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Real-time polymerase chain reaction (PCR) detection of *Trichomonas vaginalis* from urine samples of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) patients in Limpopo Province, South Africa

Tshifhiwa Constance Nangammbi[1], Sydwell Thusani Mukhadi[2] and Amidou Samie[3]

[1]Laboratory of Molecular Genetics, Department of Zoology, School of Mathematical and Natural Sciences, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa.
[2]Department of Life and Consumer Sciences, Florida Campus, University of South Africa, Private Bag X6, Florida 1710, South Africa.
[3]Molecular Parasitology and Opportunistic Infections Program, Department of Microbiology, School of Mathematical and Natural Sciences, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa.

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Trichomonas vaginalis **is a protozoan parasite that infects the human urogenital tract. This study determined the prevalence and risk factors of *T. vaginalis* infection amongst human immunodeficiency virus (HIV) infected patients in Limpopo Province using urine-based real time polymerase chain reaction (RT-PCR). Urine samples were collected from 155 patients attending three public hospitals and one private clinic in the Vhembe region. Demographic data, clinical and socioeconomic status were collected from the patients using a structured questionnaire. Total genomic DNA was isolated from urine samples using the Qiagen Blood Mini Kit and RT-PCR protocol was used for the detection of *T. vaginalis*. The overall prevalence of *T. vaginalis* in our study population was 21%. The prevalence was higher among patients less than 25 years compared to older patients. Patients who were taking antibiotics at the time of sample collection had less infection compared to patients who were not on antibiotics (24% vs. 7%; p=0.042). Low CD4 counts and early age of sexual debut appeared to be important risk factors. The high recovery rate obtained in this study demonstrates the importance of employing real-time PCR techniques in the diagnosis of the trichomoniasis in this population.**

Key words: *Trichomonas vaginalis*, opportunistic infection, sexually transmitted disease, urine, real-time polymerase chain reaction, prevalence, Limpopo province.

INTRODUCTION

Trichomoniasis is a common sexually transmitted disease (STD) caused by the protozoan *Trichomonas vaginalis* and it affects approximately 180 million people worldwide per year, making it the most common non-viral STD

*Corresponding author. E-mail: samie.amidou@univen.ac.za; samieamidou@yahoo.com. Tel: +27159628186; Fax: +27159628648.

Abbreviations: STD, Sexually transmitted disease; **HIV,** human immunodeficiency virus; **PCR,** polymerase chain reaction; **CI,** confidence interval; **OR,** odds ratios; **ARV,** antiretroviral; **STIs,** sexually transmitted infection.

agent (Schwebke et al., 2011). Diagnosed infections in women are vaginitis, urethritis and cervicitis and these infections are associated with premature birth, low birth-weight or post abortion consequences (Crucitti et al., 2011). The prevalence of *T. vaginalis* infections vary from country to country. It has been estimated that prevalence among women varied from 0.4 to 27.4% in women while among men, the prevalence varied between 0.0 to 5.6% (Swygard et al., 2004). In Sudan, the prevalence varied from 15.6 and 4.8% in different localities (Dahab et al., 2012). *T. vaginalis* infection has also been linked to the increased risk of human immunodeficiency virus (HIV) transmission (Simpson et al., 2007). In spite of the high prevalence of HIV and AIDS in South Africa, few studies have addressed the epidemiology of trichomoniasis in Limpopo Province.

Although certain sign and symptoms are predictive for trichomoniasis, the detection of the parasite is necessary to establish the diagnosis. Diagnosis of trichomoniasis based solely on clinical sign and symptoms is unreliable because the spectrum of infection is broad and other sexually transmitted pathogens can cause similar signs and symptoms (Krieger and Alderete, 2000). Different diagnostic techniques such as wet mount (Fule et al., 2012; Ozdemir et al., 2011), culture and staining (Ertabaklar et al., 2011), and polymerase chain reaction (PCR) methods (Stemmer et al., 2012) are generally used in the diagnosis of trichomoniasis. Comparison of different methods showed that wet mount microscopy and culture have a good chance of detection of *T. vaginalis* infection due to its sensitivity (60%) and specificity (100%). The use of PCR method in the detection trichomoniasis was also found to be 80% sensitive and 97.95% specific, but its availability and cost effectiveness limit its use in routine diagnostic laboratories (Patil et al., 2012).

A study done to compare a Taq Man based real-time PCR with conventional PCR, culture, and wet-mount microscopy for the diagnosis of trichomoniasis in women proved that real-time PCR test is significantly more sensitive than culture and wet-mount microscopy, although its specificity was slightly lower than these tests (Pillay et al., 2007). In addition, real-time PCR was found to be rapid and less time consuming than conventional PCR for the detection of *T. vaginalis* (Schirm et al., 2007).

Several PCR-based diagnostic assays for trichomoniasis using either vaginal swabs or urine samples from women have been described (Lawing et al., 2000). However, the method of detection used in these studies differs as well as the gene region analysed. A study done by Lee et al. (2012) strongly supported the usefulness of PCR on urine samples for detecting *T. vaginalis* and further suggested that in clinical research settings in which vaginal specimens are not available and culture conditions are not feasible, urine-based PCR may be useful for the detection of trichomoniasis.

Although, *T. vaginalis* has been reportedly being prevalence among HIV patients (Mayer et al., 2012), no studies have been conducted on the prevalence and risk factors among HIV infected patients in the Vhembe district of the Limpopo province. The purpose of this study was to detect and determine the prevalence and risk factors of *T. vaginalis* infection and also to determine the association of *T. vaginalis* with other diseases amongst HIV infected patients attending antiretroviral (ARV) treatment in Limpopo province using urine-based real-time PCR.

MATERIALS AND METHODS

Ethical consideration

The study was approved by the University of Venda Research and Publication Committee, the ethical committees of the different hospitals and the Department of Health in Limpopo. The aims and objectives of the study were clearly explained to the patients and a written consent form was obtained before the beginning of the study. Patients were mostly from the surrounding villages and were in different stages of HIV disease except for the seronegative University students. All interviews were made confidential and identifiers such as name of the patients were not collected to protect their identity.

Sample collection and DNA purification

Data on the demographic, clinical and socioeconomic status were collected from the HIV positive patients visiting the three main hospitals in the Vhembe region viz. Tshilidzini, Donald Fraser and Elim and the University of Venda clinic using a structured questionnaire. Urine samples were collected from a total of 155 patients randomly selected for the present study based on the willingness to participate in the study. Genomic DNA was isolated from urine samples and purified using the QiaAmp Blood Mini Kit (QIAGEN®, Germany) following the manufacturer's recommendations. The quality of DNA was assessed by electrophoresis.

Real-time polymerase chain reaction (RT-PCR)

A real-time PCR protocol was developed from previously described protocol by Caliendo et al. (2005). PCR primers used for the detection of *T. vaginalis* were TV forward 5'-CATTGACCACACGGACAAAAAG-3' and TV reverse 5'-CGAAGTGCTCGAATGCGA-3' primers. One hundred and fifty five (155) samples were amplified with two negative controls and a positive control. The real-time PCR master mix was prepared using the following reagents: 10 µl of SYBR green mix, 0.6 µl of each forward and reverse primer of 10 µM, 3.8 µl of nuclear-free water and 5 µl of DNA template.

Data analysis

Statistical Package for Social Science (SPSS, Inc., 2009, Chicago, IL, www.spss.com) version 18 widows program was used to analyse the results obtained. The Chi-square statistics was used to find Pearson Chi-Square significance and Likelihood ratio. Crude odds ratios (OR) (as estimates of the relative risk) were calculated

with 95% confidence interval (CI).

RESULTS

Demographic information of the study participants

Out of 155 patients recruited for the present study, 74% were females and 26% were males. Forty eight per cent (48%) of the study population were single, 33% were married, 11% were widow, and 7% were divorced. Most patients 56 (36%), were recruited from Donald Fraser hospital, while 50 (32%) were from Tshilidzini, 40 (25.8%) from Elim, and 9 (5%) from UNIVEN. About half (49%) of the study participants indicated that they had secondary education, while 12.8% had no formal education, 27% had only primary education, and 10% had tertiary education. The age of the participants varied between 18 and 78 years. About 9% were aged less than 25 years; 80% aged between 25-45 years, and 30% were above 45 years of age.

General prevalence of *Trichomonas* in the population

Real time PCR was used for the detection of *T. vaginalis* DNA from the urine samples. Figure 1 shows the amplification curve as well as the melt curves that were obtained from the light cycler 480. The overall prevalence of *T. vaginalis* in our study population was around 20%. The distribution of *T. vaginalis* was high in patients from Donald Frazer hospital (19.6%). However, the prevalence was higher among patients from Elim (30%) ($\chi^2 = 0.090$). Out of 17 widows that were tested for *Trichomonas*, none of them tested positive as compared to patients who were divorced (27%), and the results were statistically significant ($\chi^2 = 0.025$). The presence of *T. vaginalis* was also tested in males and females participants as well as in people of different age groups. Out of 114 females, 21% were tested positive and 20% out of 40 males also tested positive. As a result there was no significant difference between infection rate among males and female in our study population. In our study, individuals less than 24 years of age (23%) were found to be more infected by *Trichomonas* than individuals from any other age group. However, there was no significant difference in terms of age group. The general prevalence of *Trichomonas* is presented in Table 1. There was no significant different between the distribution of *T. vaginalis* according to number of dependents, highest educational level and water storage in the homes.

Out of 12 patients with more than 6 dependents, 5 (45%) were tested positive for *T. vaginalis*, and the results were almost statistically significant ($\chi^2 = 0.061$). *Trichomonas* was found in patients with primary 10 (26.3%) and secondary 15 (21.4%) education. However, the results were not statistically significant ($\chi^2=0.712$).

There are also no association found between water storage and *T. vaginalis*.

Effects of ARV treatment and other support system on the occurrence of *T. vaginalis* in the study population

The prevalence of *T. vaginalis* was similar among patients who were on ARV and those who were not on ARV treatment. However, patients who were taking bactrim 15 (34%, $\chi^2 = 0.002$) and prophylaxis 16 (29%; $\chi^2 = 0.045$) had more *T. vaginalis* and the association was statistically significant. Patients who were taking antibiotics at the time of sample collection had less infection compared to patients who were not on antibiotics (24% vs. 7%) and the difference is statistically significant ($\chi^2 = 0.042$) (Table 2).

The occurrence of *T. vaginalis* in the study population in relation to different symptoms

No significant association was found between *T. vaginalis* and common opportunistic infections or symptoms such as sores in genitals, diarrhoea, fever, pain, fatigue, weakness and STD. However, a significant association was observed between patients who had localized pain and *T. vaginalis* and the difference was almost statistically significant ($\chi^2 = 0.062$) (Table 3). There were 8 patients who had a CD4 count less than 50 cells /µL and 4 of these were found to carry *T. vaginalis*.

Prevalence of *T. vaginalis* among the patients in relation to the age at which they started sexual activities

The highest infections of *T. vaginalis* were found among patients who started sexual activities at the age of 22-25 years followed by those who started at the age of 12-15 years (Figure 2).

Prevalence of *T. vaginalis* among HIV patients in relation to sexual behaviour

The patients were asked about their sexual habits. Generally the prevalence of *T. vaginalis* in the study population increased with the number of sex partners since their first sexual experience (Table 4). Patients who indicated that they were abstaining during the period of interview appeared to have more infections compared to those who were sexually active. The number of sexual partners in the previous year before the survey played an important role since patients who indicated that they had more than one partner during the previous year had a higher prevalence of *Trichomonas* and the difference was statistically significant (p=0.016). Surprisingly, patients

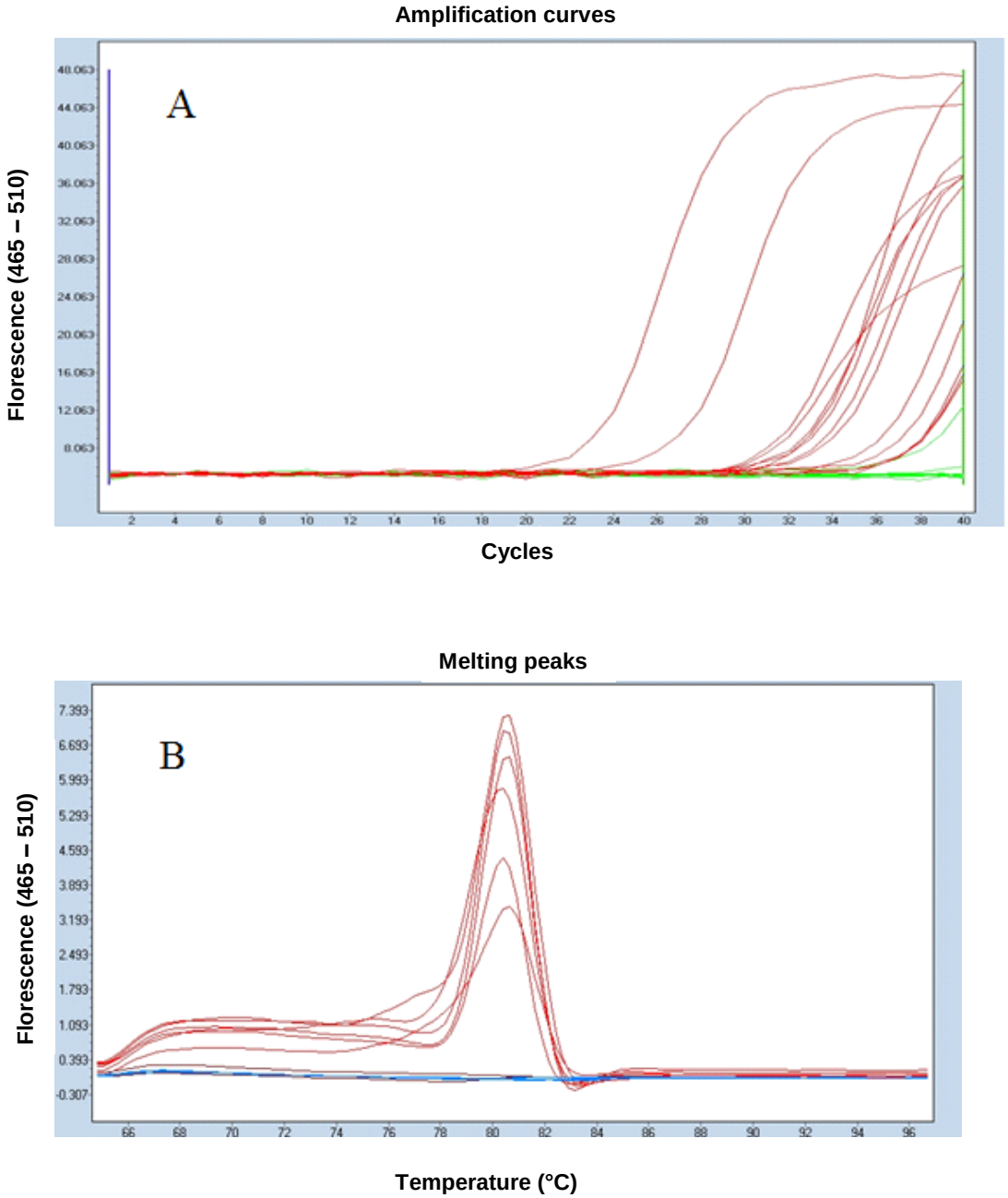

Figure 1. Graphs obtained from the light cycler 480 II after amplification of *T. vaginalis* DNA from the urine sample. A, Amplification curves; B, melting curve analysis.

who reported to have had no sexual partner in the previous year had a higher rate of infection compared to those who had sexual partners, and the difference was significant (p = 0.001) (Table 4).

Prevalence of *T. vaginalis* in relation to smoking and drinking alcohol

Patients who indicated that they were smoking had a higher prevalence of *T. vaginalis*. However, the difference was not significant while alcohol drinking did not affect the prevalence among these patients (Table 5).

DISCUSSION

T. vaginalis has been incriminated as one of the most common sexually transmitted pathogens and appears to be highly prevalent with widespread geographical distri-

Table 1. General prevalence of *Trichomonas* in the study population.

Parameter	Description	*T. vaginalis* positive (%)	Total	χ^2 (p value)	Odds ratio: 95% CI
Hospital	Donald Frazer	11 (19.6%)	56	0.054 (0.817)	2.036 (0.8-4.6)
	Elim	12 (30%)	40	2.880(0.090)	
	Tshilidzini	8 (16%)	50	0.972(0.324)	
	Univen	1 (11.1%)	9		
	Total	32 (20.6%	155		
Marital status	Single	18 (24.3%)	74	1.088(0.29)	1.515: (0.69-3.319)
	Married	11 (21.2%)	52	0.020(0.934)	
	Divorced	3 (27.3%)	11	0.303(0.582)	
	Widow	0	17	5.012(0.025)	
	Total	32 (18.2%)	155		
Sex	Female	24 (21.1%)	114	0.020(0.888)	1.060: (0.425-2.613)
	Male	8 (20%)	40		
	Total	32 (20.6%)	155		
Age	<25 Years	3 (23.1%)	13	0.104(0.450)	
	25-45 Years	17 (19.5%)	87		
	>45 Years	9 (20.9%)	43		
	Total	29 (21.2%)	143		
Dependents (People for whom the patient is responsible for)	0	4 (30.8%)	13		
	1-3	10 (17.5%)	57	0.566(0.452)	
	4-6	11 (17.5%)	63	0.708(0.400)	
	>6	5 (41.7%)	12	3.508(0.061)	3.086(0.904-10.129)
	Total	30 (20.7%)	145		
Education	Illiterate	3 (16.7%)	18		
	Primary	10 (26.3%)	38		
	Secondary	15 (21.4%)	70	1.370(0.712)	
	Tertiary	2 (13.2%)	15		
	Total	30 (21.3%)	141		
Water storage	1 Day	14 (18.4%)	76		
	2 To 4 Days	7 (24.1%)	29		
	5 Days Or More	9 (29%)	31		
	Total	30 (22.1%)	136		
Do you treat water	No	30 (21.9%)	137		
	Yes	0 (0%)	5		
	Total	30 (21.9%)	142		

bution (Bakare et al., 1999; Sorvoillo et al., 2001; Bachmann et al., 2011). Most patients with *T. vaginalis* infections are asymptomatic or mildly symptomatic and hence they are likely to continue to remain sexually active in spite of the infection. In the present study, real time PCR was used to detect *T. vaginalis* in urine samples collected from HIV and AIDS patients attending different treatment centres in Vhembe District, South Africa.

In the present study, *T. vaginalis* was detected in both males and females and the overall prevalence was 20%. This general prevalence is much higher compared to the 3.3% described in Uganda (Osinde et al., 2012) or 3% in the USA (Mayer et al., 2012), among HIV patients. In Mozambique, a prevalence of 2% was described for *T. vaginalis* (Zimba et al., 2011). In Sudan, a prevalence of 12% was described among patients with vaginal discharge (Dahab et al., 2012). In a study conducted in South Africa, (Cape Town and Johannesburg) the prevalence of *T. vaginalis* among patients with urethral or vaginal discharge varied between 19 and 34% (Mhlongo et al., 2010). The prevalence was higher among patients aged less than 25 years as compared to older patients. Similar findings were also obtained by Dahab et al. (2012). It is possible that younger individuals engage in risky sexual behaviour which might lead to an increase prevalence of sexually transmitted infection (STIs), including *T. vaginalis*. Early age at first intercourse may be a proxy for cumulative sexual exposure. Kaestle et al. (2005) observation on the rate of first intercourse on younger age people confirmed higher rate of *T. vaginalis*

Table 2. Effects of ARV treatment and other support systems on the occurrence of *T. vaginalis* in the study population.

Character	Description	*T. vaginalis* positive (%)	Total	χ^2 (p value)	Odds ratio: 95% CI
ARV	No	6 (20.7%)	29	0.000(0.986)	
	Yes	25 (20.8%)	120		
	Total	31 (20.8%)	149		
Waz -2	Unknown	3 (18.7%)	16	1.018(0.601)	
	No	27 (22.1%)	122		
	Yes	2 (11.8%)	17		
	Total	32 (20.6%)	155		
Prophylaxis	No	15 (15.8%)	95	4.003(0.045)	2.246 (1.006-5.014)
	Yes	16 (29.6%)	54		
	Total	31 (20.8%)	149		
Multi vitamins	No	14 (18.9%)	74	3.104(0.078)	3.061 (0.845-11.084)
	Yes	5 (41.7%	12		
	Total	19 (22.1%)	86		
Bactrim	No	16 (15.1%)	106	7.271(0.002)	3.013 (1.324-6.85)
	Yes	15 (34.1%)	43		
	Total	31 (20.8%)	149		
Vitamin B com	No	18 (21.7%	83	0.117(0.732)	
	Yes	10 (19.2%)	52		
	Total	28 (20.7%)	135		
Taking antibiotic	No	30(23.8%)	126	4.116(0.042)	0.237 (0.053-1.05)
	Yes	2 (6.9%)	29		
	Total	32 (20.6%)	155	154	155

Waz-2, Weight per age Z score less than-2 (These were underweight individuals).

infections as compared with older age and the effect diminished with increasing age. A study done in Ndola, Namibia observed a high rate of prevalence of trichomoniasis in adolescent girls who reported that they never had sex. It was however suggested that high prevalence of trichomoniasis in virgins was not through sexual intercourse but via shared bathing water and inconsistent use of soap (Crucittie et al., 2011). Another study done by Stemmer et al. (2012) in different age group found trichomoniasis to be highest among women aged 45 to 65. This was found to be due to *T. vaginalis* filtrating the subepithelial glands and was detected only in hormone-induced or anti-induced changes in the vaginal flora.

Even though marital status have been reported to play a significant role in the transmission of *T. vaginalis* (Osoba, 1972; Menéndez et al. 2010), in the present study no association was found between marital status and *T. vaginalis*. In fact out of 17 widows that were tested for *Trichomonas*, none of them tested positive, and the results were statistically significant (χ^2 = 0.025) unlike in the study conducted by Menendez and colleagues. The results obtained in the present study show no significant association between ARV treatment and *T. vaginalis* infections, whereas there was association with bactrim prophylaxis. Berenguer et al. (2004) reported that the use of bactrim and prophylaxis for opportunistic infections remains necessary in patients who lack access to antiretroviral therapy, in extremely immunosuppressed patients until the therapy takes effect. Our study confirm the findings of a cross-section study by Ghys et al. (1995), which reported higher rates of *Trichomonas* at lower CD4 cell levels and suggested that *Trichomonas* may be an opportunistic infection among women with HIV. Similar results were also described by Leroy et al. (1999).

The prevalence of *T. vaginalis* in relation to opportunistic infection reported by the patients was significantly associated with localised pain. According to Petrin et al. (1998), trichomoniasis is characterized by a severe discomfort with inflammation and abdominal pain. However, these may suggest that not any pain is associated with *T. vaginalis*. In the present study, most opportunistic infections were not associated with *T. vaginalis*. However, a study done by Garcia et al. (2004) reported that an association was found between trichomoniasis with other STDs. Another most interesting findings obtained from this study was the association of *T. vaginalis* infection with patients who were either abstaining and/or always using condoms. This could be explained by the fact that it may be possible that they did not engage in sexual activities because they were feeling

Table 3. Common opportunistic infections reported by the patients

Opportunistic infection		*T. vaginalis* Positive (%)	Total	χ^2 (p value)	Odds ratio: 95% CI
Sores in genitals	No	28 (21.2%)	132	0.175(0.676)	
	Yes	4 (17.4%)	23		
	Total	32 (20.6%)	155		
Diarrhoea	No	24 (20%)	120	0.135(0.713)	
	Yes	8 (22.9%)	35		
	Total	32 (20.6%)	155		
Fever	No	14 (26.4%)	53	1.250(0.263)	
	Yes	3 (14%)	21		
	Total	17 (23%)	74		
Pain	No	10 (22.7%)	44	0.004(0.951)	
	Yes	7 (23.3%)	30		
	Total	17 (23%)	74		
Localised	No	1 (6.2%)	16	3.483(0.062)	0.923(0.734-65.254)
	Yes	6 (31.6%)	19		
	Total	7 (20%)	35		
General	No	6 (25%)	24	1.193(0.275)	
	Yes	1 (9.1%)	11		
	Total	7 (20%)	35		
Fatigue	No	13 (26.5%)	49	0.877(0.349)	
	Yes	4 (16.7%)	24		
	Total	17 (23.3%)	73		
Weakness	No	12 (24.5%)	49	0.121(0.728)	
	Yes	5 (20.8%)	24		
	Total	17 (23.3%)	73		
STD	No	23 (21.1%)	109	0.000(0.989)	1.002(0.388-2.64)
	Yes	7 (21.2%)	33		
	Total	30 (21.1%)	142		
CD 4 count less than 50 cells/µL	No	15(22.7%)	66	2.781(0.095)	3.40(0.738-15.749)
	Yes	4(50.0%)	8		
Viral load<25	No	6(19.4%)	31	0.004(0.152)	0.348(0.151-0.804)
	Yes	5(20.0%)	25		

pain from *Trichomonas* infections. According to Martin et al. (1999) condom use can significantly reduce the risk of acquiring trichomoniasis. This is in contrast with Garcia et al. (2004) who reported that there was an association, although not statistically significant, between the existences of *T. vaginalis* and the use of condoms. However, our results suggest that those who reported to abstain and always using condoms could have started abstaining or using condoms after they had been infected with *Trichomonas*. Sexual contact is the principal way of *T. vaginalis* infection. However, patients reportedly having multiple partners were not associated with *T. vaginalis*. There was a significant association between the number of sexual partners in the previous year and *T. vaginalis* is surprising. Our results are therefore in line with previous study by Tunyuksel et al. (1996) who reported that *Trichomonas* is most prevalent among patients with large number of sexual partners.

In this study, we found no significant association between patients who drinks alcohol and *T. vaginalis* infection. Our results contradict a study done by Coth et al. (1991) who reported that alcohol consumption showed a positive association with *T. vaginalis* infection. Different smoking status such as past and currently smoking and the prevalence of the infection was not significantly different among HIV infected patients. In our study, patients who reported smoking were more infected with *T. vaginalis* compared to those who did not. These results correlate with those described by Naguib et al. (1966) who reported a significant association between smoking and *T. vaginalis* infection. However we did not find any association with alcohol consumption. This could be due to the limited number of patients who responded about alcohol consumption.

Socioeconomic status, such as education has been linked to the prevalence rates of *T. vaginalis* infection

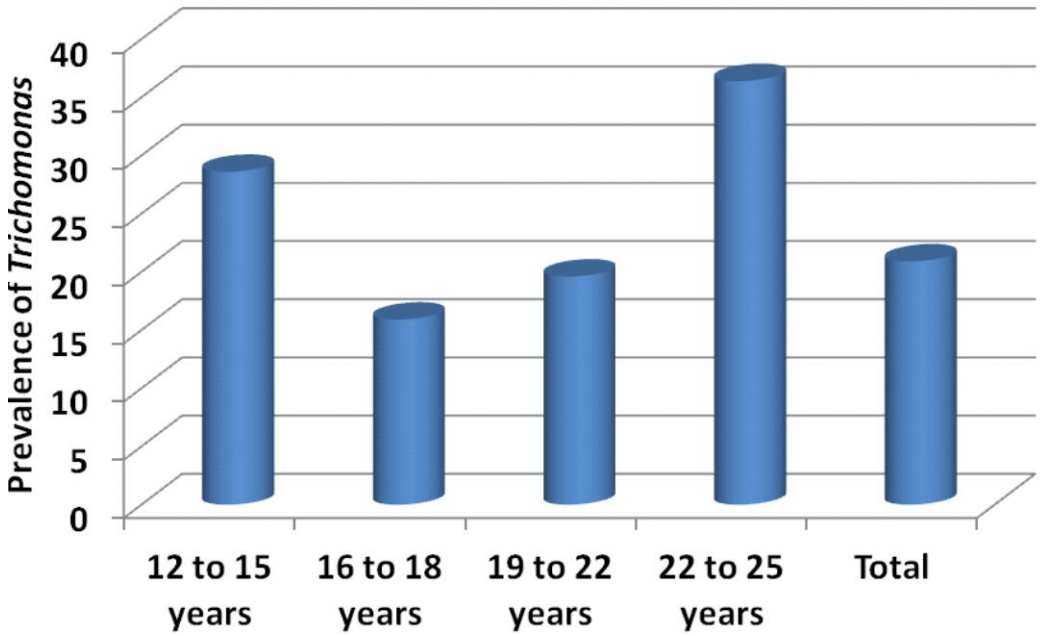

Figure 2. Prevalence of *Trichomonas* among the patients in relation to the age at which they started sexual activities.

Table 4. Prevalence of *T. vaginalis* among HIV patients in relation to sexual behaviour.

Character	Description	*T. vaginalis* positive (%)	Total	
No of partner since 1st sex	Unknown	2 (11.8%)	17	
	1	12 (18.2%)	66	
	2-3	10 (24.4%)	41	
	4-5	5 (29.4%)	17	
	6-7	2 (15.4%)	13	
	>8	1 (100%)	1	
	Total	32 (20.6%)	155	
Contraceptive	Unknown	6 (26.1%)	23	
	No	20 (18.7%)	107	
	Yes	5 (21.7%)	23	
	Total	31 (20.6%)	154	
Condom use	Unknown	1 (8.3%)	12	
	Never	8 (14%)	57	
	Sometime	6 (25%)	24	
	Always	15 (30%)	50	
	Total	30 (21%)	143	
Abstain	No	14 (16.1%)	87	
	Yes	15 (30%)	50	
	Total	29 (21.2%)	137	
One To 4 Partners since 1st sex	No	14 (20.4%)	68	χ^2=0.104, p=0.747
	Yes	16 (22.9%)	70	
No Sex partners last year	No	15 (14.2%)	106	χ^2=11.602, p=0.001
	Yes	16 (40%)	40	OR=4.044, 95%CI: 1.753 - 9.329
One sex partners last year	No	21 (29.6%)	71	χ^2=5.754, p=0.016
	Yes	10 (13.3%)	75	OR=0.366; 95%CI: 0.158 - 0.847

Table 5. Prevalence of *T. vaginalis* in relation to smoking and drinking alcohol.

Character	Description	*T. vaginalis* (%)	Total (%)
Past smoking	No	24 (20.9%)	115
	Yes	6 (25%)	24
Currently smoking	No	26 (20.3%)	128
	Yes	4 (36.4%)	11
Alcohol drinks	No	27 (21.6%)	125
	Yes	3 (21.4%)	14
	Total	30 (21.6%)	137

(Cotch et al., 1991; Quinlivan et al., 2012). In our study, patients who had lower education level were more infected by *T. vaginalis* although the difference was not significant. According to Naguib et al. (1966) and Cotch et al. (1991) women with a higher education and family income had a lower prevalence of the disease compared to those with a lower education. Fernando et al. (2012) also reported that individuals with higher education had less *T. vaginalis* infections. Our study did not find a significant association between water storage and the occurrence of *Trichomonas* infections. However, the five individuals who treated their water did not have *T. vaginalis*. This is in agreement with the report by Ukoli (1990), who reported that the spread of trichomoniasis by water undoubtedly occur, especially in areas with poor environmental hygiene. A study done on the transmission of *T. vaginalis* in adolescent girls attending school in Ndola, Zambia found high prevalence of trichomoniasis in virgins was due to non-sexual transmission of trichomoniasis via shared bathing water and inconsistent use of soap (Crucitti et al., 2011).

Generally, low CD4 counts and early age of sexual debut appeared to be the important risk factors while the use of antibiotics was protective again *Trichomonas* infections. High recovery rate obtained in this study demonstrates the importance of employing real-time PCR techniques in the diagnosis of the trichomoniasis using urine samples. It is also suggested that for clinical research settings in which vaginal swab specimens are not available and culture conditions are not feasible, urine-based PCR may be useful for the detection of trichomoniasis in HIV patients.

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