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Hepatitis B Virus Total Core Antibodies among HIV-1 Infected Hepatitis B Surface Antigen Negative Patients Attending a Tertiary Health Facility in North-central Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AJAO designed the study and wrote the first draft of the manuscript. Author TMA handled the data, performed the statistical analysis and wrote the first draft of the manuscript. Author NM designed the study. Authors NM and OJA performed the experiments. Authors DSA, OA and AOE reviewed the manuscript. Authors AOE and EE wrote the manuscript. Authors PL, HJZ, ESI and SO read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to determine the prevalence of total Hepatitis B core antibodies (anti-HBc) among HIV-1 infected adults without Hepatitis-B surface antigen (HBsAg).
Study Design: Observational cross-sectional study.
Place and Duration of Study: This study was carried out at the AIDS Prevention Initiative in Nigeria (APIN) adult HIV clinic at Jos University Teaching Hospital (JUTH), Jos, North-Central Nigeria, between August and December, 2014.
Methodology: We determined the presence of total anti-HBc for 120 HIV-1 infected patients (32 males and 88 females, with a mean age of 40.4±10.6 years). We performed serological screening for total anti-HBc and for other serological markers for each patient, then performed $CD4^+$ cell enumeration, biochemical analysis of serum for Alanine aminotransferase levels and HIV viral load assays.
Results: A total of 105, HIV-1 positive patients who were HBsAg negative were studied. Of these patients, 59 (56.2%) showed no HBV serological markers, 23 (21.9%) had total anti-HBc, and 15 (14.3%) had HB surface antibodies. Only one patient (1%) showed HBeAg while anti-HBe was detected in 20 (19.0%). The mean age of patients with anti-HBc was 41.4 ± 10.8 years which was similar to the mean age of patients who were negative for anti-HBc (*P* =.81). While no significant associations were observed between ALT levels, CD4 counts, marital or educational status and total anti-HBc of the patients (*P* =.91, *P* =.39, *P* =.78 and *P* =.44, respectively), there was a significant association between having a history of tooth extraction and total anti-HBc (*P* =.03).
Conclusion: There may be a need to assess occult HBV infection in HIV-infected individuals with isolated anti-HBc for active replication of HBV by detecting HBV DNA. Dental care practitioners must take great care to prevent possible transmission through surgical equipment used in procedures for tooth extraction.

Keywords: Hepatitis-B; isolated; total core antibodies; dental extraction; HIV; North-central.

1. INTRODUCTION

Testing for Hepatitis B virus (HBV) has been recommended as a routine for all HIV-1 infected persons by national guidelines worldwide [1]. This strategy provides treatment centers the evidence for deciding whether a patient receives vaccination against the virus or if a different course of action should be pursued. Serological testing for HBV is however made challenging in individuals who test negative for HB surface antigen (HBsAg) and HB surface antibodies (HBsAb) but positive for antibodies to hepatitis B core antigen (anti-HBc). Notwithstanding its diagnostic value as a predicting factor for other viral hepatitis and usefulness in the prevention of HBV infection transmitted by individuals without HBsAg [2], anti-HBc positive results from serological assays present several interpretative possibilities. These interpretations could range from evidence for resolution of a past HBV infection, the presence of occult infection with undetectable levels of HBsAg [3-5] to even a false positive result. The risk of HBV transmission through organ transplants has been reported to increase in cases where the surface antigen is not detected but anti-HBc is present [6]. Furthermore, HIV infection can be an immunosuppressant that increases the risk of reactivation in infected individuals [7].

Due to the public health significance of HBV transmission via blood transfusion route, several studies have considered the phenomenon of testing HBsAg negative but Anti-HBc positive within the context of blood donors [8-10]. There is, however, a paucity of studies in HIV settings, particularly in Nigeria. Our objective was therefore to determine the prevalence of total anti-HBc in an HIV-1 positive cohort attending the AIDS Prevention Initiative in Nigeria (APIN) adult HIV clinic at the Jos University Teaching Hospital (JUTH), Jos, Nigeria.

2. MATERIALS AND METHODS

We utilized an observational cross-sectional design in this study which was carried out at APIN adult HIV clinic of JUTH, located in Jos-North Local Government Area at 9°55'N 8°54'E/ 9.917°N 8.900°E in Plateau State, North-Central Nigeria. This clinic provides comprehensive HIV care services in the state and serves as a reference center for neighboring states in the North-Central zone. Only patients who had been confirmed by the clinic to have HIV infection and

enrolled into care were tested for total anti-HBc. Ethical approval for this study was obtained from the Ethical committee at JUTH.

2.1 HBV Serological Assay

HBV serological markers were detected using a one-step qualitative assay (Acon laboratories, USA) following manufacturer's instructions and results obtained were recorded in a Microsoft Excel spreadsheet database.

2.2 CD4 T Lymphocyte Enumeration

Blood specimen preparation for $CD4^+$ enumeration was performed using Partec CD4 Easy Count® Kit (Partec, Munster, Germany) using the following outlined steps: 20 µl of whole blood was added to 20 µl of CD4 monoclonal antibody (mAb). The mixture was then incubated in the dark for 15 minutes. After incubation 800 µl of Partec CD4 Easy Count® kit no lyse buffer was added to stop the reaction and analysis for the CD4 count performed with a CyFlow® counter cytometer (Partec, Munster, Germany) and data recorded in a worksheet in cell counts/ µl.

2.3 Biochemical Analysis for Serum Alanine Aminotransferase (ALT)

Assessment for serum ALT was determined using a Roche COBAS® C311 biochemical auto analyzer. Data obtained was documented in a worksheet against each patient's identification number.

2.4 HIV Viral Load Assay

HIV viral load assay was performed on a COBAS Ampliprep and Taqman to obtain data on HIV viral load following the manufacturer's instructions.

2.5 Data Analysis

Data generated from this study was analyzed using SPSS software (version 17 SPSS Inc. Chicago, IL) to obtain descriptive statistics and make statistical inferences. Continuous variables were analyzed using students t-test while categorical variables were analyzed using chi-square distribution to test for associations. We considered *p*-values <0.05 statistically significant.

3. RESULTS AND DISCUSSION

3.1 Demographic Data

Of the 120 HIV-1 positive patients who were tested, 105 were HBsAg-negative. In this group of 105 patients, 59 (56.2%) had no HBV serological markers, 15 (14.3%) were positive for HBsAb while 23(21.9%) tested positive for anti-HBc. Only one patient (1%) had HBe antigen (HBeAg) while antibodies to HBeAg (anti-HBe) was detected in 20 (19.0%) of the patients (Table 1).

Table 1. Seroprevalence of HBV serological markers in HBsAg negative HIV-1 patients

	Positive	Negative
	n (%)	n (%)
Anti-HBs	15(14.3)	90(85.7)
HBeAg	1(1)	104(99)
Anti-HBe	20(19.0)	85(81.0)
Anti-HBc	23(21.9)	82(78.1)

The mean age of HIV/HBV co-infected patients who were positive for anti-HBc was 41.4 ± 10.8 years which was similar to the mean age of patients who were negative for anti-HBc (*P*=0.811). Of the 23 who tested positive for anti-HBc, 9 (8.5%) also tested positive for anti-HBe. Univariate analysis also showed that ALT levels, $CD4^+$ T-lymphocyte counts and log viral load copies were similar in subjects who tested positive for anti-HBc and those patients who were negative for anti-HBc. Although no significant association was observed between sex, educational or marital status and anti-HBc, there was a significant association observed between a history of tooth extraction and testing positive for anti-HBc (*P*=0.03) (Table 2).

3.2 CD4 Count and Anti-HBc Data

We also tested the association between having anti-HBc and $CD4^+$ T-lymphocyte counts <200 cells/µl and the association between having elevated ALT levels ≥40IU/l. Our analysis did not show any significant association between having low CD4 counts <200 cells/µl (*P*=.86) (Table 3) or elevated ALT levels ≥ 40 IU/l (*P*=.81) and having isolated anti-HBc (Table 4).

Table 2. Demographic results for variables in anti-HBc positive HBsAg negative

Characteristic or variable	Positive (n=23)	Negative (n=82)	*P* value
Sex			
Male	7(30.4)	21(25.6)	.64
Female	16(69.6)	61(74.6)	
Age in years			
Mean ±SD	41.4 ± 10.8	40.8 ± 11.0	.81
Educational status			
No education	3(13.0)	6(7.3)	.44
Primary education	4(17.4)	24(29.3)	
Secondary education	8(34.8)	31(37.8)	
Tertiary education	8(34.8)	21(25.6)	
Marital status			
Married	13(56.5)	48(58.5)	.78
Single	2(8.7)	13(15.9)	
Widowed	8(34.8)	21(25.6)	
CD4 cell count (Cells/µl)			
Mean ± SD	259.5±240.9	211.1±172.6	.39
ALT Level (IU/mL)			
Mean ± SD	26.1±24.5	25.5±21.9	.91
No (%) of subjects	12(52.2)	23(28.1)	.03
LogVL (Copies/ml)			
Mean ± SD	9.8 ± 2.5	10.5 ± 2.2	.28

Note: ALT= Alanine Aminotransferase; VL= Viral Load;

Table 3. Association of $CD4^+$ cell counts with anti-HBc positive patients

CD4 count	Positive n (%)	Negative n (%)	Total n (%)	*P* value
< 200 Cells/µl	13(56.5)	48(58.5)	61(58.1)	.86
≥ 200 Cells/µl	10(48.5)	34(41.4)	44(41.9)	
Total	23(21.9)	82(78.1)	105(100.0)	

Table 4. Association of ALT levels with anti-HBc positive patients

ALT level	Positive n (%)	Negative n (%)	Total n (%)	*P* value
< 40 IU/l	18(78.2)	66(80.5)	84(80.0)	.81
≥ 40 IU/l	5(21.8)	16(19.5)	21(20.0)	
Total	23(21.9)	82(78.1)	105(100.0)	

3.3 Discussion

This study revealed that the prevalence of anti-HBc among HIV-1 patients attending the APIN, JUTH facility who were seronegative for HBsAg and Anti-HBs was 23.8%. Our result is in contrast to the lower prevalence that have been observed in apparently healthy blood donors in Nigeria (13%) and Korea (13.5%) [9,10], a disparity that may be because both studies were carried out in apparently healthy individuals.

One study found 10% of HIV-infected individuals without HBsAg with detectable anti-HBc alone but with HBV DNA in serum, which confirmed occult HBV infections [11]. The presence of anti-HBc in this present study may point to the possibility of an ongoing occult infection among the study subjects [11-12]. Although this may be considered a possibility, the lack of confirmation of HBV DNA in this in those individuals who were anti-HBc positive was one of the limitations of this study. It would be useful to assess occult infection in these HIV-infected individuals with

anti-HBc for active replication of HBV by detecting HBV DNA. Gandhi and colleagues have proposed another explanation for the prevalence of anti-HBc in addition to the possibility of occult infection [13]. They suggest it is possible that some subjects who have resolved HBV infection lose detectable HBsAg levels over time leaving anti-HBc as a residue. In HIV-1 infection, this loss may occur frequently because of a modified immunological response. Several studies have demonstrated that individuals co-infected with HIV and HCV are more likely to have anti-HBc than subjects with HIV alone [13-16]. The interference of other viral agents such as HCV may adversely affect the expression of HBV genes consequently leading to inhibition [7]. This suppression may not only account for HBsAg negativity but also for low or even undetectable levels of serum viral DNA which is common in most cases of occult infection [3]. In addition to raising suspicion of occult infection, Alhababi et al. [17] have recommended the use of anti-HBc to monitor the development of possible HBsAg mutants that may produce a false negative test to current assays. It may also be preferable in the long run to regards patients who are positive for isolated anti-HBc as patients with a high risk of HBV reactivation [18].

Our observation of no significant difference between $CD4^+$ cell counts of HIV-1 infected, anti-HBc positive and anti-HBc negative individuals in this study is similar to the observation made by Ghandi and colleagues [13].

There was a high frequency of study participants (56.2%) who did not have any HBV serological markers indicative of protection either through natural infection or administered vaccines. This group is considered susceptible to HBV infection with dire consequences, since within the setting of HIV infection the likelihood of developing chronic hepatitis B infection along with other long-term adverse outcomes could increase substantially [19,20].

Although it is universally agreed that the most effective way to prevent the HBV infection in HIV-infected individuals who lack anti-HBc is through the use of vaccines, these patients have decreased vaccine responses and a short duration of protection in comparison to immunocompetent individuals [21,22]. However, regulating HIV replication using antiretroviral drugs with resultant increased $CD4^+$ cell counts are associated with better responses to hepatitis B vaccination [23], investigations to find new strategies for vaccination such as increased vaccine dose, new administration routes, and addition of adjuvants will improve response rates in adults with HIV [24,25].

Although it is well established that HIV and HBV share horizontal transmission routes in adults particularly through sexual intercourse, use of contaminated needles by intravenous drug users and surgical or other related medical processes, it is often assumed in these settings that the mode of acquisition of HBV by HIV infected individuals is through sexual means. However the most significant observation of this study was the association of tooth extraction with testing positive for anti-HBc among our study participants. Previous studies have made the connection between infection with HBV and dental extraction procedures [20,26,27]. The dental clinic has been identified as an important locale where the risk of HBV and HCV infection can be increased to epidemiologically significant levels [20]. In HIV settings, the impact of such associations can have far reaching consequences such as increasing the risk of hepatologic complications. Our study raises the question of the extent to which dental related transmission account for the exposure of this HIV-positive subpopulation to HBV or HCV. We suggest that dental clinics are significant contributors to HBV infection in HIV co-infected patients possibly from the use of contaminated surgical equipment along with nosocomial infection from dental consultants who perform tooth extractions, increasing the chance of exposure to occult HBV infection among this cohort of patients. As a line of future study, we, therefore, suggest the use of well-designed observational studies to determine the extent to which dental procedures contribute to the increased risk of viral hepatitis in both HIV-positive and HIV-negative populations who utilize related transmission routes, this is in addition to other risk factors such as behavioural patterns like drug use, multiple blood transfusions, history of organ transplantation and number of sexual partners.

4. CONCLUSION

There is an urgent need to increase the educational awareness of dental practitioners and clients to HBV infection control and prevention strategies in dental clinic settings. This need is particularly essential in developing countries where the risk of infection can have

dire public health implications. In addition to this, we recommend that regulatory oversight of Federal and State ministries of health will need strengthening to ensure strict adherence of dental facilities to safety precautions. Future research on this subject should include DNA testing of HBsAg negative individuals who have positive anti-HBc. If occult infection is established in a significant proportion, it would then be necessary for the testing policy to include DNA screening of this patient sub-population. The vaccination programme against HBV is an important control strategy that needs priority attention in HIV-infected populations.

CONSENT

All authors declare that 'written informed consent was obtained from the study patients (or other approved parties) for publication of this work'.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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