Published Quarterly
Mangalore, South India
ISSN 0972-5997
Volume 6, Issue 2; Apr -Jun 2007

Original Article

Trichomonas vaginalis infection in human immunodeficiency virus-seropositive Nigerian women: The public health significance

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Citation
Uneke CJ, Alo MN, Ogbu O, Ugwuoru DC. *Trichomonas vaginalis* infection in human immunodeficiency virus-seropositive Nigerian women: The public health significance *Online J Health Allied Scs.* 2007;2:3

URL
http://www.ojhas.org/issue22/2007-2-3.htm

Open Access Archives
http://cogprints.ecs.soton.ac.uk/view/subjects/OJHAS.html
http://openmed.nic.in

Submitted Jan 10, 2007; Accepted: Nov 6, 2007; Published: Nov 10, 2007

Abstract:
Evidence from the biology and epidemiology of *Trichomonas vaginalis* suggests that this protozoan parasite may play an important role in human immunodeficiency virus (HIV) transmission dynamics, especially where heterosexual behaviour and a high prevalence of HIV obtain. The prevalence of *T. vaginalis* was evaluated among HIV-seropositive Nigerian women, in an anonymous, unlinked, cross-sectional survey. Of the total of 250 HIV-seropositive women studied using the wet mount preparations from high vaginal swab (HVS) and urine specimens, the presence of *T. vaginalis* was demonstrated in 61(24.4%) of the HVS specimens and 57(22.8%) of the urine specimens. The highest prevalence of *T. vaginalis* infection (32.6%) was recorded among individuals in the 26-30 years age category and the lowest (18.8%) among the age categories 20-25 years and above 40 years. Since the coinfection of *T. vaginalis* and HIV has public health implications for HIV prevention as it confirms the practice of unprotected sex, educational efforts must be aimed at sexually active persons and high risk groups and are best focused upon the use of barrier precautions, particularly condom use.
Key Words: *Trichomonas vaginalis*, HIV, Women, Prevalence

Introduction:
Trichomonas vaginalis is a sexually transmitted parasitic protozoan known to be responsible for an estimated 180 million new infections per year, making it the most prevalent nonviral sexually transmitted pathogen worldwide.(1,2) It can also be transmitted to neonates during passage through an infected birth canal, but the infection is usually asymptomatic and self limited.(3) Although *T. vaginalis* infection is frequently asymptomatic in adults, it can cause urethritis in men and vaginitis in women. Symptomatic women with trichomoniasis usually complain of vaginal discharge, vulvovaginal soreness, and/or irritation. Dysuria and dyspareunia are also common.(1,4) The infection has also been associated with an increase in adverse outcomes of pregnancy. Complications of trichomonal vaginitis that have been reported include premature rupture of membranes, premature labour, low birth weight, and post-abortion or post-hysterectomy infection.(4-6) Trichomoniasis has neither been the focus of intensive study nor of active control programs in the sub-Saharan Africa, including Nigeria, and this neglect is likely a function of the relatively mild nature of the disease.(7) However available evidence suggests that *T. vaginalis* may play a critical and under-recognized role in amplifying human immunodeficiency virus (HIV) transmission and, in some circumstances, may have a major impact on the epidemic dynamics of HIV infection and the acquired immunodeficiency syndrome (AIDS) in the sub-Saharan Africa.(8-10)

The Sub-Saharan Africa remains by far the worst-affected region by the global HIV/AIDS epidemic, with 25.4 million people living with HIV (Just under two thirds, i.e. 64% of all people living with HIV).(11) The HIV/AIDS epidemic is affecting the females most severely in the sub-Saharan Africa, with women and girls making up almost 57% of adults living with HIV, largely because heterosexual sex is a dominant mode of HIV transmission in the region.(11) In Nigeria, the first AIDS case was reported in 1986, and since then, the epidemic has rapidly grown and has extended beyond the commonly classified high risk group and is now common in the general population with the adult prevalence of 5% in 2003.(12) The life expectancy in Nigeria increased from 45 years in 1963 to 53 years in 1990 and was estimated to have dropped to 51 years in year 2002, largely due to the AIDS epidemic.(12) It is well established that when the prevalence of AIDS reaches 1% of the adult population, the epidemic will become difficult to constrain or reverse unless drastic and effective measures are taken.(13)

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Nevertheless, understanding the role of other sexually transmitted diseases (STDs) including trichomoniasis, in the transmission of HIV, the role of STDs in progression of HIV disease, and the role of HIV infection in alterations of natural history, diagnosis, or response to therapy of STDs is critical to the development of optimal strategies for HIV control.(14)

In many parts of the sub-Saharan Africa including Nigeria, there is paucity of information on the interrelationships between Trichomoniasis and HIV infection among women of child-bearing age. In this report, we present the findings of a hospital-based study on *T. vaginalis* infection among Nigerian women with HIV-infection. The public health significance of results is discussed as it affects the health care delivery system and the control of HIV infection in Nigeria and other parts of the sub-Saharan Africa.

Materials and Methods:

Study Area

The study location was Abakaliki the capital city of Ebonyi State, South-eastern Nigeria. The Federal Medical Centre (FMC), one of the largest health institutions and a major referral centre for HIV screening and confirmation in Abakaliki, was used for the study. Sex trade is a prominent phenomenon in many parts of the city, particularly the low income areas where many operational brothels with commercial sex workers (CSWs) are present and usually receive high patronage from men of uniform services from the police barrack and the military cantonment both located in the city. Heterosexual intercourse is the predominant sexual behaviour in the area and the prevalence of HIV infection among women attending ante-natal clinics (ANCs) in the area was 4.6%.(15)

Study Population /Sampling Technique

Female patients who were confirmed HIV-seropositive by western blot (WB) technique using the BIO-RAD NEW LAV-BLOT I kits (Bio-Rad Novapath Diagnostic Group US.), at FMC Abakaliki, from January 2003 to December 2004 were considered for the study. Prior to the WB assay, some of the subjects had a positive HIV test result as determined by HIV Tri Line Test ELISA kits (Biosystem INC, Austria), at the FMC Abakaliki, while others tested positive to HIV infection elsewhere and were referred to the hospital for confirmatory test. Individuals who had indeterminate WB results and those who declined participation were excluded from the study. All the patients were at the hospital to seek medical attention.

The study protocol was approved by the Department of Medical Microbiology, Faculty of Clinical Medicine, Ebonyi State University Abakaliki-Nigeria. Approval was also obtained from the authorities of the FMC. The approval was on the agreement that patient anonymity must be maintained, good laboratory practice/quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. All work was performed according to the international guidelines for human experimentation in clinical research.

The study was thus, an anonymous, unlinked, cross-sectional survey and following informed consent 250 HIV-seropositive women were enrolled into the study. High vaginal swab (HVS) and urine samples were obtained from each woman. Each patient was given a sterile cotton-tipped swab and instructed to insert the swab into the vagina and to swab the vaginal wall. A sterile universal specimen container was also given to each patient for the collection of first-voided urine (about 10ml). Both HVS and urine samples were analysed for *T. vaginalis* infection. The age of each subject was obtained by interview, other socio-demographic information could not be obtained due to the general reluctance of the participants to disclose such information mainly for fear of stigmatization.

Laboratory Analysis

Microscopic examination of wet mount preparations of the HVS and the urine samples was done as described

previously.(16,17) Briefly, each swab was placed in 1.5 ml of sterile phosphate-buffered saline (PBS), pH 7.2. The resulting suspension was used to produce a wet mount for direct microscopic examination. Centrifugation was performed at 37° C for 5 min at 2,000 rpm, on each urine sample. The sediment was used to make a smear on a microscope slide, stained with Giemsa at pH 6.8 for 25 minutes and observed under the microscope. Trichomoniasis was defined as *T. vaginalis* infection confirmed through direct microscopy.

Statistical Analysis

Differences in proportion were evaluated using the chi-square test. Statistical significant was achieved if $P < 0.05$.

Results

Of the total of 250 HIV seropositive women studied, laboratory analysis indicated the presence of *T. vaginalis* in 61 (24.4%, 95% CI., 19.1-29.7%) of the HVS specimens and 57(22.8%, 95% CI., 18.0-28.0) of the urine specimens (Table 1). *T. vaginalis* was found in the HVS specimen of each individual whose urine sample had the parasite. *T. vaginalis* was not found in the urine samples of five women who had the parasite in their HVS specimen. The highest prevalence of *T. vaginalis* infection (32.6%, 95% CI., 19.1-46.1%) was recorded among individuals in the 26-30 years age category, followed by those of the 31-35 years age group (29.2%, 95% CI., 18.1-40.3%). The lowest prevalence of *T. vaginalis* infection (18.8%) was observed among the women of age categories 20-25 years (95% CI., 5.3-32.3%) and above 40 years (95% CI., 7.7-29.9%) as indicated in table 2. Statistically, no significant difference was observed in the association between *T. vaginalis* infection and age (X^2 =4.42, df=4, P<0.05).

Table 1. Comparison of diagnostic methods for *T. vaginalis* using HVS and urine specimens among HIV-infected Nigerian women.

Specimen	Number examined	Number positive	Percentage positive	95% Confidence interval
HVS	250	61	24.4	19.1-29.7
Urine	250	57	22.8	18.0-28.0

Table 2. Age-related prevalence of *T. vaginalis* infection among HIV-infected Nigerian women.

Age (years)	Number examined	Number positive	Percentage positive	95% Confidence interval
20-25	32	6	18.8	5.3-32.3
26-30	46	15	32.6	19.1-46.1
31-35	65	19	29.2	18.1-40.3
35-40	59	12	20.3	10.0-30.6
>40	48	9	18.8	7.7-29.9
Total	250	61	24.4	19.1-29.7

Discussion

The findings of this study suggest that in Nigeria where heterosexual behaviour predominates, *T. vaginalis* infection may be a frequent occurrence among Nigerian women with HIV infection. The *T. vaginalis* infection prevalence of 24.4% observed in this study is comparatively higher than those recorded among HIV-seropositive women in Kinshasa, Zaire (1.9%), (8) Congo (18.6%), (18) and parts of the USA such as Missouri (11.0%) (19), Rhode Island (12%) (20), and California (17.4%).(21) However, the prevalence rates of *T. vaginalis* infection among HIV-infected Ivorian women (27%)(9) and among HIV-infected women in New Orleans, USA (36%) (22), were higher than what we observed in this study.

The coinfection of *T. vaginalis* and HIV has public health implications for HIV prevention as it confirms the practice of unprotected sex, a habit common in many settings in the sub-Saharan Africa, including Nigeria.(11,12) Although it has not been unequivocally established whether trichomoniasis is a risk factor for HIV transmission or just a marker for high-risk heterosexual activity (8), findings in a recent study from the Centers for Disease Control and Prevention, Atlanta, USA, indicated that coincubation of *T. vaginalis* isolates with acutely HIV-1-infected peripheral blood mononuclear cells enhanced HIV-1 replication.(23) Two mechanisms which have identified that could contribute to the epidemiologic association of trichomoniasis with the sexual transmission of HIV-1 were (i) *T. vaginalis* disruption of urogenital epithelial monolayers could facilitate passage of HIV-1 to underlying layers, and (ii) activation of local immune cells by *T. vaginalis* in the presence of infectious HIV-1 might lead to increased viral replication.(23) Hence the need for more vigilant efforts in the diagnosis and treatment of *T. vaginalis* in women and also in men cannot be overstated, especially in countries where heterosexual behaviour predominates, and a high prevalence of HIV obtains, as in the sub-Saharan Africa.

Although the prevalence of *T. vaginalis* infection observed in this study may be considered to be relatively high, with the HVS specimens recording slightly higher rate than the urine specimens, the possibility of underestimation of the prevalence may not be ruled out. While a positive wet mount is diagnostic, a negative wet mount does not necessarily exclude trichomoniasis.(24) Moreover microscopic examination of wet mount preparations has a sensitivity of approximately 60%.(25) The wet mount and rarely the culture method are the techniques used for routine *T. vaginalis* diagnosis in most settings in Nigeria as in other parts of the sub-Saharan Africa. Although the wet mount is only 35 to 80% sensitive compared with culture (26), the sensitivity of culture when compared with polymerase chain reaction (PCR) has been estimated to be 70%.(16) Such highly sensitive PCR and related techniques are neither routinely used nor readily available for *T. vaginalis* in Nigeria and in other parts of the sub-Saharan Africa. This is because sophisticated equipment must be available, is costly to purchase and maintain, and must be located near clean water and a reliable supply of electricity. The validity of the results obtained by these techniques strongly depends on the skills of the technicians, and their interpretation requires skills training and supervision. These conditions are often lacking in sub-Saharan Africa, at least in district-level hospitals.(27)

The use of suboptimal laboratory methods for routine *T. vaginalis* diagnosis could have far-reaching public health implication as regards HIV transmission dynamics. Substantial under-diagnosis of the infection is a major consequence and since most patients with *T. vaginalis* infection are asymptomatic or mildly

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symptomatic, they are likely to continue to remain sexually active in spite of infection. It is well established that *T. vaginalis* typically elicits an aggressive local cellular immune response with inflammation of the vaginal epithelium and exocervix with evidence of punctate hemorrhages.(7,25) In the event of HIV infection, greater numbers of both free virus and viral-infected white blood cells may increase the probability of HIV exposure and transmission by *T. vaginalis*-infected women.(21) Therefore, HIV-infected women who develop trichomoniasis are likely to be an important source of continuing HIV transmission and characterizing such individuals can assist in the targeting of prevention efforts. It is thus suggested that careful clinical examination and selective use of wet-mount examination together with wider use of more sensitive tests for subclinical infection, such as culture or direct immunofluorescent staining of vaginal fluid, could lead to improved detection and control of *T. vaginalis* infection.(26)

In this study, age-related prevalence of *T. vaginalis* infection indicated the highest occurrence among the HIV-infected women of 26-35 years old, which is in conformity with the observation made in a similar study in Los Angeles.(21) The reason for this was unclear in the present study and besides, there was no significant difference in the association between *T. vaginalis* infection and age ($P < 0.05$), as was also observed in an earlier study in Alabama, USA.(17)

Our inability to obtain sufficient socio-demographic data from subjects is a major draw back to this study. Also our study population size may not be an adequate representation of the general Nigeria population because the study was conducted in the south-eastern region which is only one of the six geo-political zones in the country. These limitations may have affected adequate interpretation of the public health implications of the findings. Further studies incorporating detailed socio-demographic parameters as well as larger study population size are advocated.

In conclusion, the need for providing proper counselling and education on sexual behaviour and genital hygiene besides treatment to control and prevent these infections is advocated. Since HIV and *T. vaginalis* are primarily spread as sexually transmissible diseases, the educational efforts must be aimed at sexually active persons and high risk groups and must be explicit regarding the behaviours that lead to the spread of both HIV and *T. vaginalis*. A significant number of both boys and girls become sexually active as teenagers and must be included in prevention strategies. Given that the level of promiscuity will often be difficult to modify within a population as is commonly the case in Nigeria, then educational campaigns are best focused upon the use of barrier precautions, particularly condom use.

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