Original Research Article

Human Immunodeficiency Virus (HIV) and *Treponema pallidum* (syphilis) co-infection in Uyo, Nigeria

.

ABSTRACT

Aim: HIV/AIDS remains a leading cause of death and disability in Sub Saharan Africa and this accounts for almost half of the world's HIV related deaths. On the other hand, bacterial sexually transmitted diseases (STDs) such as syphilis contributes to the morbidity and mortality obtained in developing countries. Co-infection of syphilis and HIV may increase the risk of HIV transmission and adversely affect reproductive health. Prompt diagnosis and treatment of STDs in HIV positive individuals can help prevent spread to their partners. There is also very little information about incidence and prevalence of HIV/Syphilis co-infection and their determinants. The aim of this study is to evaluate the HIV/Syphilis co-infection among HIV-infected individuals in Uyo, Nigeria.

Methods: A total of 176 individuals living with HIV participated in this study. The average age of the study participants was 39.1 years from a range of 6-67 years. Plasma samples obtained from the human subjects were analysed for presence of HIV and Syphilis antibodies using enzyme-Linked immunosorbent Assay.

Results: Our findings showed that the overall prevalence of HIV/Syphilis co-infection in Uyo was 1.7%. Analysis of the results revealed that the variables—sex and educational background—significantly influenced the rate of syphilis sero-positivity among the population under study. While variables- age, marital status and occupational skills non-significantly influenced the rate of syphilis sero-positivity among the population under study.

Conclusion: This study confirmed the co-infection of HIV and Syphilis in Uyo, Nigeria. Early screening of Syphilis and other STDs contributes to the control of infection and reduces the spread of HIV to partners. A number of primary preventive interventions for HIV and syphilis need to be adopted including use of condoms and medical male circumcision in order to improve sexual and reproductive health amongst individuals.

***Keywords*:** Co-infection, HIV, Syphilis, Prevalence, Nigeria

1. INTRODUCTION

Sexually transmitted diseases (STDs) are infectious diseases that can be transferred from one individual to another through sexual contact. The World Health Organization [1]

estimates that over 1 million new STDs are acquired each day. STDs have become lethal factors in the global burden of disease, with their management hampered by the diversity of pathogens, social stigmatization, and by lack of or mild symptoms [2].

Syphilis a major STD and remains an important cause of morbidity. It is considered a dangerous combination when in association with HIV [3]. Syphilis is caused by *Treponema pallidum,* a thin helical spirochete approximately 0.5nm-6.50nm [4]. Syphilis is a global public health hazard that continues to be a tragic and substantial problem in many developing countries, including Nigeria, where it is more common among the poor population and those with multiple sex partners [4, 5]. Vertical transmission from infected mother to child is also a major concern [6].

Blood transfusion increases the possibility of transmitting STDs such as Syphilis and are less likely to spread toxoplasmosis, malaria and viral infections [7]. The report of syphilis transmission through transfusion decades ago led to the recommendation of screening blood donors of syphilis by the WHO [8]. The presence of antibodies to syphilis in blood donors could be the result of non-specific reactions or an old syphilis infection. Prompt diagnosis and treatment of syphilis reduces chances of serious complications such as infertility in both genders and any adverse outcomes in pregnancy [9]. WHO suggest *Treponema pallidum* Haemagglutination Assay (TPHA) and the Enzyme Immunoassay (EIA) as specific test or Rapid Plasma Reagin (RPR) and Venereal Disease reference Laboratory (VDRL) as non-specific test for Syphilis screening [8].

Syphilis morbidity ranges from the relatively minor symptoms of the primary stage of infection to the more consequential neurological and cardiovascular effects of the tertiary stage of the disease [10]. The infection is re-emerging as a public health hazard globally particularly, among HIV infected persons [11]. The effect of the infections on each other is well documented. HIV wanes the immune system and there is a strong correlation between a weaken immune system and a higher chance of developing syphilis [11]. Conversely, the genital ulcerations and inflammation caused by syphilis are implicated as co-factor making infected individuals more likely to acquire HIV if exposed to the virus through sexual contact [12]. Syphilis-HIV co-infection provides further burden to HIV-control efforts, particularly in resource-limited countries in sub-Saharan Africa that have a high prevalence of HIV infection [13].

The urgent need for innovative research is one of the strategic plans of WHO to address the burden of STDs globally [2, 14]. A preponderance of studies of syphilis/HIV co-infection in Nigeria has been on pregnant women because of the risk of congenital transmission. There is however insufficient information on the prevalence of syphilis/HIV co-infection in the general population of Uyo, Nigeria. This study was carried to assess the prevalence of syphilis/HIV co-infection among HIV patients attending University of Uyo Teaching Hospital (UUTH), Uyo, Nigeria.

2. MATERIAL AND METHODS

2.1. Study area

The study was conducted in the University of Uyo teaching hospital (UUTH), Akwa Ibom State, Nigeria. Akwa Ibom state is made up of 31 LGA's lies between latitudes 4^{o} 32'N and 5^{o} 33'N, and longitudes 7^{o} 25'E and 8^{o} 25'E.

2.2. Study design

This is a cross-sectional study involving a cohort of HIV-infected patients attending University of Uyo Teaching Hospital (UUTH), Uyo, Nigeria and is to investigate syphilis

prevalence and its co-infection among HIV-infected patients in Uyo, Nigeria. The methods adopted in this study consists of informed consents from study participants, blood withdrawal by venepuncture, screening for suspected cases of syphilis, clinical evaluation and recording of demographic information's such as the age, marital status, occupation, sexual activity etc.

2.3. Determination of sample size for the study

The sample size for this study was determined using the established formula [15, 16]: N= [Z^2 (PQ)]/d^2. Where N is the desired sample size. **Z** = standard normal deviation at a 95% confidence interval (which was 1.96). **p** = proportion of target population (prevalence estimated at 6.4% as at 2012); this implies 6.4/100 = 0.066. **q** = alternate proportion (1-p), which was calculated as: 1 – 0.06 = 0.936. **d** = desired level of precision (degree of precision/significance). This was taken as 0.05. Then, the desired sample size (N) = 92. Hence, the estimated sample size was 92 individuals with an additional 10.0% sample (which is 9.2) to take care of study participants that may be lost to follow-up [15, 16], providing a total sample size of 101. However, A total of 176 samples were collected to take care of participants that may be lost to follow –up.

2.3. Study population

The study population includes HIV- positive male and female patients attending University of Uyo Teaching Hospital (UUTH) in Uyo, Nigeria. At most, 176 HIV-positive patients were selected and enrolled for the study (Table 1). While the entire HIV- positive individuals in Uyo, Akwa Ibom State, Nigeria was the target population to which the findings of the study were extrapolated. The demographic details relevant to the study were obtained as shown in the Table 1.

2.4. Ethical Considerations

Ethical considerations and approval for the study was sought from the Health Research and Ethics Committee of University of Uyo Teaching Hospital, Uyo, Nigeria in accordance with the ethics for research involving human subjects. This study was carried out in line with the World Medical Association (WMA) Declaration of Helsinki on the principles for medical research involving human subjects, animal subjects and identifiable human/animal material/data.

2.5. Inclusion and exclusion criteria

All HIV-infected patients were eligible for the study. HIV-infected patients who had full documentation in the registration book were included, whereas HIV-infected patients who had incomplete data like age, sex and duplicate records were excluded from the study. Those on any form of antibiotics were also excluded from the study.

2.6. Specimen Selection, Collection, and preparation

About 5ml venepuncture blood were collected in EDTA BA Vacutainer TM anti-coagulant tubes (BD, Franklin Lakes, USA). Plasma specimens were separated by centrifugation at 3000rpm (resolutions per minute) for 5 min. The plasma was stored at -20°C and used for the serological analyses.

2.7. Re-Screening of the samples for HIV antibodies

Blood samples of HIV positive individuals were collected by venepuncture method and re-screened for HIV antibodies using the Determine HIV-1/2, HIV ½ Stat Pak and ELISA Kits. Plasma was tested at the Virus Research Unit, Department of Microbiology, University of Port Harcourt, for the presence of antibodies to HIV following the respective manufacturer's instructions. HIV testing was done according to the national algorithm recommended by the Federal Ministry of Health of Nigeria. Rapid HIV tests: HIV (1 + 2) rapid test strips (Determine, Alere Co, LTD, Japan) as the screening test; and Stat-Pak (Chembio Diagnostic Systems, Inc., New York, NY, USA) as a confirmatory test for positive samples. These HIV testing methods were immuno-chromatographic assays. All samples with non-reactive results to HIV kits were considered negative. A commercially available HIV-1/2/P24/O ELISA kit (ELISA; Dia.Pro, Milano, Italy), was used as a tie-breaker. Positive and negative standard sera, accompanying the kit were included in each assay. Laboratory testing was carried out according to the manufacturers' instructions, and all tests were run using quality controls according to standard operating procedures.

2.8. Serological Analysis for *Treponema pallidum* (Syphilis)

Syphilis was analysed using Syphilis Ab version ULTRA ELISA kit (by DIA.PRO Diagnostic Bioprobes, Milano-Italy for the determination of antibodies to *Treponema pallidum* in serum. Assay Procedure was performed using the Long Incubation method (1st incubation 60 minutes, 2^{nd} and 3^{rd} incubations 30 minutes) by the manufacturer's specification as stated below: The required number of microwells was placed in the microwell holder. Leaving A1 well empty for the operation of blanking. One hundred microliter (100µl) of negative control in duplicate, 100ul of calibrator in duplicate and 100ul of positive control in single were dispensed in proper wells, followed by 100ul of each of samples according to the dispensation scheme. The strips were sealed with the adhesive sealing foil because the test was performed manually. The plates were incubated for 60 min at 37°. The plates were washed with an automatic washer for 5 cycles. One hundred microliter (100µl) Enzyme Conjugate was pipette into each well, except the 1^{st} blanking well, and covered with the sealer. The plates were incubated the microplate for 30 min at +37°C. The plates were washed with an automatic washer for 5 cycles. One hundred microliter (100µl) TMB/H2O2 mixtures were pipette into each well, the blank well included. The plates were incubated for 30 minutes at room temperature (18-24°C). It was not exposed to strong direct light as a high background might be generated. One hundred microliter (100µl) of Sulphuric Acid was added into all the wells using the same pipetting sequence as described before to stop the enzymatic reaction. Addition of acid turned the positive control and positive samples from blue to yellow/brown. The colour intensity of the solution in each well, was measured with a microplate reader at 450nm (reading) and at 630nm (background subtraction, strongly recommended), blanking the instrument on A1 well. Results were interpreted according to manufacturer's specification.

2.9 Calculation of the Cut-Off

The tests results were calculated by means of a cut-off value determined with the following formula on the mean OD450nm value of the Negative Control (NC): NC + 0.200 = Cut-Off (Co).

2.10. Interpretation of Results

Test results were interpreted as ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following: S/Co < 0.9 = Negative, S/Co 0.9 - 1.1 = Equivocal and S/Co > 1.1 = Positive. A negative result indicates that the patient has not been infected by *Treponema pallidum* or that the blood unit may be transfused. Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. A positive result is indicative of *Treponema pallidum* infection and therefore the patient should be treated accordingly.

2.11 Detection of co-infection of Syphilis and HIV

Blood samples positive to Syphilis and HIV were noted as co-infection.

2.12 Data Analysis

Microsoft Excel (Microsoft Corporation) was used to analyse the data collected. The sero-prevalence was calculated as the number of serologically positive samples divided by the total number of samples tested multiplied by a 100%. The Chi-square test was used to determine associations between demographic factors and sero-positivity of Syphilis and HIV. The level of significance was set at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Participants characteristics

A total of 176 HIV positive individuals from Uyo, Akwa Ibom State, Nigeria were recruited for the study. The age range of the HIV positive individuals who participated in the study was 6-67 years with an average age of 39.1 years. One hundred and thirteen (64.2%) of them were in the 36-67 years age range. The majority (61.9%) of the HIV-1 infected individuals were females and 38.1% were males. The male: female ratio in the present study is 1: 2. Over 60.0% of the HIV-1 infected individuals were married (60.2%, n=109), 57 (32.4%) were singles and 13(7.4%) were divorced/widows/widowers. Majority of the study participants had tertiary education (49.5%, n=87), 62 (35.2%) had secondary education, 24(13.6%) had primary education and 3 (1.7%) had no formal education. In terms of occupation, the majority were traders (31.8%), followed by civil servants (14.8%), students (10.2%), teaching (8.5%), artisans (6.8%), and 5.7% were unemployed, public servants, driving and retirees while and business (3.4%) and farmers (2.3%) were the least (Table 1).

3.1.2. Overall prevalence of HIV/Syphilis co-infections

Results showed an overall prevalence of HIV/Syphilis co-infection in Uyo was 1.7%. Table 1 shows the sero-prevalence of HIV/Syphilis co-infections amongst HIV-infected individuals in Uyo, Nigeria in relation to their socio-demographic and clinical variables.

3.1.3. Age-specific HIV/Syphilis co-infections

Higher HIV/Syphilis co-infections occurred among age groups 36-67 years (1.8%) than in age groups 6-35 years (1.6%). However, this difference was not statistically associated ($P > 0.05$, $X^2 = 9.955$) as shown in Table 1.

3.1.4. Sex-specific HIV/Syphilis co-infections

Higher HIV/Syphilis co-infections occurred among females (1.8%) than in males (1.5%). The study showed significant difference (P<0.05) between sex and HIV/Syphilis co-infections amongst HIV-1 infected individuals (Table 1).

3.1.5. Marital Status-specific HIV/Syphilis co-infections

Higher HIV/Syphilis co-infections occurred among individuals who were married (1.9%) than singles (1.8%) and divorced/widow/widower (0.0%). A non-significant difference (P=0.770; X^2 value =3.841) exists between marital status and HIV/Syphilis co-infections amongst HIV-infected individuals (Table 1).

3.1.6. Educational Status-specific HIV/Syphilis co-infections

Higher HIV/Syphilis co-infections occurred among individuals who had no formal education (33.3%) than primary education (4.2%) and secondary education (1.6%). Those with tertiary education had zero prevalence (0.0%). Significant difference (P<0.05) exist between educational status and HIV/Syphilis co-infections (Table 1).

3.1.7. Occupation-specific HIV/Syphilis co-infections

Higher HIV/Syphilis co-infections occurred among unemployed individuals (10.0%) than students (5.6%) and traders (1.8%). Other occupational groups had zero prevalence for HIV/Syphilis co-infection. A non-significant difference (P= 0.888) exists between occupational skills and HIV/Syphilis co-infections (Table 1).

Table 1: **Prevalence of HIV/Syphilis co-infections in relation to the Socio-demographical Characteristics of HIV-infected Individuals in Uyo, Nigeria**

Variables	No. Tested (%)	Syphilis (%)
Age groups (Years)		
6 – 35	63 (35.8)	1 (1.6)
36 – 67	113 (64.2)	2 (1.8)
Sex		
Males	67 (38.1)	1 (1.5)
Females	109 (61.9)	2 (1.8)
Marital Status		
Married	106 (60.2)	2 (1.9)
Singles	57 (32.4)	1 (1.8)
Divorced/Widowed	13 (7.4)	0 (0.0)
Educational Status		
Non-Formal	3 (1.7)	1 (33.3)
Primary	24 (13.6)	1 (4.2)
Secondary	62 (35.2)	1 (1.6)
Tertiary	87 (49.5)	0 (0.0)
Occupation		
Trading	55 (31.3)	1 (1.8)
Teaching	15 (8.5)	0 (0.0)

Civil Servant	26 (14.8)	0 (0.0)
Public Servant	10 (5.7)	0 (0.0)
Business	6 (3.4)	0 (0.0)
Artisans	12 (6.8)	0 (0.0)
Driving	10 (5.7)	0 (0.0)
Retired	10 (5.7)	0 (0.0)
Farming	4 (2.3)	0 (0.0)
Student	18 (10.2)	1 (5.6)
Unemployed	10 (5.7)	1 (10.0)
Total	**176 (100.0)**	**3 (1.7)**

3.2. Discussion

Syphilis infections are serious health issues facing our health sector for over decades. In Congo, the Syphilis prevalence was estimated to be 3.7% [17] and in Cameroon 9.1% [18] and in Ghana 7.9% [19]. This study was conducted to detect the presence of *Treponema pallidum* (syphilis) antibodies among HIV infected persons attending University of Uyo Teaching Hospital (UUTH) in Uyo, Nigeria, as means of establishing syphilis/HIV co-infection among the studied population. The subjects were between 6-67 years of age, of which females where in the majority (61.9%). The gender of persons in this study did agree with the findings of Aliyu *et al*. [20] and Uneke *et al*. [21] that showed that the majority of individuals with HIV attending treatment centres were female.

The prevalence of syphilis/HIV co-infection in this study is relatively low (1.7%) compared with previous studies in Nigeria. Forbi *et al*. [13] reported a syphilis-HIV co-infection rate of 3.3% among HIV-infected subjects in Keffi, Nasarawa State, North Central Nigeria. Uneke *et al*. [21] reported a prevalence of 14.0% among 250 HIV-positive patients in Abakaliki, Ebonyi State, Nigeria. Iyalla *et al*. [22] reported a prevalence of 25.3% among patients attending Rivers State University Teaching Hospital, Port Harcourt, Nigeria. The disparity may be attributed to fact that HIV infection may reduce the specificity of syphilis testing in some populations [23] and the differential effects of sexual behaviour among the study population [24].

The 1.7% reported in the present study is higher than the 0.8% reported by Okonko et al. [25] in Ibadan, Nigeria, the 0.1% reported in studies conducted in Port Harcourt, Nigeria [26], the 0.3% reported in Eritrea [27] and very similar to the 1.7% reported in a study Enugu [28]. The present findings deviated from findings of other previous studies. Okonko et al. [26] also reported that no co-infections of HIV—syphilis. Hussain et al. [29] also reported that none were found to be co-infected with HIV-syphilis, HBV—syphilis, or HCV—syphilis.

The 1.7% reported in the present study is lower than the prevalence of HIV/syphilis (6.6%) reported in Port Harcourt [30], the 2.61% reported in Ile-Ife [31], the 3.1% reported in Calabar by Okoroiwu *et al.* [32], and the 7.5% reported in Ghana [19], and the 12.4% reactivity reported in Ilorin, Nigeria [33], within Nigeria. It is believable that some individuals may have a higher skill of contracting syphilis. The difference in the prevalence can be a result of the differences in the health care system, the test method used in the study and risk factors in the different study locations. It is true that individual risk of contracting syphilis depends on their lifestyle choices. Being a nosocomial infection, syphilis can be spread by unscreened blood during transfusion [33].

Subjects aged 36-67 years had more cases of syphilis/HIV co-infection (1.8%) with a higher prevalence than in the age group 4-35 years (1.6%). Though with youth exuberance one would expect this value to be high in age group 4-35 years. The difference in the prevalence between these age groups is greatly influence by number of persons within the group 64.2% to 35.8%. Although this study only covered two age groups, it disagreed with the findings of

Uneke *et al*. [21] which revealed that patients <40 years had a relatively high rate of syphilis/HIV co-infection. It also disagrees with that of Nwankwo *et al*. [34] who reported age range (27-38 years) as having the highest prevalence of syphilis and that of Adewuyi et al. [30] who reported a similar age range (31-35 years) as having the highest prevalence of syphilis in their respective studies.

Men are affected more frequently with primary or secondary syphilis than women. This difference has varied over time. Male-to-female ratios of primary and secondary syphilis increased from 1.6:1 in 1965 to nearly 3:1 in 1985. After, the ratio decreased, reaching a nadir in 1994-95 [35]. In this present study, females had higher prevalence of syphilis/HIV co-infection (1.8%) compared to males with 1(1.5%). Again, the disparity can be attributed to the larger of females, 106 as compared to 67 males screened during this study. This finding is however in consonance with other studies that reported a higher prevalence of syphilis/HIV co-infection in females than in males [21, 30, 36]. In contrast, Forbi *et al*. [13] reported that males were more exposed to HIV/Syphilis than females in North-central Nigeria, while Shimelis *et al*. [37] reported a higher sero-prevalence of syphilis in HIV-positive males in an Ethiopian study. Okonko et al. [25] reported that more males had syphilis than females in their study.

Higher HIV/syphilis co-infections were observed among individuals who were married (1.9%) than singles (1.8%). This finding is however in consonance with other studies that reported a higher prevalence of HIV/syphilis co-infection in married subjects [30, 38]. Again, the disparity can be attributed to the larger of married subjects, 106 as compared to 67 unmarried (singles, divorced/widow/widower) screened in this study. However, the marital status of these subjects in this study had no significant association with HIV/syphilis co-infection.

Schooling is one of the most important variables to measure the socio-economic status and its effects on the health status of a population [38]. We found that HIV-infected individuals who had no formal education (33.3%) and those who attended up to the primary school (4.2%) showed a significantly higher prevalence of syphilis positivity compared to other educational class (secondary 1.6% and Tertiary 0.0%). This is suggestive of an association between syphilis infection and educational status. Though bulk of the HIV-infected individuals had tertiary education (49.5%). This may reflect the low overall awareness of these individuals about the risks involved and measures to prevent STIs [38, 39]. This finding is however in consonance with other studies that reported a higher prevalence of HIV/syphilis co-infection in subjects with low educational levels [38, 40-41]. In contrast, Adewuyi et al. [30] reported something different. The study has shown that low educational level is an independent risk factor for syphilis infection [38]. The study by Opone et al. [42] also reported low educational status as a risk factor for contracting syphilis. Mutagoma et al. [40] also reported that subjects with lower education had a higher prevalence of syphilis (1.2%) compared to others (0.4%).

An increase in the level of education of men and women will generally improve their socioeconomic status and might thus lead to a reduction in the prevalence of this disease [41]. However, higher HIV/Syphilis co-infections occurred among unemployed individuals (10.0%) than students (5.6%), traders (1.8%), other occupational groups (0.0%). This agrees with that of Adewuyi et al. [30] who reported lowest sero-prevalence of Syphilis among those with high skills. This might be as a result of the nature of their work. The low sero-prevalence of HIV/syphilis co-infection among the high skill individuals might be because they are well informed of the skill and spread these infections, as they are mostly educated [30, 38, 40-41].

Analysis of the results revealed that the variables—sex and educational background—significantly influenced the rate of syphilis sero-positivity among the population under study. While variables- age, marital status and occupational skills non-significantly influenced the

rate of syphilis sero-positivity among the population under study. This agrees with what has been previously reported by some authors [29, 30]. Forbi *et al*. [13] similarly reported no significant effect of gender and age on syphilis/HIV co-infection.

As with other previous studies [38, 40], this study equally has some limitations. Firstly, limited sample size and restriction to only one tertiary hospital, which makes the results not necessarily representative of the HIV-infected population of Akwa Ibom State, Nigeria. Another limitation is that the diagnosis was exclusively based on serological tests, no clinical examinations were performed in order to check signs of active disease. Also, recently acquired infections can present a small concentration of antigens and antibodies that aren't detected yet by either VDRL and ELISA and therefore may have been diagnosed as negative. In addition, although convenience sampling has been found to be adequate for quasi-representative sampling in hidden populations, other sampling methods could have been used to improve representativeness. Finally, the cross-sectional design of this study limits its capacity to establish causality, but we believe that these limitations did not significantly affect the final interpretation of study findings.

4. CONCLUSION

The present study has confirmed the prevalence of syphilis among HIV-infected persons in Uyo, Nigeria to be 1.7%. Syphilis/HIV prevalence is low in this study. This study achieved its objectives of detecting the prevalence HIV/syphilis co-infection among HIV-infected individuals in Uyo, Nigeria and their relationship with the recorded demographic factors. Screening the high-risk population for syphilis would aid early detection of the infection and treatment. Syphilis should continue to be part of the baseline investigation for patients screening for HIV.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this study. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Hospital Research Ethics Committee of University of Uyo Teaching Hospital (UUTH) and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

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