (*p* value)
	HBV-DNA (Positive) (n=7)		HBV-DNA(Negative) (n=28)		
	n	%	n	%	
Age (years)					
Min-Max	21 -53		22-55		$Z_{(MW)}$=0.352
Median (IQR)	35.0 (28.00-38.00)		31.50 (28.00-39.25)		*p*=0.732
Sex					
Male	6	85.71	20	71.43	χ^2=0.598
Female	1	14.29	8	28.57	$p_{(FE)}$=0.648
Marital status					
Single	4	57.14	15	53.57	χ^2=0.029
Married	3	42.86	13	46.43	$p_{(FE)}$=1.000
Occupation					
Not –working	5	71.43	15	53.57	χ^2=0.729
Working	2	28.57	13	46.43	$p_{(FE)}$=0.672
Vaccination					
Vaccinated	1	14.29	4	14.29	NA
Non –vaccinated	6	85.71	24	85.71	
Alcohol intake					
Alcoholic	1	14.29	3	10.71	χ^2=0.071
Non –alcoholic	6	85.71	25	89.29	$p_{(FE)}$=1.000
CD4 count (cells/mm^3)					
Min-Max	26 -967		101-1167		$Z_{(MW)}$=1.217
Median (IQR)	247.00 (80.00-550.00)		506.50 (252.00-616.75)		*p*=0.229
CD4 count (cells/mm^3)					
≥500	2	28.57	14	50.00	χ^2=1.036
< 500	5	71.43	14	50.00	$p_{(FE)}$=0.415

MW: Mann-Whitney U test FE: Fisher's Exact test NA: Non-applicable statistics (due to exact match)

DISCUSSION

In the current study, HBsAg was detected in 13 (6.60%) HIV infected patients. Interestingly, it was higher than that previously recorded in the same setting as the present study in 2014; where Aly found HBsAg positivity in 1.30% of the HIV patients.[15]

In the present research, 5 (2.54%) HIV patients had HBsAg as the sole viral marker. In a Brazilian study, HBsAg has been reported to be the sole HBV marker in one (0.3%) HIV-patient.[16] Possibilities include recent infection, acute infection or remote past infection with undetectable other markers. Out of those with sole HBsAg marker, only one patient was vaccinated. This can be explained by a non-response to HBV vaccine given to a previously chronically infected HBV patient or transient antigenemia following recent vaccination.

In the present work, anti-HBs was positive in 70 (35.53%) of HIV-patients. Again, this was higher than that recorded in the same setting by Aly; anti-HBs was seropositive in 17.8% of HIV patients attending Alexandria Fever Hospital. The dual presence of both markers was also higher than the 7% previously reported by Aly.[15]

Anti-HBc was detected in 82 (41.62%) of the studied HIV cases indicating previous exposure to HBV, of whom 8 (9.76%) were HBsAg positive. This may reflect a carrier state or current infection. Anti-HBc as being previously mentioned for other markers, showed an increase than the 21.0% detected by Aly among the HIV patients at Alexandria Fever Hospital.[15] Anti-HBc was detected in 29.20% and 27.3% of HIV infected patients in Nigeria and Brazil respectively.[17,16]

Anti-HBc was the sole HBV marker in 35 (17.77%) HIV patients, which is close to 17.50% (71 out of 405) and the 16% (38 out of 240) reported in Italy and Boston, respectively.[18, 19] Isolated anti-HBc was detected in 12.7% in the study carried out by Aly[15] However, this percent is lower than what has been described in previous studies of HIV-positive patients, namely; the 26.80% detected in India,[20] and the 28.30% in Taiwan.[21] This high percent of sole anti-HBc in these countries may be explained by their high endemicity of HBV with subsequent higher prevalence of chronic and past HBV infection. On the other hand, Bautista-Amorocho et al., reported isolated anti-HBc in 5.1% of HIV patients in Colombia.[22] The sole presence of anti-HBc can be attributed to one of the following: resolved remote infection with undetectable anti-HBs, false positive anti-HBc, "low level'' chronic infection and last; resolving acute infection. The current results regarding anti-HBc are in line with other studies highlighting anti-HBc as the sole serological surrogate of

OBI, yet anti-HBc alone does not necessarily indicate high frequency of OBI.[23,24]

In an attempt to resolve the cause of sole anti-HBc detected in this study, HBV-DNA was investigated. It was positive in 7 (20%) among the 35 previously mentioned patients indicating OBI. No data were available for OBI among HIV patients in Egypt so as to compare the results of the present work. However, a strikingly higher percent was declared by a study in Morocco revealing OBI in 68.4% of HIV patients with isolated anti-HBc. Morocco is considered an area of intermediate HBV endemicity similar to Egypt. [25] This great variation in OBI prevalence may be explained in relation to the selection of the studied population in the Moroccan study which differs from the present one. They were antiretroviral treatment naïve HIV patients who haven't received HBV vaccine. In addition, the overall percent detected in HBsAg negative patients was 58%. Furthermore, the investigators used a quantitative technique in determining HBV-DNA.

Relatively lower OBI rates have been reported elsewhere. A percent approaching the present one has been recorded recently by Saha et al., among HIV positive patients in India.[20] They found OBI in 17.8% of patients with isolated anti-HBc. A higher percent of 24.5% was previously reported also from India (intermediate HBV endemicity) by Gupta and Singh in 2010.[5] An OBI prevalence of 25% and 13.6% has also been reported in two studies from Iran.[26, 27] On the other hand, still in areas with intermediate HBV endemicity, Altinbaş et al. reported no OBI among the investigated HIV patients in Turkey. They suggested that this might be due to suppression of viral replication due to the anti-retroviral therapy received by the enrolled patients or a true absence of OBI.[28] Although sub-Saharan Africa is known for high HBV prevalence, yet Attia et al., found OBI in 21.3% HIV patients in Cote D'Ivoire who had isolated anti-HBc.[29]

In Cameroon HBV-DNA was detected in 20 out of the 337 HBsAg negative samples (irrespective of prior anti-HBc positivity) giving an OBI prevalence of 5.9%.[30] Similar prevalence was reported in a study conducted in Italy.[18] In the same context, a Colombian study documented an OBI in 8.7% of HIV patients.[22] Higher OBI prevalence of 26.8% was recorded in Sudanese HIV patients.[10]

In Egypt, OBI has been studied in different populations with a varying prevalence. High prevalence of OBI has been reported among chronic disease patients as seen in a study on chronic liver disease patients (58.3%).[31] Among immunocompromised patients, OBI was found to reach 62.5% in patients with HCC.[32] Nevertheless, these rates cannot be compared to that found in the current work, as OBI has been sought for in cases with isolated anti-HBc which was not the issue in the aforementioned studies. Yet, they, together with the present study underscore the high OBI prevalence among vulnerable groups. In the conducted work, OBI was more prevalent in male participants (85.71%) Similarly, in Nigeria OBI was more prevalent among males (75%) as compared with female (25%).[17] Nevertheless, a female predominance of 85.00% was reported among the OBI patients in Cameroon.[30]

Further comparison between sole anti-HBc positives with and without detectable HBV-DNA showed that the OBI group was associated with much lower median CD4 count (247.00 *vs* 506.50 cells/mm^3, respectively). The present work showed that 71.43% of OBI patients had CD4 count less than 500 cells/mm^3, in contrast to 50% in the negative group. However, this result was not statistically significant. This is consistence with other findings on OBI showing a similar association.[33,34] In Netherlands, Cohen Stuart et al., found that the CD4 counts of HIV infected patients with OBI were significantly lower than those without OBI (105±157 cells/mm3 *vs.*, 323±299 cells/mm3). No occult HBV was observed in patients with CD4 counts >500 cells/mm3.[33] In a Nigerian study[34], the association of HIV/HBV co-infection with lower CD4 T-cell counts was demonstrated.

Previous studies have made controversial findings regarding factors associated with OBI. While some investigators have reported a significant association[5,33], the current study together with others have not made this finding.[30,35] Diagnostic algorithms for definition of OBI in the different studies may be the explanation.

It is noteworthy to mention that 85.71% of HIV patients with OBI were non-vaccinated against HBV. A Sudanese study carried out in 2014 reported that 99% of OBI/HIV patients were non-vaccinated.[10] In addition, no HBV markers were detected in 79 (40.10%) of HIV patients recruited in the present work placing them at risk for subsequent HBV infection. These finding highlight the significance of continuing prevention of HBV among HIV patients through vaccination.

The results of the current work were interpreted taking into considerations several limitations. First, the HBV DNA viral loads used to identify OBI were not available as the semi nested conventional PCR was the technique adopted. Secondly, the results of this study couldn't be generalized to the whole HIV population tested as only those with isolated anti-HBc were subjected to HBV-DNA testing owing to resource constraints. Thirdly, due to culture and administrative restrictions, there was a difficulty in obtaining detailed information from the enrolled HIV patients.

CONCLUSION AND RECOMMENDATIONS

In conclusion, the results of the present work underline the importance of continued screening of HIV positive patients for HBV infection using HBsAg and anti-HBc, and follow up of those with sole anti-HBc for further testing for OBI. This practice would guide correct choice of drug combination before initiation and during HAART. In addition, monitoring HIV patients for their immune status against HBV, with emphasis on vaccination coverage for those who are susceptible is mandatory. Finally, further studies for investigating OBI in HIV patients who are

negative for HBsAg regardless of their anti-HBc status are recommended. We would like to thank the staff members of the virology laboratory at Alexandria Fever Hospital.

Conflict of Interest

The authors declare that they have no conflict of interests.

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