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Prevalence of Hepatitis B virus surface antigen among HIV/AIDS patients attending national tuberculosis and leprosy training centre, Saye-Zaria, Nigeria

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ABSTRACT

Background: Despite the public threat posed by hepatitis B virus and human immunodeficiency virus (HIV) infections in Nigeria, the data on the frequency of their co-infection are scanty in some locations. **Methodology**: In this study, 120 individuals living with HIV receiving care and treatment at the National Tuberculosis and Leprosy Training Centre (NTLTC) in Saye-Zaria, Nigeria, who consented were enrolled in the study to provide information about their socio-demographic characteristics using structured questionnaire. From each patient, 5 mL of venous blood sample was collected and centrifuged using ROTOFIX 32A at 40RPM/RCF×100 for 30minutes. The plasma was used for the determination of HIV status (Alere Determine HIV-1/2 rapid test kit), quantification of HIV-1 RNA (AmpliPrep/COBAS TaqMan) and detection of HBsAg (OnSite HBsAg rapid test kit); the data were analyzed using SPSS. **Results**: The results confirmed that all the patients recruited were HIV positive (100%), and the sero-prevalence of the HBsAg among them was 10.8%. The variables statistically identified as significant ($p \leq 0.05$) in relation to the infection were high HIV viral count, never being transfused with blood, intravenous injection with illicit drugs and history of the infection in the family, as well as unprotected sex and irregular use of HAART. **Conclusion**: The prevalence of the HBV infection among HIV patients is within the same range as previously reported in the country; there is a need to create more awareness on the dangers associated with unprotected sex, sharing of sharp objects, intravenous injection with illicit drugs and suboptimal commitment to HAART.

Introduction

Human Immunodeficiency Virus (HIV) infection continues to be one of the leading causes of illness and mortality globally. Worldwide, liver disorders are a leading cause of morbidity and death for those living with HIV [1]. The World Health Organization (WHO) defines a chronic hepatitis B virus (HBV) infection as having positive hepatitis B surface antigen for at least six months within the body system [2]. Hepatitis B virus infection affects 240 million people globally with the highest prevalence in East Asia and sub-Saharan Africa (SSA) [3]. It is reported that about 2 million people are living with HIV in Nigeria [4], and about 19 million people are estimated to be infected with hepatitis B virus [5]. And the HIV/HBV co-

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infection rate was estimated to be 10% in Nigeria [6]. Globally, it is reported that 4 million people living with HIV are also co-infected with HBV [7]. Because the risk factors and modes of transmission for these two illnesses are identical, they coexist. Individuals who are co-infected with HIV and HBV have reduced rates of spontaneous HBV clearance, and some researchers suggest that the co-infected maybe ten times more likely than the general population to develop chronic HBV infection. Human Immunodeficiency Virus also makes people with chronic HBV more susceptible to liver cirrhosis [3]. Human Immunodeficiency Virus infection and Hepatitis B Virus (HBV) have a mutually harmful effect in that HIV infection hastens the HBV-related liver illness, and HBV infection has been shown to exacerbate HIV infection management, hinder CD4 recovery, and hasten immunologic progression [1]. The risk of liver-related mortality has been found to be 2-3 times in HIV-HBV co-infected patients than in HIV mono-infected patients [3].

The most populous country in Africa is Nigeria, and studies have found that different groups have different prevalence rates of HBV and HIV infections. However, few studies have assessed the prevalence and risk factors for HBV infection among HIV-positive patients in Nigeria. This co-infection which may lead to the progression of HBV infection to other serious medical complications, including death in the HIV patients [1].

Additionally, in Zaria Kaduna State, Nigeria, there is no currently published data on HIV-HBV co-infection to the best of our knowledge. In light of this background, it becomes essential to determine the sero-prevalence of the co-infections of the two viruses in the study area, as well as generating epidemiological data that can be helpful in reviewing the preventive and treatment policy guidelines.

Therefore, the aim of this study is to determine the prevalence of hepatitis B virus surface antigen among HIV/AIDS patients attending National Tuberculosis and Leprosy Training Centre, Saye-Zaria, Nigeria.

Methods

Study area

The study was conducted at National Tuberculosis and Leprosy Training Centre, Saye-Zaria, Nigeria. This referral hospital is located within Saye village, Zaria local government area of Kaduna State, Nigeria. Services such as antenatal, laboratory, HIV counselling and testing, dermatological and outpatient, amongst others are rendered at the NTLTC.

Study population

This laboratory-based study was carried out by examining 120 HIV positive patients, 18 years and above on active antiretroviral therapy accessing care at the National Tuberculosis and Leprosy Training Centre, Saye-Zaria, Nigeria.

Ethical consideration, consent and criteria

Ethical approval letter with reference number: NTR/TRG/ZA/182/VOLIV was secured from the management of National Tuberculosis and Leprosy Training Centre, Saye-Zaria, Nigeria. Consent was also obtained from each of the HIV patient enrolled in the study. The inclusion criteria covered HIV patients on highly active antiretroviral therapy from 18 years and above who gave consent; while the exclusion criteria included HIV negative patients and HIV patients who were not on HAART.

Data collection and sample size determination

A structured questionnaire was designed to obtain socio-demographic characteristics to analyse their possible relationship with infection. The questionnaire was issued to each of the patients who gave consent after counselling, completed, and returned before collection of sample. The least sample size to be used in the study was determined using the formula below as described by Naing *et al.* [8]:

$N = Z^2pq/e^2$

Where N = sample size

Z = standard normal distribution at 95% confidence interval = 1.96

P = prevalence = 3.5 % (0.035) in the previous study [9].

q = 1- p; 1- 0.035= 0.965

e = allowable error (0.05)

$N = 1.96^2 \times 0.035 \times 0.965/0.05^2 = 52$

However, the sample size was increased to 120 to improve coverage in the study.

Sampling technique

Systematic sampling technique was employed, considering that on the average, 300 individuals per month that fulfilled the inclusion criteria received services from the facility. Thus, 3600 (300x12) were the estimated population that

receive HAART services over the sampling period of 12 months.

Individuals sampled = y/n^{th} ;

Where y= Population = 3600

n= sample size= 120

Individual to be selected = 3600/120= 30

Thus, the 30^{th} persons amongst those fulfilled the inclusion criteria were selected for the study

Sample collection

From each of the 120 patients enrolled in the study, 5mL of venous blood was collected aseptically between November 2022 and October 2023. The samples were collected using a disposable sterile vacutainer needle into EDTA (ethylenediamine tetra-acetic acid which is an anticoagulant) containers. The samples were centrifuged using ROTOFIX 32A at 40RPM/RCF×100 for 30minutes. The plasma was carefully aspirated into 2 mL cryo vials and then stored at 2 ^{o}C for further analysis [10].

Human Immunodeficiency Virus status rescreening test

All the blood samples collected were rescreened for HIV, using Alere Determine HIV-1/2 (Alere medical Co. Ltd) rapid test kits according to WHO standard, outlined by FMOH,(2010b) which is an in vitro immune-chromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2 in human plasma. Following the manufacturer's instructions, the protective foil cover of the test kit was removed and the kit labelled with the specimen identifier. Using Pasteur pipette, 50µl of the plasma was then added to the sample pad. The result was read after 15 minutes. Appearance of a single red line on the control region indicated a negative result. A positive result is indicated by appearance of a red line each on the control and the test regions (patient window site). An invalid result is indicated by the absence of red line on the control region. Inherent quality controls were used to validate the results.

Determination of HIV viral load count

The HIV-1 RNA was quantified using AmpliPrep/COBAS TaqMan amplification technique. Plasma was allowed to attain room temperature, vortexed using a thermo mixer and then1100µl of plasma was drawn from the cryo vials container and transferred into S-tube using an auto-pipette. The tubes were then arranged into S–rack together with low positive, high positive and negative control. The rack was then placed into the COBAS ampliprep/ COBAS Taqman machine chamber for automated specimen preparations, automated reverse transcription, PCR amplification and detection of HIV-1 target RNA and HIV-1 Quantitation Standard (QS) Amored RNA. And finally, the machine printed the result on printed paper automatically.

Determination of HBsAg

Onsite HBsAg rapid test kit (CTK BIOTECH, USA) was used for the detection of HBsAg. The test strip was removed aseptically from the sealed pouch and immersed in the sample as soon as possible with the arrow ending pointing towards the specimen; it was not immersed above the maximum line. The strip was taken out after 10seconds and was laid flat on a non-absorbent flat surface. Finally, the result was read after 15minutes. Appearance of two distinct red lines one on the control (C) region and the other on the test (T) region indicated a positive result. The intensity of the red colour in the test line region varies depending on the concentration of HBsAg present in the specimen. Therefore, any shade of red line in the region (T) was recorded positive. A negative result was indicated by appearance of a red line on the control region (C) with no appearance of apparent red or pink line on the test region (T). An invalid result was indicated by the absence of red line on the control region and this was due to insufficient specimen volume or incorrect procedural techniques. Inherent quality controls were used to validate the results.

Statistical analysis:

Results and data from the questionnaires were analysed using SPSS version 20 (IBM Corporation Armonk, New York, United States) to evaluate associations between socio-demographic variables and positivity for HBV and presented as tables and figures.

Results

The results obtained from the screening, re-affirmed that all the patients recruited in this study were HIV positive (100%) as indicated in Figure 1.

The overall prevalence of HBsAg infection among the people living with HIV is 10.8% (13/120). In relation to HIV viral load count, the prevalence was lower among those (6/70: 8.6%) with low viral load (<10,000copies/mL) compared to those (2/13: 15.4%) with high viral load

(≥100,000 copies/mL), and the differences observed were statistically significant (p= 0.000) **(Table 1)**.

Additionally, in relation to age (years) the highest (1/6:16.7%) prevalence of the HBsAg infection was recorded among the age group 60 years and above, while the lowest (1/19: 5.3%) was observed within the age group 18-31 years **(Table 2)**, however the differences observed were statistically insignificant (p=0.955). With regards to gender, the prevalence was higher in females (8/46: 17.4%) than in males (5/74: 6.8%), but the differences observed were statistically insignificant (p=0.359). The prevalence in relation to marital status, was highest among divorced (3/15: 20%), and lowest among the singles (1/19: 5.3%), but the differences observed were statistically insignificant (p=0.324). For educational status, the highest (3/10: 30%) and lowest (1/55:1.8%) prevalence rates were observed in primary school certificate holders and those attended tertiary institutions respectively, however the statistical observations were statistically by chance (0.583) (**Table 2**)

The HBV infection in relation to the pre-exposing factors revealed higher prevalence (5/79:63.3%) in those that were once transfused with blood, compared to those that were not (8/40: 20%) and the observed difference was statistically significant (p=0.000). Intravenous injection with illicit drugs was observed as predisposing factor responsible for higher prevalence (3/11: 27.3%), compared to those that were not practicing it (10/109: 9.2%), and this observed difference was statistically significant (p=0.000). Similarly, having history of the infection in the family was observed as responsible for higher prevalence (8/40: 20%) compared to those that did not have the history of the infection in their family (5/80: 6.25%), and the difference is statistically significant (p=0.000). Furthermore, unprotected sex was found to be responsible for higher prevalence (12/69:17.4%) in comparison to those that were not engaged in such practise (1/51:2.0%), and this observation was statistically significant (p=0.0000). The difference in close prevalence rates between those that answered 'yes' on whether they used to share sharp objects (8/77: 10.4%) and those that answered 'no' (5/43: 11.9%) was statistically by chance (p=0.020). Lastly, those that answered 'yes' on whether they were regular on HAART had the lower prevalence (12/117: 10.3%) compared to the higher prevalence (1/3: 33.3%) in those answered 'no', and the difference was statistically significant (p=0.003) **(Table 3)**.

Figure 1. HIV Status of the Individuals enrolled in the Study.

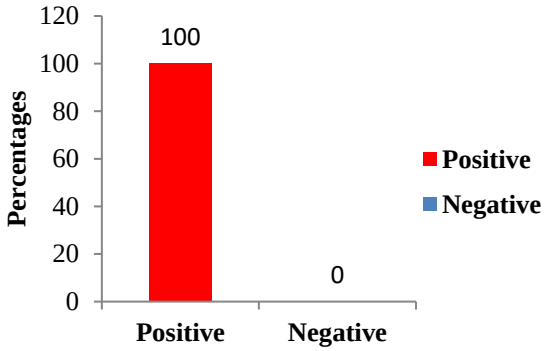

Table 1. Prevalence of HBsAg in relation to HIV viral load count among HIV/AIDS patients.

Viral Load (copies/mL)	No. examined (n=120)	No. positive (%)	Chi-Square	Df	p-value
Low	70	6(8.6)	121.870	3	0.000
Medium	21	2(9.5)			
High	13	2(15.4)			
Invalid	16	3(18.8)			
Total	120	13 (10.8)			

Key: Low= <10,000; Medium= 10,000-99,999; High= ≥ 100,000; Statistical association (p=0.000); Df= Degree of freedom

Table 2. Prevalence of HBsAg in relation to some demographic factors among HIV/AIDS patients.

Age Group (years)	No. Examined (n=120)	No. Positive (%)		Chi-Square Value		Df	p-value
Age (years)							
18-31	19	1 (5.3)		5.077		3	0.955
32-45	52	5 (9.6)					
46-59	43	6 (14.0)					
60 and above	6	1(16.7)					
Gender							
Male	74	5(6.8)		2.052		1	0.359
Female	46	8(17.4)					
MS							
Married	66	7(10.6)		6.963		3	0.324
Divorced	15	3(20.0)					
Single	19	1(5.3)					
Widowed	20	2(10.0)					
ES							
Informal	25	5(20.0)		4.701		3	0.583
Primary	10	3(30.0)					
Secondary	30	4(13.3)					
Tertiary	55	1(1.8)					

Key: MS= Marital status; ES= Educational status; Df= Degree of freedom

Table 3. Prevalence of HBV infection in relation to some predisposing factors among HIV/AIDS patients.

Variables	No. examined (n=120)	No. Positive (%)	Chi-Square	Df	p-value
History of B.T					
Yes	79	5(63.3)	125.143	1	0.000
No	40	8(20.0)			
Injection with illicit drugs					
Yes	11	3(27.3)	123.357	1	0.000
No	109	10(9.2)			
Family history					
Yes	40	8(20%)	120.216	1	0.000
No	80	5(6.25%)			
Unprotected sex					
Yes	69	12(17.4)	120.512	1	0.000
No	51	1(2.0)			
Sharing of sharp objects					
Yes	77	8(10.4)	120.065	1	0.020
No	43	5(11.9)			
Regular on ART					
Yes	117	12 (10.3)	120.252	1	0.003
No	3	1 (33.3)			

Key BT= Blood transfusion, ART= Antiretroviral therapy, Statistical association ($p<0.05$)

Discussion

The overall prevalence of the HBV infection reported in this study is alarming, because it implies that at least one out every ten people living with HIV (PLWHIV) may end up facing rough ride of HBV infection thereby compounding the outcome of progression of the HIV infection. The immune deficiency condition of the subjects is worrisome as it may facilitate the progression of the infection into liver cirrhosis, hepatic carcinoma and other complications. The current prevalence of 10.8% reported in this study is in agreement with 12.5% prevalence reported from north western Nigeria by Hamza *et al.*[11], 9.2% reported by Akindigh *et al.* [12] in north central Nigeria, and 11.5% observed in 2012 in a health facility in north-central Nigeria [13].This study also observed that HIV patients on HAART with high viral load count had a statistically significant higher prevalence of HBV than those with low VLC. This implies that those whose system was battling with high viral load of HIV may concurrently be facing another life threatening challenge of liver, posed by HBV infection. The driving factor here may be the immune system whose strength is boosted by eliminating HIV in peripheral circulation using the antiretroviral drugs, as reported by Alxender and Rosenthal [14].

From the current findings, the prevalence of the HBV infection among the subjects increases with age. This may be associated with many factors such as depletion of immunity (why the prevalence is higher in the 60 years old and above) and exposure to social versus (why the prevalence is higher among 32-45years) among others. The result is in agreement with the findings of Okocha *et al*. [15] and Omatola *et al*. [9], who similarly reported increased in the prevalence of HIV-HBV co-infection with increase in the age group of the subjects, in their independent findings. Similarly, Kolou *et al* [16] reported highest prevalence of HBV infection among individuals within socially active age (20-39 years) compared to other groups.

From our study, both males and females have almost similar chances of acquiring the HBV infection upon the same degree of exposure. This is because the higher prevalence in females in the study was statistically by chance, which is in conformation with the work of Bao *et al* [17], who reported statistically insignificant higher prevalence of HBV infection in females among HIV patients in China. Another point of concern in our findings with respect to gender is that 61.7% of those participated in the study were males, implying that proportion of males attending the HAART clinic is higher than that of females, which may be an indication that females are still shying away from the service, have less awareness about the service or their HIV status. This is in agreement with similar findings reported where less utilization of HIV infection management approaches were observed more in females [18], but contrary to findings in Tanzania which reported more females utilizing the HAART than males [19]. The difference may be due to variation in culture.

Although, marriage is a social barrier to having multiple sexual partners and therefore a protective factor against acquiring HBV infection; from our findings, the marital status was neither preventive nor a risk factor of the infection. Perhaps it is because the HIV positive status of the individuals had influenced their attitudinal change for practicing preventive measures across all the categories. Similarly, educational status was not found to play a role in acquiring or prevention against the HBV infection in our findings. However, recording lower prevalence in those that have acquired academic tertiary institution certificate may be indicative of possible correlation between education and awareness about the HBV infection, hence had taken some preventive measures. This agrees with the findings from a previous study by Okoye *et al*. [20]. Educated persons are often more aware of preventive measures against infectious diseases, and this may have accounted for the observed pattern of the result. The prevalence of HBV, however, was not significantly affected by educational status in this study. Omotola *et al.* [9], similarly reported higher HIV-HBV co-prevalence among individuals with low educational status compared to those that have higher educational status with significant statistical difference.

The observation of having statistically significant higher prevalence among those that had never been transfused with blood, is an indication to the possibility that people that were healthy enough to had never experienced the blood transfusion are likely to be negligent in taking preventive measures against blood borne diseases, thereby putting themselves at risk of such infections. These findings are in agreement with findings previously reported [20]. Using intravenous illicit drugs by injection was observed as statistically significant risk factor of acquiring HBV infection. This may be due to a

multiple use of the syringe-needles which is a potential web of HBV infection transmission. These findings are in agreement with the report of Bao *et al* [17], where prevalence of HBV among HIV individuals was reported as statistically significant in some part of China. Despite the enlightenment on the dangers of unprotected sex, our study established that many patients might have acquired the HBV infection through such practices. Perhaps, the practice remains a risk factor not just to singles with multiple sex partners, but also married individuals who unsuspectedly contract the infection from their married partners. This report is in agreement with the work of Roberts *et al* [22], in which unprotected sex was implicated as responsible for 95% of HBV transmission.

According to our findings, sharing of sharp objects is not currently practiced frequently enough to be a risk factor of transmission, however, it is still practiced. The current finding is in agreement with the previous study where sharing of sharp objects was reported to be related with higher prevalence of HBV infection statistically by chance [9]. Furthermore, there is statistical evidence indicating that regular use of antiretroviral drugs may have a possible role in prevention against the HBV infection among HIV patients. This may be due to the ability of the drugs to reduce the HIV load in blood circulation, thereby giving chance to immune system to rejuvenate and possibly fight other infections including HBV. This finding is in agreement with previous report which shows that normal intake of antiretroviral drugs was statistically related to low prevalence of HBV antiginemia [23].

Conclusion

HBsAg was detected with the prevalence of 10.8% (13/120) among HIV infected patients attending NTLTC Saye-Zaria. Having high HIV viral load was recorded as predisposing factor for HBV infection. Differences were observed in prevalence across the age groups, gender, marital and educational status, but none was statistically significant.

The study also recorded never been transfused with blood, injection with intravenous illicit drugs, history of the HBV infection in the family and irregularity in the use of the Highly Active Antiretroviral Therapy (HAART) as predisposing factors for a high prevalence of the HBV infection in HIV patients across their categories, and the observation were statistically significant ($p=\leq 0.05$).

The study unveiled the possibility of concurrent transmission of HIV-HBV co-infection, which could increase hopelessness in the prognosis of each of the infection compared to when it happens singly.

Limitaion of study

The study could not follow up the prognosis of the HIV-HBV co-infection, in comparison to those that had HIV mono infection, to be able to observe the possible outcomes of the two different status. Furthermore, the study design did not allow determination of which infection occured first, limiting our ability to assess whether some HBV infection might have resolved spontaneously if acquired earlier, rather than persisting due to prior or conccurrent HIV infection.

Recommendations

1. Routine screening of PLWHIV for HBsAg should be considered before the initiation of HAART so that proper treatment can be offered to the co-infected individuals in order to improve quality of life and reduce morbidity, especially since HBV infection is now treatable with administration of interferon, anti-HBV nucleoside and so on.
2. There is a need to create more awareness on the danger of unprotected sex, sharing of contaminated sharp objects and importance of adhering to HAART prescription.
3. Human Immunodeficiency Virus management policy should include HBV vaccination as a critical preventive strategy against the HBV infection.

Conflicts of intrest

The authors declare that they do not have any conflict of interest.

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Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors contributed significantly to the work. All authors reviewed and approved the final version of the manuscript.

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