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Vaginal trichomoniasis among HIV patients attending primary health care centers of Jos, Nigeria

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ABSTRACT

Objective: To determine the prevalence of trichomonal infection in HIV/AIDS and non-HIV control group of patients in a population of women with abnormal vaginal discharge. **Methods:** We conducted a simple cross-sectional study. Primary health care centers in Jos metropolis and Jos University Teaching Hospital, during December 2006 to December 2007. Seven hundred high vaginal swabs were collected; 350 from HIV positive and another 350 from HIV-negative control group of patients with abnormal vaginal discharge attending primary health care centers in Jos metropolis and analysed for microscopy and culture in Jos University Teaching Hospital. Data on epidemiologic indices from the patients, using structured interviewer-administered questionnaires were collected. **Results:** The rate of trichomoniasis among all participants in the study was 17% ($n=120/700$). The prevalence rate of trichomoniasis among persons with HIV was 24% while it was found to be 10.3% among HIV negative controls. The difference was statistically significant ($\chi^2 = 23.172$; $df=1$; $P<0.05$). The rate of co-infection of *Trichomonas vaginalis* (*T. vaginalis*) in bacterial vaginosis was 42% ($n=50/120$), while it was 24% ($n=29/120$) in candidiasis. The singles had a 35% high rate of trichomonal infection. The infected women had a median age of 26 years, and a median number of 3 intra-vaginal sex partners per week. **Conclusions:** There was a significant statistical difference in prevalence of *T. vaginalis* between HIV/AIDS group and non-HIV(control) group of patients in the study ($P<0.05$). Local HIV prevention strategies should target such women with trichomonal infection for intervention efforts, especially in HIV endemic area of sub-continent of Africa to further reduce the burden of HIV in the population.

1. Introduction

Trichomonas vaginalis (*T. vaginalis*) has been incriminated as one of the common sexually transmitted genital pathogens and is found to be associated with approximately 20% of all cases of non-gonococcal urethritis[1]. It is transmitted primarily by sexual intercourse and causes vaginitis in women and non gonococcal urethritis in men. Trichomoniasis is widely distributed all over the world and remains a common infection among female patients attending sexually transmitted disease clinic[2]. In developing tropical Africa, a

prevalence rate among the female patients range 5–37 percent. The world health organization (WHO) estimate of 180 million cases of Trichomonal infection are acquired annually world wide and it has been found to increase the risk of transmission of HIV and predispose pregnant women to premature rupture of membranes and early labor[3]. Majority of female patients harboring this organism present with vaginal discharge, which is usually frothy, greenish yellow and offensive. Abnormal vaginal bleeding, such as postcoital bleeding, often has been recorded due to cervicitis caused by *T. vaginalis* or by associated pathogens. In a few cases of infected women on routine speculum examination, revealed small punctate cervical hemorrhages with ulcerations; referred to as “colpitis macularis” or “strawberry cervix”, this sign is highly specific for trichomoniasis[2].

There is evidence that genital inflammation associated with

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vaginal trichomoniasis increases the transmission of HIV[4]. HIV transmission and trichomoniasis share in common risk behaviors, such as sexual promiscuity and poor condom use. Other common risk behaviors include multiple sex partners, multiparity, women married at early age, during pregnancy, poor personal hygiene, and low socio-economic status[5]. Co-infection of *T. vaginalis* with HIV may result in epidemiological synergy with prolonged or augmented infectiousness of both infections.

Trichomoniasis has neither been the focus of intensive study nor of active control program in Nigeria, and the neglect is likely a function of the relatively mild nature of the disease. However available evidence suggest that *T. vaginalis* may play a critical and under recognized role in amplifying HIV transmission and in some circumstances, may have a major impact on the epidemic dynamics of HIV infection and AIDS in the sub-Saharan Africa[2].

World Health Organisation (WHO) estimated that the total number of people living with HIV in 2004 was 35.9 to 44.3 million. Since the first Nigerian case of HIV was diagnosed in 1986, an estimated 2.9 million Nigerians have become infected with HIV[6]. In the Federal Ministry of Health(FMOH) nation wide surveys among women attending antenatal clinics, the HIV prevalence has steadily increased from 1.85% in 1991 to 4.1% in 2010[7]. It is now well established that the presence of sexually transmitted infections greatly facilitates the transmission and acquisition of HIV between sexual partners[4].

Therefore, there is a need for study of the prevalence of Trichomoniasis in effort to check HIV transmission in this locality. Laboratory support is necessary for a differential diagnosis or to confirm the clinical diagnosis of Trichomoniasis.

2. Materials and methods

2.1. Study area

The study was done in primary health care (PHC) clinics in Jos, Plateau state of Nigeria. Jos has a moderate population of about 3.5 million and enjoys a near temperate type of climate with attendant tourist activities. There are thriving mining activities, brothels, local beer parlors, tourist hotels and some higher education institutions.

2.2. Study design

All patients aged 0–60 years with abnormal vaginal discharge who gave their consent were enrolled into the study in PHC centers of Jos metropolis. The patients who refused to participate were assured of no punitive measures or denial of treatment. The patients enrolled for the study were screened for HIV at the PHC clinics and the positive cases were sent for confirmatory test at APIN (AIDS Preventive Initiative in Nigeria)

laboratory in Jos University Teaching Hospital (JUTH), free of charge. Free condoms were given as an incentive. HIV positive and non-HIV negative(control) patients with abnormal vaginal discharge were recruited into the study during the clinic days and the nature of the study explained to them in the language they best understood through an interpreter from December 2006 to December 2007. All participants were assured of strict confidentiality of responses. Structured interviewer-administered questionnaire was then introduced to the patients who gave their written consent. The questionnaire included serial number; date; age; address; educational status; marital status; parity; last menstrual period; type of work; place of work; any contraception at enrollment?(Yes/No); if yes,(type)?; frequency of vaginal intercourse/week; number of sex partners/week; any Condom use?; if yes, how frequent (none, occasional, regular)?; type of sexual practice; any abnormal vaginal discharge?(Yes/No); if abnormal discharge, what color, odor, duration and frequency? Post-coital bleeding?(Yes/No). The patients were given health education and counseling, and genital samples were later collected from them.

2.3. Laboratory procedure

High vaginal swabs were collected following aseptic precautions[8]. The swabs were immediately sent to the genital bench of medical microbiology and parasitology laboratory of Jos University Teaching Hospital where they were processed according to standard procedures[8]. MACROSCOPY–Physical examination for the nature, color, consistency of the vaginal discharge was done. A characteristic fishy odor was intensified by adding a drop of 10% potassium hydroxide (KOH) to the vaginal fluid on the examination speculum (WHIFF Test). The PH of the vaginal fluid was measured by touching the PH indicator paper strips to the charged vaginal swabs. MICROSCOPY–Wet Mount: A sample of vaginal fluid from a swab-stick was mixed on a glass slide with a drop of normal saline and covered with a cover-slip. Ten fields were examined under high power (×40) objective for actively motile pear-shaped flagellates (*T. vaginalis*), for yeast like cells(for *candida* spp.) and for clue cells (used in diagnosis of *Bacterial vaginosis* (*B. vaginosis*)).

Giemsa stained smears were scanned at 100× objective to detect violet, pear shaped trophozoites, a little larger than the surrounding pus cells with characteristic morphologic features of *T. vaginalis*, including flagella and undulating membrane.

Gram-stained smear. Vaginal smears were Gram-stained. (a) Microscopic evaluation of Gram-stained smear at high power (×40) objective revealed Clue cells, usually representing at least 20 percent of vaginal epithelial cells in BV. (b) At oil immersion power (×100) objective, Gram-stain scores of 0–10 were assigned on basis of standardized criteria (Nugent); Scores of 0–3 were considered normal, 4–6 borderline for BV and 7–10 diagnostic of BV.

At oil immersion power($\times 100$) objective, Gram–negative diplococci inside polymorphonuclear cells were typical of *Neisseria gonorrhoeae*. Gram stain however, has limitations in investigations of gonococcal infection in female genital system because of the presence of other *Bacteria* that would mimic morphological appearance of gonococci.

Culture: Culture for *T. vaginalis* was performed using Nutrient broth glucose serum medium[9] at 37 °C for 5 d. Wet mount of sediment from the bottom of the Bijou bottles containing the medium was examined for motile trophozoites of *T. vaginalis* from 48 h to 5 d of incubation .

The samples collection, transportation and processing including microscopy and culture were carried out according to recommended standards[8].

The data generated were analysed using SPSS 11.0 statistical software; *chi*–square(χ^2) was used to compare association between proportions and $P < 0.05$ were considered to be significant. Approval of the study protocol was obtained from ethic committee of Jos University Teaching Hospital. Further permission was obtained from local government councils and the primary health care departments of Jos.

3. Results

The baseline demographic characteristics of the subjects with *T. vaginalis* infection as recorded are shown in Table 1. The mean age at risk was 26 years. Patients who engaged in intravaginal sexual activities 4 times per week with 3 different sexual partners were the most at risk. Of 35% trichomoniasis risk were patients of single marital status, the separated (28%), the divorced (23%), the married women (10%) and the widows (4%). Pregnant mothers constituted a 60% ($n=72/120$) *T. vaginalis* risk, while non pregnant women had a 40% ($n=48/120$) risk. The nature of vaginal discharge in *T. vaginalis* infection was as follows, yellow color(42%), white(27%), green (15%), clear(12%), frothy(30%), malodorous(65%) and blood stained (4%) (Table 1).

Three hundred and fifty (50.0%) of the 700 patients with abnormal vaginal discharge recruited in the study were confirmed HIV reactive by western blot while the remaining 50% (350/700) was HIV non– reactive as control. A total of 120 *T. vaginalis* organisms were detected in the 700 samples, constituting 17% of the abnormal vaginal discharge specimens (Table 2).

T. vaginalis diagnosed with microscopy alone by wet mount was 12(10%), while the total of 120 were diagnosed through culture. In a total 120 case of trichomoniasis; HIV/AIDS and non–HIV patients contributed 70% (84/120 cases) and 30% (36/120cases) respectively (Table 2). The prevalence rate of trichomoniasis among persons with HIV was 24% while it was found to be 10.3% among HIV negative controls. The difference was statistically significant ($\chi^2 = 23.172$; $df=1$; $P < 0.05$) (Table 3).

Co–infection of *T. vaginalis* in a clinical condition of *B.*

vaginis was recorded in a 42% ($n=50/120$) case, trichomoniasis in Candidiasis 24% ($n=29/120$), trichomoniasis alone 24% ($n=29/120$) and in other mixed infections(e.g. *N. gonorrhoea* etc.) was 10% ($n=12/120$) (Figure 1).

Table 1

Demographic characteristics of subjects with trichomoniasis among the study population.

Variable	Median (range) or percent (proportion)
Age (years)	26 (0–60)
Vaginal intercourse per week	4
No. of sex partners per week	3
Marital status	
Singles	35% (42/120)
Separated	28% (34/120)
Divorced	23% (28/120)
Married	10% (12/120)
Widow	4% (5/120)
Pregnancy	
Pregnant mothers	60% (72/120)
Non pregnant women	40%(48/120)
Nature of vaginal discharge	
White	25% (30/120)
Yellow	37% (44/120)
Clear	12% (14/120)
Frothy	30% (36/120)
Greenish	22% (26/120)
Malodorous	65% (78/120)
Blood stained	4% (5/120)

Table 2

Distribution of *T. vaginalis* in patients with abnormal vaginal discharge in the primary health care clinics of Jos–Nigeria, December 2006–December 2007.

Age in years	Abnormal vaginal discharge	<i>T. vaginalis</i> in HIV/AIDS	<i>T. vaginalis</i> in non–HIV	<i>T. vaginalis</i> of vaginal discharge	Other agents of vaginal discharge
0–10	6	–	–	–	6
11–20	173	3	3	–	170
21–30	279	80	60	20	199
31–40	210	36	21	15	174
41–50	30	1	–	1	29
51–60	2	–	–	–	2
Total	700	120 (100%)	84 (70%)	36 (30%)	580

Table 3

Prevalence of *T. vaginalis* in HIV positive persons and HIV negative control with abnormal vaginal discharge

	HIV positive (%)	HIV Negative (%)	TOTAL
<i>T. vaginalis</i> positive	84 (24)	36 (10.3)	120 (17.1)
<i>T. vaginalis</i> negative	266 (76)	314 (89.7)	580 (82.9)
TOTAL	350	350	700

Chi–squared (χ^2) statistic the value = 23.172, $df=1$, $P < 0.05$.

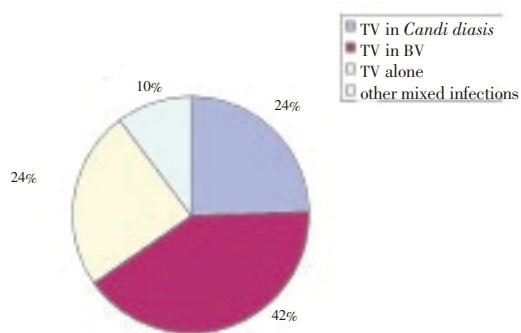


Figure 1. Co-infection of *T. vaginalis* (TV) in candidiasis and bacterial vaginosis (BV) and other mixed infections diagnosed among patients with abnormal vaginal discharge in primary health care clinics, Jos–Nigeria, December 2006–December 2007.

4. Discussion

The prevalence rate of trichomoniasis among the general population with abnormal vaginal discharge in the study was 17% ($n=120/700$). It was found to be 24% and 10.3% among HIV positive group and the HIV negative control respectively. A similar work done in eastern Nigeria on prevalence of *T. vaginalis* in HIV sero-positive women recorded 24.4%[2]. A high incidence of 74.5% trichomoniasis was recorded in Lagos, south-western Nigeria in patients with abnormal vaginal discharge[10] while in Ilorin, north central Nigeria a low rate of 4.7% was found among pregnant women attending antenatal clinics[11]. Another study done in east Africa, had a 16% prevalence of trichomoniasis reported in a population sample of women attending urban antenatal clinic in Mwanza, Tanzania[12]. In New Orleans (USA) an incidence of 36% trichomoniasis was found in HIV infected women[13]. Disparity in incidence of trichomoniasis recorded in some of the above works could be explained from the view point of improved diagnostic tools and/or increase incidence of the practice of high risk unprotected intra-vaginal sexual activities in some geographic locations.

In our study, the prevalence rate of trichomoniasis in HIV positive group vis a vis the HIV negative control was 24% and 10.3% respectively with a statistically significant difference. 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, findings in a study from the Centers for Disease Control and Prevention (CDC), Atlanta, USA[2,14], indicated that co-incubation of *T. vaginalis* isolates with acutely HIV-1 infected peripheral blood mononuclear cells enhanced HIV-1 replication. Two mechanisms which have been identified could be as follows; (a) *T. vaginalis* disruption of urogenital epithelial monolayer could facilitate passage of HIV-1 to underlying layers and, (b) activation of aggressive local cellular immune response

with inflammation of the vaginal epithelium and exo-cervix with evidence of punctuate hemorrhage by *T. vaginalis* in the presence of infection of HIV-1 might lead to increased viral replication. Hence, there is need for more vigilant efforts in diagnosis and treatment of trichomoniasis in both women and men, especially in countries where heterosexual behavior predominates and a high prevalence of HIV obtains, as in the sub-saharan Africa.

In our study, intravaginal intercourse of four times per week involving women with three sex partners constituted a high risk of trichomoniasis. The mean age at risk was 26 years, singles 35% and pregnant mothers constituted 60% risk. This corroborates findings by Okpara *et al* who reported that single (unmarried) pregnant women were more infected which pre-supposes that unmarried women are unattached thus free to indulge in more sexual activities probably involving multiple sexual partners. This study also revealed a 42% co-infection of *T. vaginalis* in a clinical condition of *B. vaginosis*, 24% in Candidiasis, 10% in other mixed infections and trichomoniasis alone 24%. Related studies have recorded a statistically significant association between trichomoniasis, prostitution, no condom use and co-infection with other STDs[16–18]. All the above listed factors are also recorded to promote HIV transmission in other notable work[4,19]. In other words, both trichomoniasis and HIV share in some common risk behaviors.

A ten percent rate ($n=12/120$) of *T. vaginalis* was diagnosed in the present work by microscopy alone (wet mount), while a total case of 120 were diagnosed through culture. The wet mount microscopy is used routinely in most settings in Nigeria for *T. vaginalis* diagnosis, and this has an average sensitivity of 60 to 80 percent for detection of the motile *T. vaginalis* organisms[20,21]. Culture of *T. vaginalis* using a Diamond medium, which has a sensitivity of 91 to 100 percent, is considered to be far superior to wet mount examination, however, the culture technique is more expensive than wet mount examination and requires two to seven days to obtain results[20,21]. Careful clinical examination and selective use of wet-mount examination together with wider use of more sensitive tests for subclinical infection, such as culture of vaginal fluid, could lead to improved detection and control of *T. vaginalis* infection.

A number of patients with abnormal vaginal discharge refused to be screened for HIV in the study. Occasional power failures and occasional delay in specimen transportation from primary health care centers to the teaching hospital microbiology laboratory all constituted a limitation in the study.

There is a strong need for more research to verify the hypotheses behind the trichomoniasis–HIV mechanism. Trichomoniasis should be acknowledged as a public health threat and government funded control programs are advocated to combat the endemicity of trichomoniasis and HIV especially in the highest afflicted population of African sub-continent.

In conclusion, the present study has therefore, shown that the rate of trichomoniasis is higher in HIV/AIDS group than in general population. We also observed that intra-vaginal intercourse and sexual promiscuity are important risk factors to both trichomoniasis and HIV transmission.

HIV/AIDS is an important public health problem in Nigeria. Nigeria has an average HIV prevalence of 4.1% among women attending Antenatal care and has the third highest burden of HIV after India and South Africa. Jos, Nigeria has an HIV average prevalent rate of 5%.

It is imperative that as a part of efforts to curtail the burden of HIV/AIDS, all relevant factors associated with its transmission should be explored.

T. vaginalis infection is a sexually transmitted infection (STI) that has unsafe intravaginal promiscuity risk factors, which constitutes a part of HIV risk factors. In addition, the presence of trichomoniasis increases the chances of HIV transmission through inflammatory cellular reaction of vaginal epithelium and exo-cervix with evidence of punctate haemorrhages. *T. vaginalis* also causes disruption of urogenital epithelium.

Trichomonas infection is therefore, of public health importance because HIV transmission is high in trichomoniasis and all effort to contain it should be explored.

Conflict of interest statement

We declare that we have no conflict of interest.

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