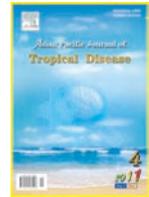




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading doi:10.1016/S2222-1808(11)60066-2

Trichomonas vaginalis in HIV/AIDS subjects in Nigeria

Nweze EI^{1*}, Mouneke GN²¹Department of Microbiology, University of Nigeria, Nsukka, Nigeria²Department of Parasitology, School of Medical Laboratory Sciences, University of Nigeria Teaching Hospital, Enugu, Nigeria

ARTICLE INFO

Article history:

Received 18 September 2011

Received in revised form 25 September 2011

Accepted 20 October 2011

Available online 28 December 2011

Keywords:

Trichomonas

HIV/AIDS

CD4⁺ lymphocytes

Nigeria

Trichomonas vaginalis

Parasitic infection

Sexually transmitted disease

Prevalence

ABSTRACT

Objective: To determine the prevalence of *Trichomonas vaginalis* (*T. vaginalis*) in HIV/AIDS patients attending two different hospitals in southeast Nigeria. **Methods:** We collected 970 urine samples from HIV/AIDS patients attending two different hospitals in southeast Nigeria. Samples were processed by microscopy and cultural methods. **Results:** Out of the 970 screened, 355 (36.60%) were positive for *T. vaginalis*. Subjects with the least CD4⁺ count in the range of 40–140 cells/mL had the highest number of positive samples (180, 50.70%), while those in the range of 480–580 cells/mL had the least value (2, 0.56%). Those in the rural areas had a higher number of positive samples (155, 38.75%) than their urban counterparts (200, 35.09%) with respect to the total number examined in each group but this was not statistically significant ($P > 0.05$). Out of the 355 positive cases, the university undergraduate students' group had the highest percentage incidence of 53.00% followed by the low-income group with 47.08%. **Conclusions:** It can be concluded that the occurrence of *T. vaginalis* increases with decrease in the CD4⁺ counts in HIV/AIDS patients in Nigeria. Since *T. vaginalis* may be an important cofactor in promoting the spread of HIV and, in some circumstances, may have a major impact on the epidemic dynamics of HIV, there is a need to take measures to check the spread of this parasitic infection.

1. Introduction

Trichomonas vaginalis (*T. vaginalis*) is a pathogenic protozoan parasite which causes trichomoniasis. It is also associated with other sexually transmitted diseases (STDs). Infection with *T. vaginalis* is one of the most common STDs worldwide^[1] with an estimated 174 million new cases a year, of which 154 million occur in resource-limited settings/countries^[2,3]. This figure is comparable to a global prevalence of 92 million cases of *Chlamydia trachomatis* and 62 million cases of *Neisseria gonorrhoea*^[4]. Majority of male infections and up to one-third of female infections are asymptomatic. This apparently underestimates its significance. However, apart from the association of *T. vaginalis* with a number of significant reproductive health sequelae including pelvic inflammatory disease and adverse outcomes of pregnancy, it has also been implicated in facilitating the sexual transmission of HIV^[5].

Despite a relative paucity of studies on the prevalence and incidence of trichomoniasis especially in Western Africa and Nigeria in particular, recent publications from other parts of the world suggest that *T. vaginalis* is one of the most common sexually transmitted infections (STIs) in several countries. For instance, in the United States, an estimated 7.4 million new cases occur annually^[6] while in African countries, the rates of trichomoniasis in women are high and that in men is expected to be high despite that it is not yet documented^[7,8]. Despite this high prevalence and widespread geographic distribution, *Trichomonas* has not been the focus of intensive study nor of active control programs. This neglect is obviously a result of the relatively mild nature of the disease, the lack of effect on human fertility, and the historic absence of association with adverse birth outcomes although recent data suggest a possible causal role in low birth weight and prematurity. Several disease conditions have been studied in relation to HIV/AIDS and CD4⁺ T-lymphocyte count, for example, studies of HIV/AIDS and T-lymphocyte counts in relation to opportunistic fungal infections in Nigeria have been investigated by a group of researchers that included one of us^[9], but studies in relation to *T. vaginalis* and HIV/

*Corresponding author: Nweze Emeka I, MSc, PhD, Department of Microbiology, University of Nigeria, Nsukka, Nigeria.

E-mail: nwezemeka@yahoo.com

Foundation Project: This work was financially supported by Mezech Limited (grant No. MNL/PGS/1/06-07).

AIDS are scarce, especially in Nigeria and this study is probably the first one to be documented. The relationship between the two is important since *T. vaginalis* may be an important co-factor in promoting the spread of HIV and, in some circumstances, may have a major impact on the epidemic dynamics of HIV in Africans/African-American communities[9]. Furthermore, in countries where HIV/AIDS occur in epidemic proportions such as Nigeria and other parts of sub-Saharan Africa, such studies will not only help in strategic planning and control strategies but provide additional useful information for clinicians and government agencies for policy implementation strategies. It is common knowledge that the incidence of HIV/AIDS has continued to increase globally in recent years especially in developing countries. For instance, the world's highest prevalence of HIV infection is found in sub-Saharan Africa, with an estimated 24.7 million adults and children older than the age of 15 years living with HIV/AIDS[10]. Also, pediatric HIV and HIV infections generally have been increasing in Nigeria over the years, with 74520 new pediatric cases estimated in 2006 alone[11]. With many patients not able to buy antiretroviral drugs or source from government hospitals free of charge, the death toll has significantly continued to increase. It has been reported that improved management of STDs decreases HIV incidence. We present the prevalence of *T. vaginalis* in HIV/AIDS subjects sampled in southeastern Nigeria. The effect of CD4⁺ lymphocyte counts, locality and social status on the prevalence of *T. vaginalis* is also discussed.

2. Materials and methods

2.1. Study area and subjects

The area studied is the commercial nerve center and state capital of Ebonyi state in southeastern Nigeria. Nine hundred and seventy samples were pooled between October 2006 and March 2007 from confirmed HIV/AIDS patients attending two public referral hospitals: the Federal Medical Center (Hospital I) and the Ebonyi State University Teaching Hospital (Hospital II) in Abakiliki, Ebonyi State. These patients visit both hospitals regularly as may be directed by the clinician as part of the monitoring of the HIV progression and collection of free antiretrovirals. On such visits, CD4⁺ lymphocytes counts were performed and used in estimation of HIV progression. A questionnaire was designed and relevant data were filled in by each literate patient. Similar data were retrieved from illiterate patients by oral interview in local dialect and filled in the questionnaire. Additional lacking information thereafter was retrieved with the kind permission and assistance of the doctors and nurses in these hospitals. Data obtained included information on the CD4⁺ lymphocytes counts, the resident (locality) and the social status of each patient that participated in the study.

Appropriate permissions and consent were obtained for the study by first explaining the full intention of the study to each participant.

Thereafter, those who agreed to participate in the study gave informed consent. Out of all requests for participation in the study, only 20 refused to participate.

2.2. Laboratory procedures and identification of *T. vaginalis*

A 10 mL aliquot from each urine sample was centrifuged at 3000 rpm for 10 minutes soon after collection. A drop of the resuspended pellet was put onto a glass slide as a wet mount preparation and examined under light microscopy for the detection of motile protozoa with the characteristic morphology of *T. vaginalis*. A second swab from the pellet was used to inoculate a culture medium for *T. vaginalis* (InPouch TV, BioMed Diagnostics, San Jose, CA, USA). This was examined under light microscopy for the presence of characteristic motile trichomonads on 3 and 7 days. Samples were considered to be infected with *T. vaginalis* if either the wet preparation or the culture was positive for the organism. Infected subjects who gave consent were later treated by qualified medical personnel.

2.3. Data analysis

Results were analysed using the *Chi* square test. Values less than 0.05 were considered to be statistically significant.

3. Results

The result showed that out of 450 subjects sampled at the Ebonyi State Teaching Hospital for the occurrence of *T. vaginalis*, 170 (37.78%) persons were found to be infected. It was slightly higher than 35.58% (185/520) obtained from the second study center, the Federal Medical Center, Abakiliki. The total percentage occurrence in both centers was therefore 36.60% (355/970). Table 1 showed the general occurrence of *T. vaginalis* in HIV/AIDS subjects and their corresponding CD4⁺ T-lymphocyte counts. Table 2 showed a corresponding table for healthy patients. In Table 1, the T-lymphocyte counts were put in six groups: 40–140, 150–250, 260–360, 370–470, 480–580 and 590–690 cells/mL, respectively. In general, 180 patients were tested positive for trichomoniasis out of the 300 screened in the range of 40–140 cells/mL. This gave the highest percentage of 50.70. It was followed closely by those in the range of 150–250 cells/mL with a percentage value of 30.99 (110/210). The percentage subsequently decreased with increasing CD4⁺ T-lymphocyte counts with a little exception in the range of 590–690 which was higher (1.4%) than the 0.56% obtained in the 480–580 range. Table 1 showed that decrease in the CD4⁺ T-lymphocyte counts led to an increase in the occurrence of the parasite and it was statistically significant ($P < 0.05$). Data

Table 1

Age, sex and CD4 lymphocytes distribution in HIV/AIDS patients [n (%)].

No of patients	Sex		Age range (years)	CD4 count (cells/mL)	Positive
	Male	Female			
300	140	160	17–55	40–140	180 (50.70)
210	115	95	19–47	150–250	110 (30.99)
110	65	45	22–52	260–360	40 (11.27)
100	47	53	16–62	370–470	18 (5.07)
50	24	26	17–67	480–586	2 (0.56)
200	95	105	20–52	590–690	5 (1.40)
970	486	484	–	–	355 (36.60)

Table 2

Age, sex and CD4 lymphocytes distribution in non- HIV/AIDS population [n (%)].

No of patients	Sex		Age range (years)	CD4 count (cells/mL)	Positive
	Male	female			
95	45	50	17–55	400–470	5 (12.50)
80	41	31	19–47	480–586	9 (22.50)
65	35	30	22–52	590–690	2 (5.00)
110	47	63	16–62	700–850	14 (35.00)
70	45	25	17–67	860–990	7 (17.50)
50	25	25	20–52	1010–1150	3 (7.50)
470	238	224	–	–	40 (8.50)

Table 3Occurrence of *T. vaginalis* in HIV/AIDS patients according to social status.

Patients' social status	Hospital I			Hospital II			Average percentage infection
	No screened	No infected	% Infected	No screened	No infected	% Infected	
Uneducated	110	35	36.75	120	50	41.67	36.75
LIE	140	58	47.08	110	58	52.73	47.08
MIE	70	10	15.48	60	10	16.67	15.48
Students	100	46	53.00	70	42	60.00	53.00
Unemployed	40	22	39.16	30	7	23.33	39.16
Others	60	14	15.48	40	3	7.50	15.48
Total	520	185	–	450	170	–	–

LIE: low income earners; MIE: middle income earners.

in Table 2 also supported this assertion. Locality appeared not to have any effect on the occurrence of this parasite ($P>0.05$) as out of the 400 rural samples screened, 155 (38.75%) were infected. It was comparable to that obtained in urban subjects 35.09% (200/570). Table 3 showed the spread of positive cases among six categories/groups: uneducated class, low-income group, middle-income group, students, unemployed group and others. The students group appeared to have more cases. With a percentage occurrence of 60.00% (42/70), while the low income class and the uneducated population had a percentage occurrence of 52.73 and 41.67, respectively. The rest classified as 'others' had the lowest value of 7.50%.

4. Discussion

This study investigated the occurrence of *Trichomonas* infection among HIV/AIDS subjects attending two referral hospital/centers in southeast Nigeria. The result showed an overall prevalence of 36.60%. This figure appears to be relatively high but is in tandem with published reports.

Adetokunbo previously reported a prevalence rate of 29.1% among pregnant women in Lagos, in the Western flank of Nigeria[12] while some other authors reported a prevalence of 37.6% in Jos, Northern Nigeria in a similar group of women[13]. However, none of these studies determined the HIV status of the women enrolled in their respective studies but tagged them as apparently healthy subjects. Nevertheless, our data are in better agreement with the later study. Nigeria is a diverse country with a population of over 150 million persons, so the socioeconomic and cultural diversity of the population may be responsible for the different prevalence rates observed in different geographical regions of Nigeria. Infection rates with *Trichomonas* in sub-Saharan African women are among the highest in the world, with a WHO estimated prevalence of 14%. We hypothesize that this value is no longer realistic. Some authors reported a higher prevalence in Kenya (60%) and Democratic Republic of Congo (55.1%)[14]. Among African-Americans living in the United States, it was only 20.1%. Community based studies in Tanzania and Uganda have found a prevalence of 25% and 24%, respectively, of trichomoniasis in rural women from the general population. In our study, the prevalence value is

quite higher than all these. This might be due to the fact that we screened only HIV/AIDS patients while the other studies investigated the general population. It is likely that when these investigations are repeated in these countries using HIV/AIDS subjects, the figures will be higher than what we obtained in our study. Apparently, the rate of infection varies from one individual to another as well as the number of samples used and methods adopted to analyze and screen the patients for the parasite. Thus, the higher prevalence of *T. vaginalis* in our study may be due to the fact that we used two methods of diagnosis. Studies in pregnant women have found that a combination of wet mounts and culture is required to effectively detect *T. vaginalis*[14,15] although culture is generally taken to be the gold standard diagnostic method. Recently, several PCR based techniques have also been used. However, the World Health Organization estimated global prevalence figures of this parasite based on a wet mount microscopy with a sensitivity range of 60%–80%. Recent data, using PCR suggest that its sensitivity may be lower (35%–60%), thus underestimating global prevalence of trichomoniasis. We agree that PCR has allowed a greater understanding of the global epidemiology of *T. vaginalis* but believe the method we have used is still reliable especially in poor resource settings where PCR procedure may be lacking. The number of wet mount positive, culture negative samples in our study was higher than other studies where all or most wet mount positives are detected by culture[14,16–18]. This may be due to the fact that we took our cultures from urine sediment which is a less sensitive method than cultures from the urethra[19,20]. Also, few delays between the time and condition of collection and analysis of samples may also have been a factor. However, the use of both methods of diagnosis increased the reliability of our data. Several authors[21–23] jointly agreed that high prevalence of trichomoniasis is mainly due to promiscuity, sexual infidelity, indiscriminate sex, lack of education and awareness, poverty and ignorance. Interestingly, we also think that these factors also encourage HIV/AIDS transmission. We found that one or more of these factors contributed to the increased prevalence of trichomoniasis in the patients examined. Greater number of the parasite (50.7%) was recovered from subjects with the least range of CD4⁺ T-lymphocyte count of 40–140 cells/mL, which was the least range obtained in the study. Our finding is in agreement with other reports that studied similar relationship of this sort with other disease conditions in HIV/AIDS patients. For example, a recent report showed an increased isolation of *Candida* infections in HIV/AIDS patients with low CD4⁺ T-lymphocyte counts in Nigeria subjects[24,25]. Some other authors[26–28] shared our view that this parasite is one of the factors that can transmit HIV infection. The reason for this may be obviously due to the depletion of the body defensive cells in those with lower CD4⁺ T-lymphocyte counts which reduces the body's protection and increases proliferation of the parasite or the pathogen in the body. It could be hypothesized that the existence of *T. vaginalis*

in HIV/AIDS could aggravate the body's immunity and consequently reduce the life span of the patient. Available data have confirmed that *T. vaginalis* is the most common sexually transmitted infection (STI) among African women and Africa-Americans[6] and may play a prominent role than other STI in augmenting the spread of HIV in this high-risk group. In this study, the students group had a higher infection rate compared to the rest of the other groups. The reason for this may be due to the fact that apart from being a sexually active group, most students keep multiple sexual partners and in some cases, may engage in prostitution. All these are factors which promote the transmission of the parasite especially with low immune status.

Although the general principles for the control of venereal disease apply, there are many steps that can be taken to enhance *T. vaginalis* detection and treatment which will help decrease the prevalence of trichomoniasis and other related diseases. Improving access to *T. vaginalis* and other STD clinical services is very vital especially to HIV/AIDS patients. Furthermore, basic clinical services must be readily available to all sexually active adults and adolescents. This is especially lacking in most developing countries and among illegal residents of most developed countries. Opportunities to identify and treat asymptotically infected persons should be taken wherever possible. This includes when persons are seen for problems during emergency visits, family planning, routine physicals, vaccination visits and non-health settings like schools and prisons. The services should be a routine part of quality care for HIV/AIDS patients and other persons with infection since *T. vaginalis* increases the chance of HIV transmission and/or progression. Presumptive treatment should be provided at very nominal cost for partners of those with infection. Efforts aimed towards reducing the incidence of this infection can be achieved first, by continuing and extending wide spread *Trichomonas* screening and treatment programs in indigenous communities in Nigeria and other developing countries. Providing free antiretroviral drugs to HIV/AIDS patients who cannot afford them is a nice idea. This is particularly important with our finding in this study that the infection persists more in patients with decreased lymphocyte counts. Additional effort where possible, is required to include free treatment for patients with *Trichomonas* infection. While good evidence based on the implementation may be lacking for some of these recommendations, the long standing prevalence of trichomonal infection in HIV/AIDS patients and other categories of uninfected (non-carriers of HIV/AIDS) is totally unacceptable and needs to be properly addressed using the best available diagnostic technology. For instance, the PCR-based identification is said to be more sensitive[2,5] but lacking in many laboratories in developing countries. With a relatively modest screening and treatment programme, the prevalence of *Trichomonas* can be conveniently reduced. Governments of developing countries and donor agencies

should also be encouraged to contribute more by equipping referral centers with the state of the art diagnostic tools as diagnosis appears to be more important in patients who can afford the cost of treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We wish to thank the hospital personnel in both hospitals who helped to make this study possible. Our thanks also extend to the former undergraduate students of the first author at the Department of Applied Microbiology, Ebonyi State University, Abakiliki, Ebonyi State, Nigeria for helping in sample collection. The study was supported by funds received from Mezech Limited.

References

- [1] Cobo ER, Eckmann L, Corbeil LB. Murine models of vaginal trichomonad infections. *Am J Trop Med Hyg* 2011; **85**(4): 667–673.
- [2] Johnston VJ, Mabey DC. Global epidemiology and control of *Trichomonas vaginalis*. *Curr Opin Infect Dis* 2008; **21**: 56–64.
- [3] Van Der Pol B, Kwok C, Pierre–Louis B, Rinaldi A, Salata RA, Chen PL, et al. *Trichomonas vaginalis* infection and human immunodeficiency virus acquisition in African women. *J Infect Dis* 2008; **197**: 548–554.
- [4] Walker CK, Sweet RL. Gonorrhea infection in women: prevalence, effects, screening, and management. *Int J Womens Health* 2011; **3**: 197–206.
- [5] Miller MR, Nyirjesy P. Refractory trichomoniasis in HIV–positive and HIV–negative subjects. *Curr Infect Dis Rep* 2011.
- [6] Sutcliffe S, Newman SB, Hardick A, Gaydos CA. Prevalence and correlates of *Trichomonas vaginalis* infection among female US federal prison inmates. *Sex Transm Dis* 2010; **37**(9): 585–590.
- [7] Mavedzenge SN, Pol BV, Cheng H, Montgomery ET, Blanchard K, de Bruyn G, et al. Epidemiological synergy of *Trichomonas vaginalis* and HIV in Zimbabwean and South African women. *Sex Transm Dis* 2010; **37**(7): 460–466.
- [8] Crucitti T, Jespers V, Mulenga C, Khondowe S, Vandepitte J, Buvé A. *Trichomonas vaginalis* is highly prevalent in adolescent girls, pregnant women, and commercial sex workers in Ndola, Zambia. *Sex Transm Dis* 2010; **37**(4): 223–227.
- [9] Nweze E, Nkpuma V, Okafor J. Current fungal prevalence in HIV/AIDS patients in Nigeria and the susceptibility of the isolates to conventional antifungal agents and medicinal plant extracts used in the locality to treat/control secondary infections due to these organisms. *Int J Infect Dis* 2008; **12**(2): S24.
- [10] UNAIDS. *Report on the global AIDS epidemic: overview of the global AIDS epidemic*. [Online] Available from: http://data.unaids.org/pub/EpiReport/2006/04–sub_Saharan_Africa_2006_EpiUpdate_eng.pdf. [Accessed on 7 October, 2011]
- [11] Federal Ministry of Health Nigeria. *National HIV/syphilis seroprevalence sentinel survey among pregnant women attending antenatal clinics: technical report*. Abuja: Federal Ministry of Health; 2006.
- [12] Adetokunbo OL, Gilles HM. *A new short textbook of preventive medicine for the tropics: protozoal infection: trichomoniasis*. 3rd ed. Ibadan: Bounty Press Limited; 1990, p. 96–97.
- [13] Ogbonna CI, Ogbonna IB, Ogbonna AA, Anosike I. Studies on the incidence of *Trichomonas vaginalis* amongst pregnant women in Jos area of Plateau State, Nigeria. *Angew Parasitol* 1991; **32**: 198–204.
- [14] Pastorek JG, Cotch MF, Martin DH, Eschenbach DA. Clinical and microbiological correlates of vaginal trichomoniasis during pregnancy. *Clin Infect Dis* 1996; **23**: 1075–1080.
- [15] Draper D, Parker R, Patterson E, Jones W, Beutz M, French J, et al. Detection of *Trichomonas vaginalis* in pregnant women with the InPouch TV culture system. *J Clin Microbiol* 1993; **31**: 1016–1018.
- [16] Borchardt KA, Hernandez V, Miller S, Loaiciga K, Cruz L, Naranjo S, et al. A clinical evaluation of trichomoniasis in San Jose, Costa Rica, using the InPouch TV test. *Genitourin Med* 1992; **68**: 328–330.
- [17] Borchardt KA, Al–Haraci S, Maida N. Prevalence of *Trichomonas vaginalis* in a male sexually transmitted disease clinic population by interview, wet mount microscopy and the InPouch™ TV test. *Genitourin Med* 1995; **71**: 405–406.
- [18] Fouts AC, Kraus SJ. *Trichomonas vaginalis*: reevaluation of its clinical presentation and laboratory diagnosis. *J Infect Dis* 1980; **141**: 137–143.
- [19] Shafir SC, Sorvillo FJ, Smith L. Current issues and considerations regarding trichomoniasis and human immunodeficiency virus in African–Americans. *Clin Microbiol Rev* 2009; **22**(1): 37–45.
- [20] Trintis J, Epie N, Boss R, Riedel S. Neonatal *Trichomonas vaginalis* infection: a case report and review of literature. *Int J STD AIDS* 2010; **21**(8): 606–607.
- [21] Wilkerson RG. Trichomoniasis in emergency medicine. [Online] Available from: <http://emedicine.medscape.com/article/787722–overview>. [Accessed on 7 October, 2011]
- [22] Miller M, Liao Y, Gomez AM, Gaydos CA, D’Mellow D. Factors associated with the prevalence and incidence of *Trichomonas vaginalis* infection among African American women in New York city who use drugs. *J Infect Dis* 2008; **197**(4): 503–509.
- [23] Johnston VJ, Mabey DC. Global epidemiology and control of *Trichomonas vaginalis*. *Curr Opin Infect Dis* 2008; **21**(1): 56–64.
- [24] Nweze EI, Ogbonnaya UL. Oral candida isolates among HIV–infected subjects in Nigeria. *J Microbiol Immunol Infect* 2011; **44**: 172–177.
- [25] Chaudhari HS, Singh PP. Comparative drug susceptibility study of five clonal strains of *Trichomonas vaginalis* in vitro. *Asian Pac J Trop Med* 2011; **4**(1): 50–53.
- [26] Khan MS, Unemo M, Zaman S, Lundborg CS. HIV, STI prevalence and risk behaviours among women selling sex in Lahore, Pakistan. *BMC Infect Dis* 2011; **11**: 119.
- [27] Kissinger P, Amedee A, Clark RA, Dumestre J, Theall KP, Myers L, et al. *Trichomonas vaginalis* treatment reduces vaginal HIV–1 shedding. *Sex Transm Dis* 2009; **36**: 11–16.
- [28] Peterson K, Drame D. Iatrogenic transmission of *Trichomonas vaginalis* by a traditional healer. *Sex Transm Infect* 2010; **86**: 353–354.