

Case Study

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

Abstract

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

Aim: We aimed to determine the prevalence and associated risk factors of isolated 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 method. Data were analysed using software within SPSS packages.

Result: Of 188 HIV-infected patients, 22 subjects had isolated anti-HBc giving a prevalence of 11.7 %. Co-infection of HIV-HBV in males (3.23%) was differed significantly from that of females (8.3%). Co-infection was highest in the 30 - 39 years age group (9.34%), while no case of co-infection was recorded in the 10-19 years. Co-infection rate was highest among long distance drivers (4.17%) followed by civil servants (3.13%) and housewives (2.08%). Prevalence of isolated anti-HBc in Ikole Ekiti HIV-infected individuals was 51.1%, 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 as positive anti-HBc with both negative HBV surface antigen (HBsAg) and anti-HBV surface antibody (anti-HBs), is frequently observed in HIV-positive patients [1]. Nevertheless, the clinical significance 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). 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 refers to presence of either IgG or IgM and test result does not discriminate between the subclasses. Its presence in the blood indicates prior or current hepatitis B infection [3]. 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 in response to acute hepatitis B virus (HBV) infection, appearing predominantly as IgM anti-HBc at about 6 to 8 weeks after 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 receiving treatment in the hospital. Ethical approval was sought 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 patients with age range of 7 -75years and mean age of 37years were tested for Anti HBc IgM. This number was made up of 43 males and 145 females. Of these, 22 patients were positive for Anti HBc IgM, giving an overall prevalence of 11.7%. Anti HBc IgM seroprevalence rate among the males was 3.13%, while it was 8.3% among the females. The mean age (range) of the Anti HBc IgM positive patients was 36years. Coinfection rate was highest in the 21 -30 years age group (9.34%), followed by 1-10 (1.04%) and > 56 (1.04) while no case of co-infection was recorded in those 11 - 20 years. In relation to marital status, married had the highest rate of co-infection (9.37%), while those who were married had 2.08%. The average CD4 count of AntiHBc positive and AntiHBc negative HIV patients was 378.5cell/mm³ and 479.0cell/mm³ respectively. The overall CD4 count of the study population was 459.8cell/mm³. HBV co-infection rates among different occupational groups. Co-infection rate was highest among long distance drivers (4.17%) followed by civil servants (3.13%) and housewives (2.08%).

1.4 DISCUSSION

This study showed Anti HBc IgM prevalence rate of 11.7% among HIV infected patients. This is similar to the findings of Sirisena *et al.*, 2002 but much lower than the 15% obtained 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, the prevalence of isolated anti-HBc was highest in persons aged 71–80 years (22.7%) and lowest in persons aged 1–30 years (9.4%). [8]. Unlike our observations, one study reported HIV-positive patients 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 our study did not differentiate carriers of AntiHBc IgM from those with active infection, it is known 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. The rate of co-infection between males and females did not differ significantly ($p > 0.5$), with males having 3.4% and females having 8.3%. This is in contrast to the findings of Baba *et al.*, 1990 who found a significantly higher AntiHBc IgM seroprevalence in males than in female. The reason for this disparity is not clear.

Age distribution showed that the highest rate of AntiHBc IgM positivity (41.6%) was in the 20 -55 years age group. In keeping with the endemic nature of HBV in this environment, there is relatively AntiHBc IgM positivity in at the age groups 0 -12 years and >56years but no cases was seen recorded 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. 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. 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.

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