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Detection of Hepatitis B Virus among HIV Positive Fresh Undergraduate Students in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author IOO designed the study, wrote the protocol, performed the laboratory analysis and wrote the first draft of the manuscript. Authors SAO, CO and TIC managed the analyses of the study. Authors SAO and TIC managed the literature searches and performed the statistical analysis. Author IOO supervised the whole study which, author CO used as part of her B.Sc. Project in the Department of Microbiology, University of Port Harcourt, Port Harcourt, Nigeria. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was carried out to ascertain hepatitis B virus (HBV) and human immunodeficiency virus (HIV) co-infection among fresh undergraduate students of the University of Port Harcourt, Rivers State, Nigeria.
Study Design: Cross-sectional study.
Place and Duration of Study: Lulu Briggs Health Center, University of Port Harcourt, Nigeria, between June 2014 and July 2015.
Methodology: The study was conducted on fresh undergraduate students of the University of Port Harcourt (43 male and 57 female) accessing HIV services at Lulu Briggs Health Center, University of Port Harcourt, Nigeria, who consented to be part of the study. A total of 100 patients were recruited for the study. All 100 blood samples were HIV positive and then screened using HBsAg one-step Hepatitis B surface antigen (HBsAg) test strip and HBsAg one Ultra ELISA kit for the detection of HBsAg.

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Results: HBsAg was detected in 2(2%) of the subjects. Patients of the age group 16-20 years had the highest percentage of HIV/HBV co-infection (6.5%). HIV/HBV co-infection was dominant among males 1 in 43(2.3%) than in females 1 in 57(1.8%). However, age (p= 0.1031) and sex (p= 0.8399) were not statistically associated.
Conclusion: This study confirmed the presence of HIV/HBV co-infection in the study population. This highlights the necessity to create awareness of HBV prevention and transmission among HIV positive individuals.

Keywords: HIV; HBV; co-infection; prevalence; Nigeria.

1. INTRODUCTION

Hepatitis B virus (HBV) causes a potentially life-threatening systematic disease primarily involving the liver [1]. It is simply an inflammation of the liver, causing cirrhosis, fibrosis, and hepatocellular carcinoma. The disease is a major global health concern commonly transmitted perinatally or through exposure to infected blood, leading to about one million deaths annually from liver diseases [1,2].

Hepatitis infection either acute or chronic is defined by the presence of HBV surface antigen (HBsAg). HBsAg is produced in excess during viral replication. HBsAg appears in the bloodstream days or weeks after infection, often long before signs of liver damage are evident [3]. Without intervention, a mother who is positive for HBsAg confers a risk of passing the infection to her offspring at the time of birth [4]. Chronic infection is more likely to develop in infants born with HBV [5].

Globally, about 37.9 million persons living with HIV, with 1 in every 25 adults living with the virus in Africa [6]. Generally, once a person becomes infected with HIV, the course of the disease may be great, some rapidly progressing into AIDs and death within a few years if untreated. This is further complicated with the presence of HBV, as results from countries with highly active antiretroviral therapy (HAART) suggesting liver diseases associated with HBV plays a major role in deteriorating health and increasing the mortality rate in persons living with HIV [7]. Both viruses share certain epidemiological characteristics, with HBV hinted to fasten the progression of HIV, even to AIDS [8].

Out of the total number of persons living with HIV, about 7.4% are co-infected with HBV WHILE 1% of persons living with HBV are also infected with HIV [1]. HIV and HBV co-infection are relatively common because both viruses share common modes of transmission and probably because HBV produces a protein that has been shown to promote the replication and transcription of HIV as *in vitro* studies suggest [9-12]. Co-infection of HBV with HIV largely modifies the natural course of HBV infection along with its management, as it increases HBV replication levels and the risk of hepatotoxicity in addition to drug interactions [13,14].

HIV and HBV are highly prevalent in Africa and both infections are endemic in Nigeria [1,10]. The adverse effects of HIV/HBV co-infection necessitate the investigation among fresh undergraduate students of University of Port Harcourt, Nigeria, as this will be useful in the control and prevention of hepatitis B virus transmission especially among patients who are HIV positive.

2. MATERIALS AND METHODS

2.1 Study Area and Population

Lulu Briggs Medical Centre is located within the University Park of the University of Port Harcourt, along the East-West Road, Obio-Akpor local government area of Rivers State, Nigeria. The institution provides primary health care for members of the university community. The study was conducted on fresh undergraduate students of the University of Port Harcourt (43 males and 57 females) accessing HIV services at Lulu Briggs Health Center, University of Port Harcourt, Nigeria, who consented to be part of the study. A total of 100 patients were recruited for the study.

2.2 Sample Collection

Blood samples were collected from HIV positive fresh undergraduate students of the University of Port Harcourt, at Lulu Briggs Health Center, University of Port Harcourt, Nigeria and used in this study. Serum was obtained from the collected blood and directly analyzed.

2.3 Inclusion and Exclusive Criteria

Consenting fresh undergraduate students of the University of Port Harcourt accessing HIV services at Lulu Briggs Health Center, University of Port Harcourt, Port Harcourt, Nigeria who were not on medications for any conditions, were included in the study irrespective of age. Patients who failed to give their consent were excluded from the study.

2.4 Sample Analysis

Serum from the collected blood samples of recruited patients was tested for the presence of HBsAg using HBsAg one-step Hepatitis B surface antigen test strip (an *in-vitro* rapid diagnostic kit produced by ACON Laboratories Inc., Californi, United States) and HBsAg one Ultra ELISA kit (produced by Dia.Pro. Diagnostic Bioprobes Srl., Milano, Italy). The HBsAg one-step Hepatitis B surface Antigen test Strip serum/plasma) is a qualitative, lateral immunoassay for the detection of HBsAg in serum or plasma. All tests were run using quality controls according to standard operating procedures as instructed by the manufacturer.

ELISA tests were performed according to the manufacturer's instructions. The required numbers of strips were placed in the plastic holder and wash them once to hydrate wells. The wells for controls, calibrator and samples were carefully identified. The A1 well was left empty for blanking purposes. One hundred and fifty microliters (150 µl) of the negative control was dispensed in triplicate, 150 µl of the calibrator in duplicate and then 150 µl of the positive control in single followed by 150 µl of each of the samples. The presence of samples in the well was carefully checked by the naked eye or by reading at 450/630 nm. One hundred microliters (100 µl) of the diluted enzymatic conjugate was dispensed in all wells, except for A1, used for blanking operations. Following the addition of the conjugate, the colour of the samples changed from yellowish to pink/red and then the microplate was incubated for 120 minutes at 37°C. When the first incubation was over, the microwells were washed using ELISA microplate washer (Model ELx50, BioTek Instruments, USA). Two hundred microliters (200 µl) of chromogen/substrate was dispensed into all the wells, A1 included. The microplate was protected from light and incubated at 18-24°C for 30 minutes. Wells dispensed with the positive control, calibrator and positive samples turned from clear to blue. One hundred microliters (100 µl) of Sulphuric acid was dispensed into all the wells to stop the enzymatic reaction, using the same pipette sequence as in steps above. Addition of the acid solution turned the positive control, the calibrator and positive samples from blue to yellow/brown colouration. The colour intensity of the solution in each well was measured using a 450 nm filter (reading) and a 630 nm filter, blanking the instrument on A1 using ELISA microplate reader (Model ELx808i, BioTek Instruments, USA).

The test results were calculated using a cut-off value determined on the mean OD450 nm value of the negative control (NC) with the following formula: NC + 0.050 = Cut-Off (Co). Test results are interpreted as ratio of the sample OD450 nm (S) and the Cut-Off value (Co), mathematically S/Co, according to the following: < 0.9 = negative, 0.9 – 1.1 = equivocal and > 1.1 = positive. A negative result indicated that the patient is not infected by HBV and that the blood unit may be transfused. Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample; the blood unit should not be transfused. A positive result was indicative of HBV infection and therefore the patients should be treated accordingly or the blood unit should be discarded.

2.5 Statistical Analysis

Data from serological results were analysed using descriptive statistics such as frequencies and percentages to estimate the overall prevalence and its significant relationship with age and sex. Chi-square test was used to determine relationships between the variables and the prevalence of HBsAg. The p-value was considered significant at 0.05. All analyses were conducted using complex samples analysis of the Statistical Package for the Social Sciences (SPSS), IBM version 22.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Overall prevalence of HIV/HBV co-infection

During the study, a total of hundred HIV-positive patients were screened for HBV to confirm co-infection. All participants were conversant with their HIV status. Only two samples tested

positive to HBsAg to give a prevalence of 2% (Table 1).

3.1.2 Correlation of HBV and HIV co-infection in relation with age

In the age group of 16-20 years, a total of 31 samples were tested of which two (2) tested positive, thus giving the highest prevalence of 6.5%. Age groups of 21-25 years and 26 and above showed a prevalence of 0.0%. However, this difference is not statistically associated (p= 0.1031) (Table 2).

3.1.3 Correlation HBV and HIV co-infection in relation to sex

Table 3 shows the prevalence of HBV and HIV co-infection in relation to sex. Males HIV patient were more infected with HBsAg (2.3%) than females (1.8%). Sex was not statistically associated (p= 0.8399).

3.2 Discussion

This study investigated the frequency of HBV (indicated by a positive test for HBsAg) and HIV co-infection among fresh undergraduate students of the University of Port Harcourt, accessing HIV services at Lulu Briggs Health Center, University of Port Harcourt, Rivers State, Nigeria. Out of the 100 HIV patients that participated in this study, the number of females was more than the number of males. This is in agreement with Aliyu et al. [15] on the sex distribution of individuals with HIV attending treatment centres, which shows a disparity in favour of the female gender.

The prevalence of HBV/HIV co-infection in this study (2%) is lesser than the prevalence of 16.4% reported for the Niger Delta region [11] where this study was conducted. A potential explanation for this disparity could be because the present student covered a smaller study population. HBV infection in HIV-positive subjects is more common than that with Hepatitis C virus (HCV) but more attention has been paid over the years to HCV infection because its risk if causing chronic disease is higher [16]. Increased prevalence of HIV may translate to an increase in HBV prevalence since HBV has similar routes of transmission as HIV. Furthermore, co-infection with HBV and HIV seem to demonstrate a correlation between these two infections which could influence the evolution of these diseases [17].

The highest frequency of HBsAg amongst the HIV positive subjects was observed in the age group 16-20 years. Results from previous studies showed that age has always proved to be the most important factor in all epidemiological studies of HIV and HBV [11,15,18,19]. In this study, the different seroprevalence of HBsAg in the various age groups indicates that age plays an important role in the prevalence rates. The high prevalence rate of HBsAg among HIV positive subjects aged 16-20 years can be attributed to the peak age of sexual experimentation among young adults. These

Table 1. The overall prevalence of HIV/HBV co-infection

Infection	No Positive (%)
HIV/HBV	2(2.0)
HIV alone	98(98.0)
Total	100(100.0)

Table 2. Prevalence of HIV/HBV co-infection in relation to age

Age	No. tested	No positive (%)	Chi-square Test
16 – 20	31	2(6.5)	p= 0.1031
21 – 25	58	0(0.0)	
26 and above	11	0(0.0)	
Total	100	2 (2.0)	

Table 3. Prevalence of HIV/HBV co-infection in relation to sex

Sex	No. tested	No. positive (%)	Chi-square Test
Male	43	1(2.3%)	p= 0.8399
Female	57	1(1.8%)	
Total	100	2 (2.0)	

findings were not consistent with similar work carried out by Avwioro et al. [11] which found that individuals within the ages of 31-50 years had the highest prevalence. Such disparity in HBV/HIV co-infection concerning age can be explained by the fact that fresh undergraduates are usually younger than the average age of sexually active people in the general population.

Males HIV patient were more infected with HBsAg (2.3%) than females (1.8%) in this study. Ekanem et al. [20] similarly reported that males were more likely to be co-infected (55.2%) than females (44.8%). This contrasted with the study by Avwioro et al. [11] which reported that females are more infected with HBsAg (5.0%) than males (3.5%) and Aliyu et al. [15] which reported a prevalence of 11.5 to 14.8 in favour of the females.

4. CONCLUSION

Prevalence of HIV/HBV co-infection among fresh undergraduate students accessing HIV services in Lulu Briggs Health Center is low (2.0%). Among the two variables (age and sex) evaluated, none were statistically associated. Because of the complications associated with having both infections, all newly diagnosed HIV patients should also be screened for HBV, to improve the management of co-infection.

CONSENT

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

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the University of Port Harcourt Research Ethics committee and have therefore been performed following the ethical standards laid down in the 1964 Declaration of Helsinki. Ethical clearance was obtained from the University of Port Harcourt Research and Ethics committee (UPH/R&D/REC/04) and approvals were obtained from the management of Lulu Briggs Health Center.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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