

Prevalence of Anti-HBc in HIV patients on ART in Ekiti, Nigeria

Abstract

Background: Antibody to hepatitis B core antigen (anti-HBc) is now found to be frequent in HIV-infected patients, and it may be a serology marker of occult hepatitis B.

Aim: This study aimed to determine the prevalence and risk factors associated with anti-HBc among HIV-infected patients in Ikole Ekiti, Nigeria.

Study Population and Duration: HIV/AIDS individuals visiting Institute of Human Virology of Nigeria, Specialist Clinic, Ikole Ekiti between November 2012 and April 2013 were included in this study.

Methodology: One hundred and eighty eight HIV samples were tested for anti-Hepatitis B core antibody by Enzyme Linked Immunosorbent Assay (ELISA) {ANTICORASE MB-96 [TMB] from General Biologicals Corporation}. Data were analysed using software within SPSS packages.

Result: Twenty two (11.7%) of 188 HIV-infected patients had isolated anti-HBc. Co-infection of HIV-HBV in males (3.23%) differed significantly from that of females (8.51%). Highest coinfection (9.57%) was found among adult age group of 20 - 55, while no co-infection was found in teenager age group (13-19 years). Based on occupation, long distance drivers had highest coinfection of 8 (4.26%) while student had lowest prevalence of 2 (1.06%). Isolated anti-HBc among HIV individuals was found in 22 (11.7%) which was significantly high.

Conclusion: Isolated anti-HBc could be associated with occult infection. Thus, all HIV-infected patients should be screened for anti-HBc before starting antiretroviral therapy.

Keywords: HBV, HIV, Anti-HBc coinfection.

1. Introduction

Despite an uncommon serological pattern in the general population, isolated anti-hepatitis B virus (HBV) core antibody (anti-HBc), defined by positivity of anti-HBc marker in absence of both HBV surface antigen (HBsAg) and anti-HBV surface antibody (anti-HBs) markers, is usually seen in HIV-positive patients [1]. Nevertheless, the clinical importance and implications of isolated anti-HBc detection remain not well defined in the HIV-positive population. Hepatitis B core antigen (HBcAg) is an intracellular antigen expressed by infected hepatocytes that is

not detectable in serum, but antibodies are produced in response to HBcAg peptides (smaller fragments of the HBcAg) [2]. These antibodies (anti-HBc) can be detected throughout the course of HBV infection and are a marker of natural exposure to HBV; anti-HBc is absent in those who have been vaccinated. Core antibody is generally in the form of IgM during acute infection or flares of HBV and predominantly in the form of IgG during chronic or resolved infection. [2]

Total anti-HBc implies the presence of either immunoglobulin (IgG or IgM) and provided that test result does not discriminate between the subclasses. Its presence in the blood indicates prior or current hepatitis B infection [2]. It does not confer protection against HBV. Anti-HBc antibodies are markers of acute, chronic, or resolved HBV infection and remain detectable for life. These can be present in the absence of both HBsAg and anti-HBs antibodies, during the convalescent period following acute hepatitis B before the appearance of anti-HBs antibodies, or in patients who have resolved infections but lost detectable anti-HBs antibodies. Anti-HBc antibodies are, therefore, detected in anyone who has been infected with HBV [3]

Hepatitis B core antibody (anti-HBc) is directed against the hepatitis B core antigen (HBcAg) peptides. The anti-HBc is the earliest antibody to develop about 6 to 8 weeks after infection in response to acute hepatitis B virus (HBV) infection. The anti-HBc typically persists for life, but after about 6 months the total anti-HBc mainly consists of IgG anti-HBc (4). For patients with resolved acute HBV infection, the IgM anti-HBc is not usually detectable after 6 months. With chronic HBV infection, the IgM anti-HBc can remain detectable at very low levels, even years after infection [4].

When routine HBV serology is requested, most laboratories report total levels of anti-HBc, which can consist of a combination of IgM and IgG antibodies. Accordingly, providers must specifically request measurement of IgM anti-HBc if they suspect acute HBV infection. In addition, some patients with isolated anti-HBc will have detectable hepatitis B e antibody (anti-HBe), but most routine hepatitis testing does not include anti-HBe.

1.2 Materials and Methods

The study populations were confirmed HIV patients attending IHVN Clinic in Nigeria. The patients were mainly Yoruba and almost all of them were on Antiretroviral Treatment (ART) in the hospital. Ethical approval was obtained for from Ministry of Health, Ekiti State. Informed consent was obtained from each study subject before administration of questionnaire. From 200 HIV positive samples collected from IHVN Clinic in Nigeria, 188 HIV samples were randomly selected for the project. 4ml of venous blood was collected from each participant aseptically by venepuncture from the cubital fossa into sterile plain bottles containers using a standard procedure. The blood was allowed to clot and centrifuged at 3000 rpm for 5 minutes to separate the serum. The serums so extracted were stored in plain bottle at -20 °C until tested.

Required number of strips in the folder was placed and wells for standard and samples were identified carefully. Samples were diluted in ratio 1:101 by dispensing 1ml of sample diluents into a disposable tube and then 10µl sample; both were mixed on vortex before use. A1 + B1 were left empty for blanking purpose. 100µl of calibrators, dissolved control serum and diluted samples were pipetted in duplicate respectively. Microplate was incubated for 60mins at 37°C. After the 1st incubation, microwells were washed. In all the wells except A1+B1, 100µl of enzyme conjugate was pipetted.

Microplate was incubated at 37°C for 60mins. After the 2nd incubation, microwells were washed. 100µl of chromogen/substrate was pipetted into all the wells (A1+B1 inclusive). Microplate protected from light was incubated at room temperature (18-24°C for 20 mins. 100µl of sulphuric acid was pipetted into all the wells to block the enzymatic reaction. Addition of the stop solution turned the positive control and positive samples from blue to yellow. Colour intensity of the solution in each well was measured using 450nm filter reading.

1.3 RESULTS

A total of 188 HIV infected individuals comprising of 43 males and 145 females with mean age 37yrs (7-75) years were investigated for Anti HBc IgM. Twenty two (11.7%) of 188 HIV individuals patients were positive for Anti HBc IgM with 3.19 % and 8.51 seroprevalence rate among the males and female respectively. The mean age of the Anti HBc IgM positive patients was 36years. Highest coinfection rate of 9.57% was found in 20-55 years age group (9.57%), followed by 1-12 (1.06%) and > 56 (1.06) while no case of co-infection was recorded among teenager. In relation to marital status, single had the highest rate of co-infection (9.57%), while married had 2.13%. The average CD4 count of AntiHBc positive and AntiHBc negative HIV patients was 378.5cell/mm³ and 470 cell/mm³ respectively. The overall CD4 count of the study population was 459.8cell/mm³. Highest co-infection rate was found among long distance drivers (4.26%) followed by civil servants (3.19%) and housewives (2.13%).

1.4 DISCUSSION

This study showed Anti HBc IgM prevalence rate of 11.7% among HIV infected patients. This is an agreement with Sirisena *et al.*, 2002 but in contrary to 15% reported by Baba *et al.*, 1990 [4,6]. Older age and CD4 counts were independently associated with presence of isolated anti-HBc. Similar to these findings; two studies also found that lower CD4 counts were associated with presence of isolated anti-HBc in HIV-positive patients [7]. Patients in most of the other studies had higher CD4 counts (median CD4 counts of 200 cells/mm³). Few studies have addressed the association between age and isolated anti-HBc. In an unselected population in Germany, highest co-infection of isolated anti-HBc was found among aged of 71–80 years (22.7%) and lowest in people aged 1–30 years (9.4%) [8]. Unlike our observations, one study reported HIV-positive individuals with isolated anti-HBc were older than those tested negative for all three HBV markers (mean 36.8 vs. 34.3 years, P = 0.026) [9]. Because anti-HBs titers decline with age, it is not unexpected to observe that older age was associated with isolated anti-HBc. This further suggests factors associated with declining immunity, such as old age or low CD4 counts; also contribute to the presence of isolated anti-HBc. Although a study did not differentiate carriers of AntiHBc IgM from those with

active infection, it was reported that a significant proportion of carriers go on to develop chronic hepatitis, and some may even progress to hepatocellular carcinoma [10].

2.0 Conclusion and Recommendation

In conclusion, isolated anti-HBc was present in 11.7% of HIV-positive patients. Presence of isolated anti-HBc was associated with increase age and severe immunosuppression with CD4; suggesting compromised immunity may play a role. Co-infection rate between males (3.19%) and females (8.51%) did not differ significantly ($p > 0.5$), which is in contrast to the findings of Baba *et al.* , 1990 who found a significantly higher AntiHBc IgM seroprevalence in males than in female.

Highest rate of AntiHBc IgM positivity of age distribution of 9.57% was found in age group 20-55 years. There was relatively AntiHBc IgM positivity in the age groups 0 -12 years and >56years, but no cases was seen in 13 - 19 years age group. This is surprising, as it is known that a significant number of HBV in this environment are acquired in childhood. The most likely explanation for this observation is the very low number of subjects in those age groups [11]. Co-infection rate was higher in those who were unmarried than among the married. This may probably be related to multiple sexual partnerships and patronizing of commercial sexual workers. Occupational breakdown showed that long distance drivers had the highest co-infection rate reflecting a high-risk sexual behavior in these groups[11]. In conclusion, there is a relatively high HBV co-infection rate in our HIV-infected patients. Therefore, there is a need to screen all HIV-infected patients for HBsAg.

[1] Sun, H.-Y. ,Lee, H.-C. ,Liu, C.-E. ,Yang, C.-L. ,Su, S.-C. ,Ko, W.-C. , Lin, C.-Y. ,Tsai, J.-J. ,Wong, W.-W. ,Ho, M.-W. Cheng, S.-H. , Lin, Y.-H. , Miao, W.-J. Hung, C.-C. J. *Viral Hepat.* **17(8)**:578-587, 2010.

[2] Web definition, University of Washington. <http://depts.washington.edu/hepstud>

[3] Neau D, Winnock M, Galperinep T. Isolated antibodies against the core antigen of hepatitis B virus in HIV-infected patients. *HIV Med.* **5**:171–3,2004.

[4] Centers for Disease Control and Prevention (CDC).Revised classification system for HIV infection, 1993.

- [5] Baba M. M, Gashau W, Hassan A. W. Detection of Hepatitis B surface antigenaemia in patients with and without the manifestation of Acquired Immunodeficiency Syndrome in Maiduguri, Nigeria. *Niger. Postgrad. Med. J.* **5**: 125-128,1990.
- [6] Sirisena ND, Njoku MO, Idoko JA, Isamade E, Barau C, Jelpe D, Zamani A, Otowo S. Carriage rate of hepatitis-B surface antigen (HbsAG) in an urban community in Jos, Plateau State, Nigeria. *Nig Postgrad. Med. J.* **9**: 7-10,2002 .
- [7] Vazquez-Vizoso, F., Eiroa, P. and Ledo, L. (1994).HIV infection and isolated detection of anti-HBc. *Gastroenterology*. **106**: 823-824,1994.
- [8] Knöll, A., Pietrzyk, M., Loss, M., Goetz, W. A. and Jilg, W. Solid-organ transplantation in HBsAg-negative patients with antibodies to HBV core antigen: Low risk of HBV reactivation, *Transplantation*, **79 (11)**: 1631- 1633,2005.
- [9] Davaro, R.E., Cheeseman, S.H, Keroack, M.A., Ellison, R.T. The significance of isolated antibody to hepatitis B core antigen seropositivity in patients infected with human immunodeficiency virus. *Clin Infect Dis*; **23(1)**: 189–190,1996.
- [10] Omer E El- Fadil, (1995). Clinical Significance of markers of hepatitis B. *Hepatitis B virus (HBV) Overview*, 1995.
- [11] Mustapha, S. K., and Y. B. Jibrin. "The prevalence of hepatitis B surface antigenaemia in patients with human immunodeficiency virus (HIV) infection in Gombe, Nigeria." (2004).

Table 4.1: Showing Demographic characteristic of studied population

	Anti HBc IgM Positive	Anti HBc IgM Negative
Age group	Number (%)	Number (%)

1-12	2 (1.06%)	4 (2.13%)
13-19	0	20 (10.6%)
20-55	18 (9.57%)	122(64.9%)
>56	2 (1.06%)	20(10.6%)
Mean (Range)	36yrs (9.5-60)	37yrs(7-75)
Male	6 (3.19%)	36(19.1%)
Female	16(8.51)	130(69.1%)
Marital status		
Married	4(2.13%)	142(75.53%)
Single	18(9.57%)	24(12.8%)
CD4 Count		
<200	4	14
200-499	6	62
>500	12	90
Mean (cell/mm ³)	378.5	470

163

164

165

166

167 **Table 4.2: Distribution of Anti HBc IgM positivity in HIV infection among occupational**
168 **groups**

Occupation	No of Positive	Percentage positive
------------	----------------	---------------------

Businessmen	2	1.06
Civil servants	6	3.19
Drivers	8	4.26
Housewives	4	2.13
Students	2	1.06

169

170