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# The Prevalence and Risk Factors for *Trichomonas vaginalis* Infection amongst Human Immunodeficiency Virus-Infected Pregnant Women Attending the Antenatal Clinics of a University Teaching Hospital in Lagos, South-Western, Nigeria

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## Abstract

**Background:** *Trichomonas vaginalis* infection is the most prevalent non-viral sexually transmitted infection worldwide. Interactions between this infection and human immunodeficiency virus (HIV) may cause adverse pregnancy outcomes such as preterm labour, premature rupture of membranes, chorioamnionitis, low birth weight and post-abortion sepsis. **Aims:** This study was aimed to determine the prevalence and risk factors of *T. vaginalis* infection amongst HIV-positive pregnant women attending antenatal care at the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. **Subjects and Methods:** This was an analytical cross-sectional study in which 320 eligible participants which included 160 HIV-positive (case group) and 160 HIV-negative (control group) pregnant women were recruited at the antenatal clinic of LUTH. A structured pro forma was used to collect data from consenting participants after which high vaginal swabs were collected, processed and examined for *T. vaginalis*. The association between categorical variables was tested using the Chi-square test and Fisher's exact test where applicable. All significances were reported at  $P < 0.05$ . **Results:** The prevalence of *T. vaginalis* infection amongst HIV-positive and HIV-negative pregnant women were 10% and 8.1%, respectively ( $P = 0.559$ ). Significant risk factors for *T. vaginalis* infection in the HIV-positive pregnant women were early coitarche ( $P < 0.005$ ) and multiple lifetime sexual partners ( $P = 0.021$ ). There was no relationship between the *T. vaginalis* infection and the immunological markers of HIV infection. **Conclusions:** While this study does not provide grounds for universal screening of pregnant women for *T. vaginalis* infection as a tool of reducing HIV acquisition, especially in pregnancy, campaign to create better sexual health awareness should be commenced as a way to contributing to the reduction in *T. vaginalis* infection during pregnancy and perinatal transmission of HIV.

**Keywords:** Coitarche, high vaginal swabs, perinatal transmission, sexually transmitted infection

## INTRODUCTION

Sub-Saharan Africa is the worst-affected region by the global human immunodeficiency virus (HIV)/AIDS epidemic, with about 25 million people living with HIV.<sup>[1,2]</sup> This is 64% of the worldwide population of people living with HIV.<sup>[1,2]</sup> The HIV/AIDS epidemic affects mostly women in the Sub-Saharan Africa, with women and girls making up almost 57% of adults living with HIV and this are large because heterosexual sex is a dominant mode of HIV transmission in the region.<sup>[1,2]</sup>

Studies now suggest that *Trichomonas vaginalis* plays an important but underreported role in increasing sexual acquisition and transmission of HIV.<sup>[3]</sup> *T. vaginalis* is a major

cofactor in the amplification of HIV transmission.<sup>[4]</sup> Persons with *T. vaginalis* infection are more likely to develop HIV infection than the normal population due to the disruption of the epithelial monolayer cells, leading to microulcerations of inhabited tissues and increased passage of the HIV.<sup>[3,5]</sup> *T. vaginalis* also induces immune activation, replication and

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cytokine production which lead to increased viral replication in HIV-infected cells.<sup>[3,5]</sup> *T. vaginalis* is a protozoan parasite, and it is the most prevalent non-viral sexually transmitted infection worldwide. It causes the curable sexually transmitted disease called trichomoniasis.<sup>[6-12]</sup> According to the World Health Organization (WHO), the worldwide prevalence was 180 million cases<sup>[13]</sup> and to put this number into relative terms of other curable sexually transmitted infections, the global incidence of syphilis, chlamydia and gonorrhoea as reported by the WHO were 12 million, 92 million and 62 million, respectively.<sup>[13]</sup> The majority of cases of trichomoniasis are localised in regions of low income or lack of resources for health care.<sup>[13]</sup> In the developing Africa countries, the prevalence rate ranges from 5% to 37%.<sup>[4,5,14-16]</sup>

*T. vaginalis* trophozoite is 'an oval, parasite with five flagella and an axostyle project which may be used for attachment to surfaces and may also cause the tissue damage noted in *Trichomonas vaginalis* infections'. The infection is usually asymptomatic<sup>[15]</sup> and is mainly reported in women.<sup>[6,17,18]</sup> A shift in the normal acidity milieu of the vagina to a more alkaline one promotes *T. vaginalis* growth. The disease is characterised in female patients by frothy-greenish, foul-smelling vaginal discharge, vulvovaginal irritation, dysuria and lower abdominal pains.<sup>[5,17,19,20]</sup> The classic 'strawberry' appearance of the cervix and vagina (spots of punctuate haemorrhage) is seen in only 10% of women with *T. vaginalis*.<sup>[20]</sup>

*T. vaginalis* infection in pregnancy has been shown to be associated with adverse pregnancy outcomes.<sup>[21]</sup> These pregnancy outcomes include preterm labour, premature rupture of membranes, chorioamnionitis, low birth weight and post-abortion sepsis.<sup>[1,21-23]</sup> It may also increase the vertical transmission of HIV due to a disruption of the vaginal mucosa.<sup>[24]</sup> Apart from the use of antiretrovirals, it has been postulated that routine detection and treatment of *T. vaginalis* infection in the HIV-positive pregnant women may help reduce mother-to-child transmission of the virus, HIV shedding and subsequently improve birth outcome. There is currently a paucity of studies on *T. vaginalis* infection in the HIV-positive pregnant woman in the Sub-Saharan African region. This study was, therefore, aimed to determine the prevalence and determinants of *T. vaginalis* infection in HIV-positive pregnant women at the Lagos University Teaching Hospital (LUTH) and to also explore the relationship between markers of HIV disease and *T. vaginalis* infection amongst these HIV-positive pregnant women.

## SUBJECTS AND METHODS

### Study design and setting

This was an analytical cross-sectional study carried out amongst women attending the antenatal clinic of the LUTH from January to August 2016. LUTH acts mainly as a referral centre for other government-owned and private hospitals in the state. It is on the mainland of Lagos State which has a population of over 9 million inhabitants. The hospital has an

established obstetrics and gynaecology department that also offers services relating to the prevention of mother-to-child transmission of HIV/AIDS (PMTCT). The annual antenatal clinic attendance and delivery rates in the hospital are 3000–3500 and about 2200, respectively.

### Study population and recruitment criteria

The study population included known HIV-positive pregnant women aged 15–49 years who presented to the antenatal clinics for booking and antenatal care, as well as newly diagnosed HIV-positive pregnant women above, detected through the screening programme of the institution. HIV-negative women (matched for age [ $\pm 3$  years] and parity [ $\pm 1$ ]) who presented to the antenatal clinics during the same period served as controls. Excluded from the study were pregnant women who have had treatment for *T. vaginalis* infection or vaginitis in the index pregnancy and women who were administered metronidazole tablets in the past 4 weeks before recruitment.

### Sample size determination and sampling techniques

The minimum sample size (N) for each of the comparative group was obtained from the analytical formula for two independent groups.<sup>[25]</sup>

$$N = \frac{P1(100 - P1) + P2(100 - P2) X (Z\alpha + Z\beta)^2}{(P1 - P2)^2}$$

Where, is the prevalence of *T. vaginalis* infection amongst pregnant HIV-positive women (9.4%) and P2 is the prevalence of *T. vaginalis* infection amongst pregnant HIV-negative women (1.9%) from a previous Nigerian study;<sup>[26]</sup>  $Z\alpha$  is the unit normal deviate corresponding to the desired Type I error rate (1.96); and  $Z\beta$  is the desired statistical power (0.80). Therefore, N was calculated as 136, and with 20% provision for attrition and uncompleted follow-ups, the sample size calculated for each study group was 160. A total of 320 eligible women were recruited into the study using the consecutive sampling method after obtaining informed written consent from each participant. A total of 160 HIV-infected pregnant women were recruited with an equal number of HIV-negative controls (matched for age and parity) around the same the study period (January to August 2016).

### Data collection

Data were collected from each participant using a structured pro forma and from their medical and laboratory records at the medical records department and AIDS Prevention Initiative in Nigeria (APIN) clinic of the hospital, respectively. As a standard of care, pregnant women who presented to the antenatal clinics for booking had provider-initiated counselling and testing using the opt-out technique. Laboratory diagnosis of HIV infection amongst pregnant women was done using the serial rapid HIV testing algorithm as recommended by the current national guidelines for prevention of mother-to-child transmission of HIV and the national guidelines for HIV counselling and testing.<sup>[27,28]</sup> A screening test kit (determine) was used initially and if negative the ultimate test result was

reported as negative and no further testing done. However if positive, a second rapid test kit (Unigold) was used. A third or tie-breaker test (Statpak) was done only when there was discordance between the first two rapid tests. The result of the third, tie-breaker test was reported as the ultimate result. The most recent CD4+ cells' counts and viral load levels of the HIV-positive pregnant participants were copied from their antenatal clinic and/or APIN laboratory records.

### Methods of sample collection and laboratory identification of *Trichomonas vaginalis*

High vaginal swab samples were then collected from each participant with the aid of a sterile disposable Cusco speculum and the sample carefully transported in a Stuart transport medium to the microbiology laboratory of the hospital for analysis within 1 h of collection. As described in details previously published by in Cheesbrough,<sup>[29]</sup> wet mounts of all swab samples were made in sterile normal saline on clean slides, covered with a cover slide and examined under the low power ( $\times 10$ ) and high power ( $\times 40$ ) magnifications for the presence of motile trichomonads. Giemsa staining was also carried out as described by Mason *et al.*<sup>[30]</sup> by making smear of the secretion on a slide, fixing it in absolute methanol for 1 min and then allowed to dry. Diluted Giemsa stain was poured on the smear and allowed to stain for 10 min after which it was washed, air dried and examined under microscope with oil immersion ( $\times 100$ ) magnification for the presence of trichomonads.

### Outcome variables of interest and data management

The study end points were the prevalence of *T. vaginalis* infection amongst both the HIV-positive and HIV-negative pregnant women and the risk factors for *T. vaginalis* infection amongst these women. Data analysis was carried out using SPSS Statistical Package 22.0 for Windows (IBM corps. Armonk, New York, United States) and descriptive statistics were computed for all relevant data. All the quantitative data were tested for normality of distribution using the Kolmogorov–Smirnov normality test. The location and spread of continuous variables were described by the mean and standard deviation (SD), respectively (if normal in distribution), or by the median and interquartile range (IQR), respectively (for skewed distribution). They were presented as mean  $\pm$  SD or median (IQR) as appropriate. The association of *T. vaginalis* infection and HIV seropositivity in the pregnant women were tested using Chi-square and Fisher's exact test where applicable to know the difference. All significance was accepted at  $P < 0.05$ .

### Ethical approval

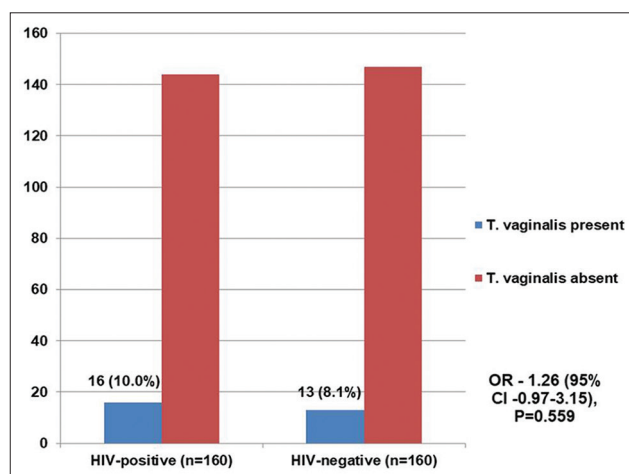
The study was carried out after obtaining approval from the Health Research and Ethics Committee of the LUTH, Lagos, Nigeria (Approval number– ADM/DCST/HREC/APP/517). Ethical principles according to the Helsinki Declaration were considered during the course of the research. Most of the human subjects were adults and those below the age of 18 years were regarded as 'emancipated minors' who were legally able

to give informed consents by themselves. 'Emancipated minor' is a person that is not of legal age to give consent (below 18 years of age in Nigeria) for a research study but who by virtue of marriage, pregnancy, being the mother of a child whether married or not, or has left home and is self-sufficient can be allowed to do so. All the participants read and signed an informed consent form before enrolment in the study; the investigators ensured strict confidentiality of all participants' information. The biological samples were collected and sent for analyses at no cost to the participants and efforts were made to minimise discomfort to the participants during the sample collections and, all participants were given equal attention and optimal care throughout the study and they stand to benefit from the policy that may eventually emanate from the findings of this study. Pregnant women who were diagnosed with *T. vaginalis* infection during the course of the study were treated free-of-charge together with their partners using oral metronidazole 500 mg twice daily for 7 days. They were advised to abstain from sexual intercourse for the 7 days treatment period.

## RESULTS

Table 1 shows that mean age ( $\pm$ SD) of the HIV-positive and negative pregnant women in the study was  $29.3 \pm 6.1$  and  $30.0 \pm 5.3$  years, respectively. There was no statistically significant difference in the mean age between the two groups ( $P = 0.110$ ). The median (IQR) was 2.0 (1.0–4.0) each in the case and control groups, respectively ( $P = 0.244$ ). There were also no significant differences in the marital status ( $P = 0.252$ ), religion ( $P = 0.219$ ), educational level ( $P = 0.097$ ) and ethnic group ( $P = 0.347$ ) of the HIV-infected pregnant women when compared to their HIV-negative controls.

The overall prevalence of *T. vaginalis* infection in the study was 9.1% (29/320). As shown in Figure 1, there was no statistically significant difference in the prevalence of *T. vaginalis* infection amongst the HIV-positive pregnant women and their HIV-negative counterparts (10.0% vs.



**Figure 1:** Distribution of participants with *Trichomonas vaginalis* infection ( $n = 320$ )



**Table 1: Sociodemographic characteristics of human immunodeficiency virus-positive and human immunodeficiency virus-negative pregnant women (n = 320)**

Characteristics	Study participants		P
	HIV positive (%)	HIV negative (%)	
Age (years)			
15-19	3 (1.9)	3 (1.9)	0.110
20-24	34 (21.2)	34 (21.2)	
25-29	47 (29.4)	47 (29.4)	
30-34	33 (20.6)	33 (20.6)	
35-39	33 (20.6)	33 (20.6)	
40-44	10 (6.3)	10 (6.3)	
Mean age±SD	29.3±6.1	31.8±5.3	
Parity			
0	29 (18.1)	29 (18.1)	0.244
1-4	102 (63.8)	102 (63.8)	
≥5	29 (18.1)	29 (18.1)	
Median (IQR)	2.0 (1.0-4.0)	2.0 (1.0-4.0)	
Marital status			
Single	5 (3.1)	2 (1.3)	0.252 <sup>a</sup>
Married	155 (96.9)	158 (98.7)	
Religion			
Christianity	88 (55.0)	83 (51.9)	0.219
Islam	72 (45.0)	77 (48.1)	
Education			
Primary	5 (3.1)	2 (1.3)	0.097 <sup>a</sup>
Secondary	55 (34.4)	41 (25.6)	
Tertiary	100 (62.5)	117 (73.1)	
Ethnicity			
Yoruba	94 (58.8)	104 (65.0)	0.347 <sup>a</sup>
Igbo	53 (33.1)	39 (24.4)	
Hausa	1 (0.6)	2 (1.3)	
Others	12 (7.5)	15 (9.3)	

<sup>a</sup>Fisher's exact test. IQR: Interquartile range, HIV: Human immunodeficiency virus, SD: Standard deviation

8.1%,  $P = 0.559$ ). However, on further analyses of the possible risk factors for *T. vaginalis* infection amongst the HIV-infected pregnant participants [Table 2], we did not find any statistically significant associations between *T. vaginalis* infection and vaginal douching ( $P = 0.342$ ) and inconsistent use of condom ( $P = 0.174$ ). We, however, found about 20-fold risk of *T. vaginalis* infection in women who had early coitarche ( $P < 0.005$ ) and about 9-fold risk in those who had more than one-lifetime sexual partners ( $P = 0.021$ ). In Table 3, we analysed the relationship between the markers of HIV infection and *T. vaginalis* infection in our pregnant participants and we found no statistically significant associations between the *T. vaginalis* infection and these markers, i.e. viral load ( $P = 0.648$ ) and CD4+ cells counts ( $P = 0.560$ ).

## DISCUSSION

The distribution of the prevalence of *T. vaginalis* infection in our study is almost similar to the reported prevalent

distribution in a similar study carried out at the University of Ilorin Teaching Hospital.<sup>[26]</sup> In the Ilorin study, the prevalence of *T. vaginalis* infection was 9.7% amongst the HIV-positive pregnant women and this is similar to the 10.0% prevalence obtained in our study. However, we reported a wide variation in the prevalence amongst the HIV-negative participants (8.1%) compared to the 1.9% prevalence reported from Ilorin. This could be because of better detection of the infection in our study due to the use of further staining with Giemsa unlike in the Ilorin study where the only method of detection of the infection was by the wet mount. Our study did not find any significant association between HIV-infection in pregnancy and *T. vaginalis* infection in contrast to most previous studies carried out within and outside Africa where such relationship was detected.<sup>[31-33]</sup>

These observed differences in the rates of infection in various studies could be due to variation in geographical location, community settings, population and diagnostic techniques.<sup>[17]</sup> Therefore, direct comparisons are difficult and cannot be generalised.<sup>[17]</sup> The finding from this study could imply that *T. vaginalis* infection does not play a big role in the acquisition of the HIV amongst women in this environment and vice versa. A plausible explanation for this could be the abuse of metronidazole tablets for other ailments noted amongst most people in our environment. However, there is a need for a more robust study with larger sample size to substantiate this finding in the future.

Even though the practice of douching seems to be quite common in our environment,<sup>[34]</sup> our study was not able to prove that vaginal douching was a significant risk factor for *T. vaginalis* infection. In a survey done in the United States between 2001 and 2004 by Sutton *et al.*, they found douching to be a risk factor for *T. vaginalis*.<sup>[35]</sup> Other studies suggested that certain feminine hygiene practices, such as douching and application of powder to the genitals, were significantly associated with *T. vaginalis* infection.<sup>[35]</sup> These hygiene practices have significant negative effects on the vaginal microflora and may increase the risk of acquiring *T. vaginalis* infection.<sup>[35]</sup> Condom use has been shown to reduce the prevalence of sexually transmitted disease generally and this was corroborated in a previous study that the use of condoms and depot medroxyprogesterone were associated with a reduced incidence of *T. vaginalis* infection.<sup>[32]</sup> We, however, failed to demonstrate such relationship in this study, but we cannot make any definitive inference from this as the number of participants who used condom consistently was rather small (35.6%). This was not surprising as majority of the participants were married (97.8%) and thus may not be expected to be using condom on a regular basis irrespective of their HIV status.

We found an association between *T. vaginalis* infection and early age at coitarche amongst the HIV-positive participants. This finding was similar to that of a study conducted in Gona, Brazil<sup>[32]</sup> and corroborated in a somewhat similar study by Sutton *et al.* in the United States.<sup>[35]</sup> Early coitarche

**Table 2: Risk factors for *Trichomonas vaginalis* infection amongst the human immunodeficiency virus-infected pregnant participants (n=160)**

Risk factors	<i>Trichomonas vaginalis</i> infection		OR (95% CI)	P
	Present (%)	Absent (%)		
Douching				
Yes	2 (16.7)	10 (83.3)	1.91 (1.07-7.32)	0.342 <sup>a</sup>
No	14 (9.5)	147 (91.9)	1.00 (reference)	
Condom use				
No	13 (12.6)	90 (87.4)	2.21 (1.43-11.01)	0.174 <sup>a</sup>
Yes	3 (5.3)	54 (94.7)	1.00 (reference)	
Age at coitarche (years)				
≤18	12 (28.6)	30 (71.4)	21.82 (9.27-32.06)	0.0003 <sup>a</sup>
>18	4 (3.4)	114 (96.6)	1.00 (reference)	
Number of lifetime sexual partners				
≥2	15 (13.9)	93 (86.1)	9.122 (4.03-14.85)	0.021 <sup>a</sup>
<2	1 (1.9)	51 (98.1)	1.00 (reference)	

<sup>a</sup>Fisher's exact test. OR: Odds ratio, CI: Confidence interval**Table 3: Relationship between markers of human immunodeficiency virus infection and *Trichomonas vaginalis* infection amongst human immunodeficiency virus-positive pregnant participants (n=160)**

HIV infection markers	<i>Trichomonas vaginalis</i> infection		OR (95% CI)	P
	Present (%)	Absent (%)		
Viral load (copies/mL)				
≥1000	2 (13.3)	13 (86.7)	1.35 (0.76-9.24)	0.648 <sup>a</sup>
<1000	14 (9.7)	131 (90.3)	1.00 (reference)	
CD4+ cells count (cells/mm <sup>3</sup> )				
<500	13 (11.0)	105 (89.0)	1.61 (0.83-7.11)	0.560 <sup>a</sup>
≥500	3 (7.1)	39 (92.9)	1.00 (reference)	

<sup>a</sup>Fisher's exact test. OR: Odds ratio, CI: Confidence interval

increases the likelihood of having a higher number of lifetime sexual partners which may in turn increase the chances of acquiring sexually transmitted infections.<sup>[36]</sup> In keeping with this hypothesis, our study demonstrated a significant positive relationship between *T. vaginalis* infection and increased number of lifetime sexual partners.

Whether immune status of HIV-positive women, as reflected by the disease markers, influences the acquisition of *T. vaginalis* infection is still debatable. We found no significant association between *T. vaginalis* infection and the immunological status of the HIV-infected pregnant women in this study. This is in keeping with the findings of other studies done in Sub-Saharan Africa<sup>[37]</sup> and South America.<sup>[32]</sup> However, a study done in North Carolina, USA showed that HIV-positive women with viral load <400 copies/mL were less likely to have *T. vaginalis* infection.<sup>[3]</sup> It was also implied that the association between high viral loads and *T. vaginalis* infection suggest that it occurred more often in women who are receiving less than adequate treatment for HIV infection or are new to care.<sup>[3]</sup> It must be taken into account that almost all the HIV-positive women in this study were on antiretroviral treatment which was provided for free. This would have had a significant impact on the improvement of their immunological status.

Our study had several limitations and these include the possibility that some participants might have unknowingly taken metronidazole tablets and so did not volunteer such information. This is a hospital-based study and findings might not be representative of the general population. There is the possibility that some participants might have *T. vaginalis* infection not detectable using the wet-mount and Giemsa staining technique. Other more accurate screening/diagnostic methods such as culture and nuclear amplification techniques are expensive and not readily available. This being a cross-sectional study may have limitation such as temporal ambiguity, i.e. a lack of certainty on whether *Trichomonas* infection preceded HIV-infection or not as the relationship between *T. vaginalis* and HIV infection can be bi-directional. The study was greatly strengthened by the combined use of wet mount and Giemsa staining methods for the detection of *T. vaginalis* trophozoites that would have ordinarily been missed by the use of direct wet mount technique alone.

## CONCLUSIONS

Even though we found out that more HIV-infected pregnant women had *T. vaginalis* infection compared to the HIV negative controls, our analysis did not to find any statistically significant difference. Important risk factors for *T. vaginalis* infection in

the HIV-positive pregnant women were early coitarche and multiple lifetime sexual partners. The immunological markers of HIV infection were not shown to have a positive influence on the rate of acquisition of *T. vaginalis* infection. While this study does not provide grounds for universal screening of pregnant women for *T. vaginalis* infection as a tool of reducing HIV acquisition, especially in pregnancy, campaign to create better sexual health awareness should be commenced as a way to contributing to the reduction in perinatal transmission of HIV.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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