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Prevalence and Factors Associated with *Trichomonas vaginalis* among HIV-1 Positive Women Attending Tertiary Hospital in Jos, Nigeria

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Abstract: *Trichomonas vaginalis* amongst other STIs facilitate the natural history of HIV infection and may play an important role in the transmission dynamics of HIV. In this study we determined the prevalence of *T. vaginalis* infection and associated risk factors among HIV-1 infected women attending antiretroviral treatment Center at Jos University of Teaching Hospital. The study was a cross sectional survey of 168 HIV-1 infected women who presented to the STI clinic with symptoms and/or without symptoms of *T. vaginalis* infection from November 2015 to January 2016 after obtained informed consent. Demographic data and high vaginal swabs were collected by trained nurse. The samples were examined by light Microscope to identify *T. vaginalis*, *Candida species* and bacterial vaginosis. Of 168 HIV-1 infected women, 87 (51.8%) were positive for *T. vaginalis* infection. The rate of co-infection of *T. vaginalis* with bacterial vaginosis, Candidiasis was 44.8% and 5.7% respectively, both showed no statistical significance, P=0.360. The highest prevalence of *T. vaginalis* infection 29.8% was among individuals in the 30-41 years age group while the lowest 3.05% was among individuals in the 54-65 years age group. In the univariate analyses; age, occupation, educational status, marital status, previous history of STI, condom use, multiple sexual partners, pregnancy, ART status and symptoms manifestations showed no significant association, but in multivariate analyses, history of STI , symptoms manifestations showed significant association with p>0.05. The high prevalence of *T. vaginalis* highlights the need for routine screening among HIV-infected women.

Keywords: *Trochomonas vaginalis*, prevalence, HIV, women, Jos, Nigeria

INTRODUCTION

Trichomonas Vaginalis (*T. vaginalis*) infection occurs worldwide with over 20 million females infected annually, and it has been known to be the most widely prevalent non-viral and its amongst the commonest curable sexually transmitted infection in the world [1]. It is relatively common pathogenic specie of both the female and male urinogenital tract and has been estimated that 10-50% of the infection in women are asymptomatic and in men the proportion may even be higher [2]. The infection causes serious morbidity in women, and it's been associated with adverse pregnancy related outcomes; such as preterm rupture of membranes, preterm delivery, low birth weight, post abortion infection and cervical cancer [3, 4]. Trichomoniasis is prevalent among people with multiple sexual partners, immunosuppressed individuals, and endemic in low socioeconomic status with prevalence rates ranges from 2-5% among women in Africa [5]. In Nigeria, few studies have reported the prevalence of the infection to range from 5.4-26.0% depending on the study population [6, 7]. The infection has been associated with an increased risk of HIV transmission in both sexes [8, 9]

The infection is characterized in female patients by frothy-greenish or yellowish foul smelling vaginal discharge accompanied with vulvo-vaginal irritation, dysuria and lower abdominal pains [10]. It is also associated with a condition known as straw berry cervix, an inflammatory reaction that mimic the cervical motion tenderness associated with pelvic inflammatory disease (PID). Prevalence in men is lower and mostly asymptomatic, thus making them potential carriers [11]. It can affect the prostate gland, seminal vesicles and epididymis leading to mucopurulent discharge, dysuria and non-gonococcal urethral disease [12]. The mode of transmission is usually through sexual contact with Vaginal or urethral discharges of infected person and transmission of organisms via artificial insemination of

infected semen is also possible. Non-sexual transmissions is rare but have been observed in cases involving contaminated moist washed clothes, specular or toilets seats [5]. Transmission to neonate during passage through an infected birth canal is also possible [13]. Studies have also revealed that *T. vaginalis* co-infection induces immune activation, specifically lymphocyte activation, replication and cytokine production, leading to increased viral replication in HIV infected cells [14].

The parasite can be detected in vaginal, prostatic or urethral secretions, and also semen and urine of infected individuals. The most common diagnostic methods used are direct microscopic examinations of wet mount preparations with sensitivity that ranges from 38% to 83%, and when culture techniques, are combined, it is more effective in establishing diagnosis than either one alone. Direct examination of wet mount preparation of clinical sample is the most rapid, most commonly used and least expensive method for identifying the protozoan parasite. Other methods include antigen detection, plastic envelope, cell culture, staining technique, Polymerase Chain Reaction (PCR) techniques, serological and DNA techniques [15]. To our knowledge, no study has been carried out on *T. vaginalis* in the HIV treatment center of Jos University Teaching Hospital (JUTH), Jos. This study aimed to determine the prevalence of *T. vaginalis* and the associated risk factors in HIV infected women attending the HIV treatment Centre at Jos University Teaching Hospital (JUTH).

MATERIALS AND METHODS

Study area and population

The study was conducted in AIDS Preventive Initiative in Nigeria (APIN) at JUTH Jos, Nigeria. The study was a cross sectional study, and 168 women diagnosed of HIV-1 aged between 18-65 years attending APIN JUTH, and those who consented were consecutively recruited from November 2015 to January 2016. Socio-demographic information was obtained from participants by use of structured questionnaire and strict confidentiality of responses as specified in the ethical clearance guideline. Pre-test counseling for sexually transmitted infections (STI) was given to each subject by a trained counselor before specimens were collected from them. The patients that came back for results were given posttest counseling and those infected were referred for treatment.

Ethical Consideration

Approval of the study protocol was obtained from institutional 'Ethic Committee' at Jos University Teaching Hospital. Further permission was obtained from the management of AIDS preventive Initiative in Nigeria (APIN) Jos.

Sample Collection and Laboratory Procedures

High vaginal swabs (HVS) were collected by a medical officer from all the 168 consenting subjects, cutting across both symptomatic and asymptomatic, drug naive and non-drug naive HIV-1 infected women. A sterilized speculum, lubricated with KY gel was gently inserted into the vagina and screwed to open the vagina, so as to see the vagina fornix clearly while the individual is in a lithotomy position. A clean sterile swab stick was then used to pick discharges from the posterior vagina fornix and was returned immediately into the rubber container to avoid contamination. 1.5ml of physiological normal saline was added to the container for preservation before it was transported to the laboratory for examination. After collection of sample using the appropriate aseptic procedure, the high vaginal swabs were macroscopically observed for color, odor and quantity of discharge. The physiological normal saline in the swab container was dislodged and aseptically poured into centrifuge tube and spin at a speed of 3000 rpm for 5 minutes. The supernatant was discarded and the sediment was kept for wet mount and stained preparation. The sediment was dislodged and two drops were placed into a ready to use slide and covered with a cover slip after appropriate labeling. The preparation was then examined under the microscope using 10X and 40X objectives with the condenser iris closed to give good contrast. *T. vaginalis* where observed as ear or round to oval shaped, motile flagellate with characteristic jerky rotating motion. Yeast like cells for the presence *Candida species* and clue cells for the presence of Bacterial vaginosis, and results were recorded appropriately. A smear of the swabs was also made on a slide, air-dried and fixed for 1minute in absolute methanol. Diluted Giemsa's stain was poured on the smear and allowed to stain for 10 minutes after which it was washed off with distilled water, air-dried and examined under the microscope with oil immersion (100X) magnification for confirmation of positive samples and study of morphological features of the parasite [16].

STATISTICAL ANALYSIS

Statistical analysis of results was done using SPSS 20.0 statistical software. Chi-square was used to compare categorical variables and the students T test was used for continuous variables. $P<0.05$ were considered to be significant. Adjusted Odd Ratio (OR) was used to describe the association between the infection and other risk factors.

RESULTS

One hundred and sixty eight (168) high vaginal swab (HVS) samples were successfully studied using wet preparation and the positive case stained by Giemsa's to confirm *T. vaginalis*. Eighty seven (87) samples of the total number were positive for *T. vaginalis* , giving a prevalence rate of 51.8%. Five (5) of the 87 (5.7%) samples had *Candia albicans*, and 39/87(44.8%) were positive for bacterial vaginosis

(Table 1). High prevalence (57.5%) was recorded among the age group of 30-41 years, 5.7% among age group 54-65 years, P> 0.05, The prevalence rate of 43.0% was found among the unemployed, employed (45.0%), P=0.455). In those with primary education (19.5%), secondary education (43.7%), tertiary (33.3%), p=0.938). The infection was higher among those that were married with a prevalence rate of 43.0%, followed by those that were single (25.3%) and widowed (22.0%), (P=0.962).

The prevalence rate was 10.3% among the pregnant women, without pregnancy 86.2%, P=0.036). The study recorded 78.2% of those with one sexual partner, 4.6%, and 1.1% of those with two and ≥3 sexual partners respectively, (P=0.878). Higher prevalence of 96.5% was recorded among those that are on ART while drug naïve subject has low prevalence 3.4% P=0.629). A high prevalence 35.6% was recorded among those that do not use condom regularly, while low prevalence 8.0% was observed among those that use condom regularly,(P=0.198). 48.3% prevalence was recorded among those with no previous history of other STIs, while 40.2% prevalence was recorded among those with previous history of other STIs, (P= 0.560).Those with history of antibiotics treatment recorded 43.7% prevalence while those without history of antibiotics treatment had 39.1%, (P=0.741) (Table 2). Table 3 shows the adjusted Odd Ratios (OR) and 95% Confidence Interval (CI) for factors associated with *T. vaginalis positivity.* Occupation (OR=3.267; 95% CI, 0.445-1.038, p=0.070),previous history of sexually transmitted infection (OR=2.536; 95% CI, 0.476-13.507, p=0.017), non-regular use of condom (OR=1.179; 95% CI, 0.541-2.568, p=0.273), ART status (OR=2.536; 95% CI, 0.476-13.507, p=0.164), history of antibiotic treatment (OR=1.472; 95% CI, 0.915-2.368, p=0.820), and noticeable symptoms (OR=1.069; 95% CI, 0.684-1.672, p=0.036).

Table 1: Prevalence of *T. vaginalis* infection co-infected with Candidiasis and Bacterial vaginosis among HIV-1 positive women

Affected patient	Number	Percentage	p-value
Patients with *T. vaginalis* only	43	25.6	0.360
Patients with *T. vaginalis* and C*andida albican*	5	2.98	
Patients with *T. vaginalis* and *Bacterial vaginosis*	39	23.2	
Total number positive	87	51.8	

Table 2: Demographical and behavioral characteristics of HIV-1 infected women attending APIN JUTH, Jos, Nigeria

Demographic variables	***T. vaginalis* Positive 87/168 (51.8%)**	**Chi-square(x^2)**	**p-Value**
Age group		2.615	0.455
18 – 29	14 (16.1)		
30 – 41	50 (57.5)		
42 - 53	18 (20.7)		
54 - 65	5 (5.7)		
Educational status		2.615	0.455
Primary	17 (19.5)		
Secondary	38 (43.7)		
Tertiary	29 (33.3)		
None	2 (2.3)		
Occupational status		0.411	0.938
Employed	39 (45.0)		
Unemployed	46 (43.0)		
Marital status		0.077	0.962
Married	46 (43.0)		
Single	22 (25.3)		
Widowed	19 (22.0)		
Pregnancy		0.036	0.036
Yes	9 (10.3)		
No	75 (86.2)		
No. of sexual partners		0.259	0.878
1	68 (78.2)		
2	4 (4.6)		

≥3	1 (1.1)		
Regular use of condom		1.659	0.198
Yes	7 (8.0)		
No	31 (35.6)		
None responders	49 (56.3)		
Previous STIs		0.339	0.560
Yes	42 (48.3)		
No	35 (40.2)		
ART status		0.233	0.629
Yes	84 (96.5)		
No	3 (3.4)		
Previous antibiotics treatment		0.110	0.741
Yes	38 (43.7)		
No	37 (39.1)		

Table 3: Select characteristics associated with *Trichomonas vaginalis* infection among HIV-1 positive women attending APIN JUTH, Jos, Nigeria

Characteristics	Odd Ratio	95% confidence interval	P value
Occupation		0.445-1.038	0.070
Employed	0.680		
Unemployed	3.267		
Previous history of STI		0.476-13.507	0.017
Yes	2.536		
No	0.389		
Use of condoms		0.541-2.268	0.273
Once	1.179		
Regularly	0.231		
ART Status		0.476-13.507	0.164
Yes	2.536		
No	0.011		
History of Antibiotic treatment		0.915-2.368	0.820
Yes	1.472		
No	0.446		
Noticeable symptoms		0.684-1.672	0.036
Symptom	1.069		
Asymptomatic	0.529		

DISCUSSION

The study showed that *T. vaginalis* infection occur frequently among women with HIV infection with a prevalence 51.8% of *T. vaginalis* infection, a similar work done in Tanzania, Lusaka and , Zambia, recorded a prevalence rates of 45- 65.5% [17,18] but is comparatively higher than that of 24% carried out on a previous survey in Jos [3] and 4.0 % in HIV negative individuals in Jos [19]. This suggests that *T. vaginalis* infection may be more frequent among Nigerian women with HIV infection. This agrees with finding that *T. vaginalis* has a relationship with HIV infection status [14], and could mean that either HIV predisposes to T. vaginalis infection or vice-versa.

However, it is also higher than reports from earlier studies carried out in Zaria, Kaduna and Abkaliki, Ebonyi which reported 9.2% and 24.4% respectively as their overall prevalence rate [20, 21]. A high incidence of 74.5% *T.vaginalis* was recorded in Lagos, South Western Nigeria in patients with abnormal virginal discharge [22]. Similar survey carried out in African reported 1.9% prevalence in Kinshasa Zaire, 18.6% Congo 27% in Ivory Coast and 8.1% in Kenya[23- 25]. In the USA the prevalence varies, Missouri with 11.0%, California 17.4%, New Orleans 36% and also in Amazonas, Brazil 4.1% prevalence. Some previous studies showed lower prevalent rates compared to the present study [26-28, 14]. These disparities in prevalence of *T. vaginalis* could be possibly due to improved diagnostic tools and or increase incidence of unprotected intra-vaginal sexual activities in some geographic locations. The observed high prevalence 51.8% in this study could be as a result of many factors: it may be due to an increase in *T. vaginalis* prevalence in the last two decades due to immunosuppression diseases such as HIV, it is a known fact that HIV–infected individuals are inherently at higher risk for STIs mainly due to the shared route of transmission [16, 29]. Other factors include; poverty,

poor personal hygiene, multiple sexual partners and unsafe sexual behavior. It also implied that there may be high sexual network that may have resulted to high transmission of *T. vaginalis* among the HIV-1 infected women [25, 29]

Among the different age group investigated *T. vaginalis* infection prevalence was highest among adults aged 30-41 years (50%). This finding corroborates earlier documented studies in USA, Meru county and Kenya [25, 30] that *T. vaginalis* prevalence is highest among women <40 years and lower >40 years old. These younger age groups are thought to be more associated with unprotected sexual behavior which increases the chances of contracting the infection compared to the older age group. This result is also in agreement with generally observed fact that the incidence of the infection is higher among women of reproductive age [31,32,14].

In this study, co-infection of *T. vaginalis* infection with bacterial vaginiosis showed high prevalence rate of 44.8% which is similar to 42% of earlier study done in Jos [3]. The co-infection could be as a result of poor personal hygiene practices, and low literacy status of the individuals.

The study showed that married subjects were more infected with *T. vaginalis* with a prevalence rate of 27.4% which is similar to the work of [33, 16], that suggested that the risk of STI transmission may be increased with regular partnerships and irregular use of condom. This may be as a result of multiple sexual partners, unfaithful sexual relationship especially in a polygamous marriage [25]. An earlier study showed that patients with secondary education have more of the infection as this corresponds to the finding of the present study which could be due to low economic empowerment that may influence their choices and makes them vulnerable to prostitution [25].

The study also observed a significant association between *T. vaginalis* infection and pregnancy but not with unemployment, pregnancy, history of other sexually transmitted infections (STIs), no use of condom, history of antibiotic treatment, ART status and multiple sexual partner ($p>0.05$). The observation of *T. vaginalis* infection among pregnant women could be due to the possibility of compromised immunity thereby increasing the odds of the infection [34].

The co-infection of *T. vaginalis* and HIV has public health implications for HIV prevention as it is associated with the practice of unprotected sex, a habit usually common in many sub-Saharan African countries including Nigeria. Although, it has not been unequivocally established whether trichomoniasis is a risk factor for HIV transmission or just a marker for high risk heterosexual activities, findings in study from the Centre for Disease Control and Prevention (CDC), Atlanta, USA [20, 32, 35], 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 that could play vital roles in the epidemiologic association of trichomoniasis with the sexual transmission of HIV-1 include; *T. vaginalis* disruption of unrinogenital epithelial monolayer could facilitate passage of HV-1 to underlying layers and activation of local cellular immune response with inflammation of the vaginal epithelium and the exo-cervix with evidence of punctuate haemorrhage by *T. vaginalis* in the presence of HIV-1 might lead to increased viral replication [31, 36]. Therefore, HIV-infected women who develop *Trichomonas* are likely to be an important source of continuing HIV transmission and characterizing such individuals can assist and be used in policy intervention strategies.

The study design is used for assessing risk factors although cross sectional is not the best design, however, it is important to determine the susceptibility of the study population by understanding the complications of *T. vaginalis* among women with HIV infection. It is a known fact that STI continue to take an enormous toll on HIV transmission and disease progression, particularly on women's reproductive health especially during pregnancy and childbirth [34]. The reported bidirectional relationship represents an important factor in sustaining the HIV epidemic in populations where *T. vaginalis*/HIV is endemic. Healthcare providers and services for HIV infected women should be improved to reduce the rates of *T. vaginalis* infection to curb HIV disease's progression. Although this could be promising, but it needs network of stakeholders in the health sector and implementation partners to key to this need to support and identify innovative interventions that address host factors such as social, behavioral, cultural and environmental that influences of the transmission pattern of STI in this group. It is also necessary to find better means of interventional measures that will effectively prevent both HIV and associated STIs.

LIMITATION

The study was done in women that are HIV infected attending AIDS clinic and may not be representative of the general population. Microscopic technique was use in the diagnosis as these may underestimate the prevalence when compare with newer techniques like immunofluorescent staining

CONCLUSION

The high prevalence of *T. vaginalis* infection among HIV women in our community is a public health risk, since HIV infection and other sexually transmitted infections (STIs) can be enhanced through the infection. Careful clinical examination and use of wet mount technique together with use of more sensitive assays for

subclinical infection such as immunofluorescent staining of vaginal fluid, could lead to improved detection and control of *T. vaginalis* infections. Provision of proper counseling, targeted sexual education and genital hygiene besides treatment so as to control these infections is advocated.

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