



Detection of *Treponema pallidum* (Syphilis) Antibodies among HIV-Infected Individuals and Sexually-active Attendees of Two Health Facilities 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 and wrote the protocol. Authors TIC, AMA and SAO managed the laboratory analyses and performed the statistical analysis of the study. Authors IOO and SAO managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study reports on the detection of *Treponema pallidum* (syphilis) antibodies in HIV infected patients in Port Harcourt, Nigeria. Screening for syphilis was carried out to determine the prevalence levels of these infections, as biological markers of risk, modes, and time functions of their transmission.

Study Design: Cross-sectional study.

Place and Duration of Study: University of Port Harcourt Teaching Hospital (UPTH) and O.B. Lulu Briggs Medical Centre, University of Port Harcourt, both in Port Harcourt, Nigeria, between August 2012 and July 2015.

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Methods: A total of 100 HIV-infected individuals and 100 sexually-active attendees were recruited for this study. Samples of blood were collected and re-screened for the presence of HIV antibodies using the Determine HIV-1/2 (Alere), HIV ½ Stat-Pak (Chembio), and HIV-1/2/P24/O ELISA kit (Dia.Pro). The same set of samples were screened for *Treponema pallidum* specific antibodies using the syphilis Ultra Rapid Test Strip (ACON(R), USA) and syphilis rapid strips (Global, USA) following the respective manufacturer's instructions.

Results: Among the 200 samples, serological reactivity was detected for syphilis in 3(1.5%). The incidence of syphilis was higher in males (2.0%) than in females (1.0%). Age, sex, and locality did not significantly ($P>0.05$) influence the rate of syphilis.

Conclusion: This study further confirms the presence of syphilis among the population studied. Routine screening of Syphilis among patients is therefore advocated.

Keywords: *Treponema pallidum*; syphilis; HIV; epidemiology; Nigeria.

1. INTRODUCTION

Sexually transmitted diseases (STDs) remain a public health problem of major significance in most of the world [1]. The incident of acute STDs is high in many countries, although the precise magnitude of the problem is not clear [2]. Some prevalent STDs include bacterial vaginosis, herpes, *Chlamydia*, trichomoniasis, gonorrhoea, HIV and syphilis [3-4]. More than 25 infectious pathogens are mainly transferred through sexual activity, and studies have shown that STDs are among the many associated variables that influence the reproductive health continuum [5]. The World Health Organization (WHO) documented the evidence that STDs such as syphilis can be transmitted vertically from mother to child during pregnancy as well as during delivery [6].

Syphilis is a sexually transmitted infection (STI) caused by *Treponema pallidum* subspecies *Pallidum*, a spirochaete bacterium well-known for its invasiveness and immune-evasiveness [7]. Syphilis is transmitted from person to person mainly by sexual intercourse or through vertical transmission during pregnancy [7]. It is one of the STDs that are prevalent in developing countries and is of public health importance [4]. The Office of Rare Diseases (ORD) of the National Institute of Health (NIH) states syphilis as a rare disease among others. This means that syphilis, or a subtype of syphilis, affects less than 200,000 people in the US population [8]. Worldwide, the incidence and prevalence of syphilis differ due to region, ethnic factors, gender and socio-economic factors [3-5]. Studies have however shown that developing and underdeveloped countries usually record higher prevalence compared with that of developed countries [4,9]. Sexually-active persons such as female sex workers (FSWs) are

exposed especially to syphilis infection. Among pregnant women, it is reported to cause fetal defects if not treated [4], and besides all the efforts to control the spread of STIs, syphilis prevalence is still rising, mainly occurring in low-income countries [7]. This study aimed to detect the presence of *Treponema pallidum* specific antibodies in sera of blood donors and sexually active patients in Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design and Study Area

This cross-sectional study was carried out on 100 HIV positive patients attending University of Port Harcourt Teaching Hospital (UPTH) located at Alakahia and 100 sexually active attendees of O.B. Lulu Briggs Medical Centre of the University of Port Harcourt located at Choba, both along East-West road, Obi-Akpo Local Government Area of Rivers State, Port Harcourt, Nigeria.

2.2 Determination of Sample Size for the Study

The sample size for this study was determined using the established formula [10-11]:

$$N = [Z^2 (PQ)]/d^2.$$

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 6.0%, reported for Rivers State as at HIV Sentinel Survey of 2010); this implies $6.0/100 = 0.06$.

q = alternate proportion (1-p), which was calculated as: $1 - 0.06 = 0.94$.

d = desired level of precision (degree of precision/significance). This was taken as 0.05.

Then, the desired sample size (N) = 87. Hence, the estimated sample size was 87 individuals with an additional 10.0% sample (which is 8.7) to take care of study participants that may be lost to follow-up [10-11] providing a total sample size of 96 approximated to 100 from each of hospital.

2.3 Study Population

Blood samples were collected from the 200 participants of different ages, sex and socioeconomic status, who attended the STI clinic of UPTH, with one or more of the complaints as enunciated by WHO in its syndromic approach for the diagnosis of STI [12] were included as subjects. The demographic details relevant to the study were obtained.

2.4 Ethical Considerations

Ethical considerations and approval for the study was sorted from the Hospital Research Ethics committee of University of Port Harcourt Teaching Hospital (UPTH) and the University of Port Harcourt Research Ethics Committee following 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 and sexually-active hospital attendees who had full documentation in the registration book were included. HIV-infected patients and sexually-active hospital attendees who had incomplete data like age, sex and duplicate records as well as those on any form of antibiotics were excluded from the study.

2.6 Specimen Selection, Collection, and Preparation

The method of sample collection employed was venipuncture technique [13]. About 3 ml of venipuncture blood was collected in EDTA BA Vacutainer TM anti-coagulant tubes (BD, Franklin Lakes, USA). Plasma specimens were separated by centrifugation at 300 rpm (revolution per minute) for 5 min. The plasma

was stored at -20°C and used for the laboratory analyses. Specimens were brought to room temperature before testing.

2.7 Serological Analysis of HIV-1 and -2 Antibodies

Blood samples of HIV positive individuals were collected by venipuncture method and re-screened for HIV antibodies using the Determine HIV-1/2, HIV ½ Stat Pak and ELISA kit. All samples with non-reactive results to HIV kits were considered negative. 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)

Each sample serum was screened for *Treponema pallidum* specific antibodies at room temperature using two syphilis Ultra-rapid test strips (by ACON(R) Laboratories Incorporated USA) and another (by Global Device, USA). The test strip was labelled correspondingly to the serum. The Syphilis Ultra-rapid test strip is a rapid chromatographic immunoassay for the qualitative detection of antibodies (IgG and IgM) to *Treponema pallidum* (TP) in whole blood, serum or plasma to aid in the diagnosis of Syphilis. The analysis of the test samples was carried out and the results interpreted as instructed by the kit manufacturer.

2.9 Data Analysis

The seroprevalence was calculated. Chi-square test was used to establish relationships between demographic factors and prevalence using Microsoft Excel spreadsheet (Microsoft Corporation). Significance level was set at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Participants characteristics

A total of two hundred subjects (one hundred from UPTH and one hundred from Lulu Briggs patients) were tested for syphilis. The age range of the subjects used in this study was 16 to 64 years (average age = 32.4 years). The male to female ratio was 1:1 (Table 1).

3.1.2 Prevalence of syphilis in HIV-infected individuals

Of the 100 HIV-infected subjects screened in this study, 2(2.0%) had antibodies to Syphilis (Table 2). Higher prevalence of Syphilis antibodies was observed in the age group 40 years and above (3.1%) than in the age group 16-39 years (1.5%). Also, a higher prevalence of Syphilis antibodies was observed in females (2.6%) than in males (1.6%). Table 2 showed the age- and sex-specific prevalence of Syphilis in HIV-infected individuals.

3.1.3 Prevalence of syphilis in sexually-active hospital attendees

Of the 100 sexually-active hospital attendees screened in this study, 1(1.0%) had antibodies to Syphilis (Table 3). Prevalence of Syphilis antibodies was observed only in the age group 16-39 years and in male sexually-active hospital attendees (2.6%). Table 3 showed the age- and sex-specific prevalence of Syphilis in sexually-active hospital attendees.

3.2 Discussion

Syphilis is still a public health problem worldwide and low-income countries have endemic rates of syphilis among their general populations while middle- or high-income countries have concentrated epidemics of syphilis in specific populations [7].

Using VDRL test reactive strips in this study, the overall prevalence of syphilis was found to be 1.5% while HIV-infected subjects had 2.0% and sexually-active hospital attendees had 1.0%. VDRL test reactive strip found significant *Treponema* antibodies in 3 of the 200 subjects in this study. Absence of significant *Treponema* antibodies in other samples may suggest a non-Syphilitic reagin antibody production or cross-reaction with endemic *Treponema* infection such as yaws, (*Treponema pertenue*), pinta (*Treponema carateum*) or Bejel (*Treponema endemicum*) [14].

Syphilis and HIV have the same mode of transmission and the same risk factors [15]. Higher prevalence of syphilis reported among HIV-infected subjects (2.0%) than the non-HIV infected subjects (1.0%) in this study is comparable to what has been previously documented. Mutagoma et al. [15] documented a higher syphilis prevalence among HIV-infected people (4.8%) than in HIV-negative study participants (0.8%) in Rwanda. Syphilis among HIV-infected people is a major health concern, but there is limited literature to describe the true burden of syphilis in resource-limited settings [15]. However, 2.0% reported among HIV-infected individuals is comparable to the 2.0% prevalence of syphilis found in India [16], the 1.98% reported in 2019 [9]. Also, the 2.0% reported among the HIV-infected subjects is higher than the 0.9% reported among HIV patients in a similar study in Rwanda [15], and 1.8% of prevalence of syphilis found, respectively, in Kenya [17].

Table 1. Age and sex distribution of syphilis among the study participants

Variables	No. tested (%)	No. positive (%)
Locations		
UPTH	100 (50.0)	2 (2.0)
Lulu Briggs	100 (50.0)	1 (1.0)
Study group		
HIV-infected individuals	100 (50.0)	2 (2.0)
Sexually-active hospital attendees	100 (50.0)	1 (1.0)
Age group (years)		
16-39	152 (76.0)	2 (1.3)
40 & above	48 (24.0)	1 (2.1)
Sex		
Males	100 (50.0)	2 (2.0)
Females	100 (50.0)	1 (1.0)
Total	200 (100.0)	3 (1.5)

Table 2. Age and sex-specific prevalence of syphilis in HIV-infected individuals

Variables	No. tested	No. positive (%)
Age group (years)		
16-39	68	1 (1.5)
40 & above	32	1 (3.1)
Sex		
Males	62	1 (1.6)
Females	38	1 (2.6)
Total	100	2 (2.0)

Table 3. Age and sex-specific prevalence of syphilis in sexually-active hospital attendees

Variables	No. tested (%)	No. positive (%)
Age group (years)		
16-39	84	1 (1.2)
40 & above	16	0 (0.0)
Sex		
Males	38	1 (2.6)
Females	62	0 (0.0)
Total	100	1 (1.0)

The 1.5% overall prevalence reported in this study is lower than the 2.63% reported in the urban areas of Akwa Ibom State, Nigeria [9], 5.0% and 10.0% reported in Yenagoa and Osogbo, respectively [18-19] in addition to the 7.5% seroprevalence reported in Ghana [20] and the 3.3% reported in Germany [21].

Similarly, the 1.0% reported among the sexually-active hospital attendees are comparable to the 1.1% reported in Osogbo, Nigeria [19]. Whereas it is lower than the 1.5% and 1.7% recorded in Benin and Ilorin, respectively [22-23] as well as 1.42% reported in the rural area of Akwa Ibom State, Nigeria [9] but is higher than the 0.1% reported by Ejele et al. [24] in Port Harcourt, Nigeria and 0.4%, recorded in Yola [25].

The prevalence of syphilis among sexually-active persons were observed only in the age group 16-39 years. This is comparable to the findings by de Souza et al. [7] who reported that the majority of FSWs who tested positive to syphilis in Brazil were between 15 and 25 years of age and Azuonwu and Timothy [4] who reported that syphilis is more prevalent among sexually-active persons aged 19-29 years [4]. This also deviated from the findings of Mutagoma et al. [15] who reported that prevalence of syphilis is higher in those aged 25-49 years (1.1%) compared to those aged 15-24 years (0.6%).

The prevalence among the genders revealed that more males (2.0%) had syphilis than their female counterparts (1.0%). Besides, the prevalence of syphilis among sexually-active persons were observed only in males (2.6%). This is contrary to the findings by Scherbaum et al. [21] in Germany who reported that female patients were 4.56 times more likely to have positive syphilis than males [21]. While among the HIV-infected subjects, higher prevalence of syphilis was observed in females (2.6%) than in males (1.6%). However, the values reported for males and females in this study is not consistent with the 3.5% among females and 3.9% among males in a population-based survey in the African [26].

Further analysis of the results revealed that none of the variables-sex, age, and locality-significantly influence the rate of syphilis positivity among the population under study. This deviates from what has been previously reported by some authors [27], Location-specific prevalence revealed higher prevalence in subjects from UPTH (2.0%) compared to those from O.B. Lulu Briggs (1.0%). But this prevalence was not significantly different. This is consistent with some previous studies. Mutagoma et al. [15] also reported that the prevalence of syphilis in an urban location in their study was not significantly different the rural locations [15]. In Kenya, the observation was quite the same even when stratified by men and women [17]. However, Mutagoma et al. [15] reported the prevalence of syphilis in HIV-positive in Kigali (11.7%) was substantially higher than other provinces in their study. Hence, signifying locational differences [15].

The disparity in the prevalence of syphilis reported in several studies may be attributed to factors such as the difference in sample size, testing method used and well as time or period of sampling. Also, access to STIs diagnosis and treatment, duration and size of studies, educational background, geographical differences and cultural and traditional practices may have contributed to this discrepancy [4]. Finally, there could be true geographical differences in prevalence [19,21].

4. CONCLUSION

The present study has confirmed the prevalence of syphilis among HIV-infected persons and sexually-active attendees of two health facilities in Port Harcourt, Nigeria. Screening the high-risk population for syphilis would aid early detection

of the infection and hence early treatment, which if initiated, would help to decrease the further spread of this blood-borne infection. There is also a need to support an approach of targeted screening of all viral infections, integrating viral hepatitis testing, counselling and referral services into the existing STD prevention and treatment services

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 Port Harcourt Teaching Hospital (UPTH) And University Research Ethics committee of University of Port Harcourt, Nigeria and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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