Original Research Article

Trichomonas vaginalis and *Candida albicans* Infections among Women of Reproductive Age in Ihiala, Anambra State, Nigeria

ABSTRACT

Background: Co-infection of *T. vaginalis* and *C. albicans* continues to present major health, social and economic problems in Nigeria.
Aim: A study to determine the prevalence and co-infection of *T. vaginalis and C. albicans* infections among women of reproductive age in Ihiala, Anambra state.
Study Design: The study was a cross-sectional survey involving 734 women from four villages in Ihiala community.
Duration: The study was conducted between January and March 2015.
Materials and Methods: Wet mounts were used to examine the high vaginal swabs of the women. Jerky movement and flagella were used to confirm the presence of *T. vaginalis.* Germ-tube test was used to confirm *C. albicans* after culturing on sabouraud dextrose agar.
Results: Of the 734 women, 150(20.4%) were positive to *C. albicans*, 7(1.0%) were positive to *T. vaginalis* and 4(0.5%) had co-infection of *T. vaginalis and C. albicans.* Women in the age group 40-49 years had the highest infection 22(37.9) of *C. albicans* and the age group 20-29 years had the lowest 96(17.0). Women in age group, 30-39 years had the highest co-infection of *C. albicans* and *T. vaginalis* 2(1.8%), while age group 20-29 years had the lowest 2(0.4%). Co-infections were highest 2(6.7%) among traders and none among civil servants and students. Co-infection of the pathogens was observed in married women only, and highest 1(3.6%) among wives of polygamous men. Co-infections of *T. vaginalis and C. albicans* was highest 1(14.3%) among the women with non-formal education and none among the women with tertiary education. Co-infections of the pathogens were observed only among non-pregnant women.
Conclusion: Since most co-infections were observed among women with no formal education and those from polygamous families, mass education on reproductive health to the women will help reduce the scourge of these infections in the study area.

Keywords: *Trichomonas vaginalis*, *Candida albicans, women, Ihiala, Anambra State.*

1. INTRODUCTION

Trichomonas vaginalis is most likely the world's most common non-viral sexually transmitted infection. It is a serious public health issue affecting women of reproductive age [1]. Poor personal cleanliness, frequent sex partners, low socioeconomic level, and underdevelopment have all been linked to a higher risk of infection [2]. Other sexually transmitted infections, such as gonorrhoea, chlamydia, and sexually transmitted viruses, are also linked to Trichomonas vaginalis infection. Other viruses, such as herpes, human papillomavirus (HPV), and human immunodeficiency virus (HIV), become more susceptible as a result of the infection (HIV) [3].

The condition is caused by a pear-shaped protozoan called T. vaginalis [4], which causes frothy-greenish-foul-smelling vaginal discharge, vulvo-vaginal irritation, dysuria, lower stomach aches, discomfort, and psychosocial distress in female patients [5,6]. Other problems include cystitis, cervicitis, and urethritis [7]. These symptoms are generally increased during menses and pregnancy. Premature rupture of membranes, premature labour, slow labour, low birth weight, abortion, post-hysterectomy infection, infertility, and neoplastic change in cervical tissues are all described *Trichomonas vaginalis* complications in pregnant women. [8,9,10].

In Nigeria, spread of Sexually Transmitted Diseases (STDs) has been blamed on increase in poverty, unemployment and violence among women [11,12]. Sexual recklessness, lack of awareness, ignorance of the public health implications, poor sanitation and poor personal hygiene are other risk factors of trichomoniasis. Prevention of *T. vaginalis* infection has not been a priority due to lack of understanding of its public health implications and lack of resources [13]. Trichomonial infections are most common in those between the ages of 16 and 35 [14], while they can also affect people in their later years [15].

Candidiasis on the other hand is associated with vaginal discharge and pruritis. The discharge appears like curded milk and deep erythma of vulva and vagina is often seen [16]. The incidence of the infection is almost doubled in pregnancy mostly in the third trimester. It has been estimated that up to 40% of pregnant women world-wide may have vaginal colonization by *Candida* species, a two-fold increase from the prevalence rate in non-pregnant women [17]. Among various pathogenic species of fungi, *Candida* is the most prominent cause of fungal infections [18]. The genus *Candida* is composed of heterogeneous group of organisms and more than 17 different species are implicated in human infections [19]. The most important pathogenic species is Candida albicans. The high frequency of STDs and reproductive tract infections is due to a combination of factors including low socioeconomic position, a lack of knowledge, a lack of diagnostic facilities, and a lack of appropriate treatment options [20,21].

The aim of this study was to investigate *T. vaginalis* and *C. albicans* infections among women of reproductive age in Ihiala. The specific objectives were to determine the prevalence of co-infection of *T. vaginalis* and *C. albicans* by age, marital status, gravidity, pregnancy status, educational status, occupational groups and contraceptive usage, and the treatment habits of *T. vaginalis* and *C. albicans* infections among women in the study area.

2. MATERIALS AND METHODS

2.1 The Study Area

The study was conducted in Ihiala community, Ihiala Local Government Area, Anambra State, Southeastern Nigeria. Ihiala is the local administrative capital of the area. The commuinty is situated between Longitude 6^0.86' East and Latitude 5^0.85' North of the equator, within the tropical rainforest zone of Nigeria. It has about 7 months of wet season period (April to October) and 5 months of dry season (November to March) with a short period of harmattan season. The relative humidity of the area is about 70% in the dry season reaching 80% during wet season [22]. The annual rainfall is between 2,250 to 2,500mm. The average daily temperature range of the area during the dry season is 27-33^{0}C and 21.1^{0}C-30^{0}C in wet season. The community is about 144m above the sea level. Ihiala has a population of 87,796 inhabitants [23]. The area is an Igbo speaking community living peacefully with people of other ethnic groups. The inhabitants are predominantly, farmers, traders, students and a few civil servants.

2.2 Study Design

The study was a cross-sectional survey of women of reproductive age in the community. Quantitative and qualitative methods were used to determine the prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates among the participants. The study was conducted from January to march 2015.

2.3 Advocacy Visit and Mobilization of Participants

The participants were mobilized through town criers and public notices made in the churches within the villages. Health education on the health implications of infections with *T. viginalis* and *C. albicans* was given to the participants. They were assured of confidentiality of individual test results. The study was conducted in line with the international guideline for experimentation [24].

2.4 Study Population and Sample Size Determination

A total of seven hundred and thirty-four (734) apparently healthy women without complains of infection were enrolled. The sample size (n) was determined using Taro Yamane formula [25] for a finite population. The formula was given as:

$$n = \frac{N}{1 + N(e)^2}$$

Where:

n = the minimum sample size
N = the population size
e = level of significance or limit of tolerable error (0.05)
1 = unity (a constant)

2.5 Sampling Technique

The women were assembled at the Akwa Health Centre, Ihiala and at Our Lady of Lourdes Hospital complex, Ihiala, for examination. Convenience sampling in which each woman was attended to as she arrived was used. Hospital examination rooms were used to provide each participant with maximum privacy. Trained nurses and laboratory scientists assisted in swab collection and examination.

2.6 Specimen Collection

(a) Collection of Biodata of the Participants

A total of 734 structured questionnaires were used to obtain the biodata of the participants such as age, village, pregnancy status, occupation, marriage structure, gravidity, educational status and marital status.

(b) Collection of High Vaginal Swabs

With the help of two medical laboratory scientists, samples of vaginal discharge were carefully obtained aseptically from the women's high vaginal area using well-labeled, sterile non-abrasive swab sticks. The vaginal swabs were tested in the medical laboratory shortly after they were collected. T. vaginalis was diagnosed using wet mounts, and *C. albicans* was cultured from swabs.

2.7 Parasitological examination of the specimens

(a) **Diagnosis of *T. vaginalis* (Wet – Mount Preparation)**

Each high vaginal swab from the women was mixed with a drop of sterile normal saline on a clean grease-free slide, covered with cover slip and examined with x10 objectives lens of the microscope. The presence of yeast cell, *T. vaginalis* and other deposits were observed. *Trichomonas vaginalis* was identified by the jerky movement, the flagella, axostyle and its oval shape. Ten percent (10%) Potassium hydroxide (KOH) was added to the wet preparation to increase the detection sensitivity of yeast cells, making the recognition of mycelia (pseudohyphae) much easier [26].

(b) **Diagnosis of *C. albicans* (Culture Method)**

Each high vaginal swab was inoculated on Sabouraud Dextrose Agar gel and incubated at the temperature of 37ºC for 48hrs. Suspected yeast colonies and positive colonies were gram stained and further sub-cultured for germ tube test [26].

2.8 Data Analysis

The statistical differences in the prevalence and co-infection of *T. vaginalis and C. albicans* in the various groups were tested using chi-square test. Chi-square statistics was computed to accompany each cross tabulation. The statistical package used was SPSS version 21.0.

3. RESULTS

Out of the 734 women, 150(20.4%) were positive to *C. albicans,* 7(1.0%) were positive to *T. vaginalis* and 4(0.5%) had co- infection of *T. vaginalis* and *C. albicans* (Table 1).

Table 1: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates among the women participants from Ihiala.

No. Examined	No. infected with *C. albicans* (%)	No. infected with *T. Vaginalis* (%)	No. co-infected with *T. Vaginalis* and *C. albicans* (%)
734	150 (20.4)	7 (1.0)	4 (0.5)

The highest prevalence of *C. albicans* 22(37.9%) was observed in age group 40-49 years while age group 20-29 years had the least 2(9.1%). The age group 30-39 years had the highest prevalence of (Table 2**).** There was a significant difference in prevalence of *T. vaginalis* and *C. albicans* in age groups (P<0.05)**.**

Table 2: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by age groups

Age Group (Years)	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
20-29	564	2 (0.4)	96 (17.0)	2 (0.4)
30-39	112	4 (3.6)	32 (28.6)	2 (1.8)
40-49	58	1 (1.7)	22 (37.9)	0
Total	734	7 (0.95)	150 (20.4)	4 (0.5)

X^2 = 3.876, df = 3, P<0.05

Ubahiekwem 3 (6.3%) had the highest prevalence of *T. vaginalis* while Ihudim village had the least 1 (3.3%). Akwa village had the highest prevalence of *C.albicans* 23 (41.1%), while the least was obsereved in Eziani, 102(17.0%). The co-infection was observed only among Ubahiekwem and Akwa village 3 (6.3%) and 1 (1.8%) respectively (Table 3). There was a significant difference in the prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates in the villages (P<0.05).

Table 3: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by location

Villages examined	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Eziani	600	0	102 (17.0)	0
Ubahiekwem	48	3 (6.3)	18 (37.5)	3 (6.3)
Akwa	56	3 (5.4)	13 (41.1)	1 (1.8)
Ihudim	30	1 (3.3)	7 (23.3)	0
Total	734	7 (0.95)	150 (20.4)	4 (0.5)

X^2 = 3.876, df = 3, P<0.05

The traders had the highest prevalence of *T. vaginalis,* 4(13.3%) and co-infection of *T. vaginalis* and *C. albicans* 2(6.7%). There was a significant difference in the prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates among women from different occupational groups (P<0.05) (Table 4).

Table 4: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by occupation

Occupation	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Students	600	0	102 (17.0)	0
Civil Servants	18	0	3 (16.7)	0
Trader	30	4 (13.3)	10 (33.3)	2 (6.7)
Farmers	86	3 (3.5)	35 (40.7)	2 (2.3)
Total	734	7 (0.95)	150 (20.4)	4 (0.5)

X^2 = 29,169, df = 3 P<0.05

The married women had the highest prevalence of *C. albicans*, and the widows had the least 1(11.5%). Only the married women had co-infection of *T. vaginalis* and *C. albicans* 4(1.7%) among the marital groups (Table 5). The prevalence of *T. vaginalis* and co-infection rates had significant difference (P<0.05) while the prevalence in *C. albicans* had no significant difference (P>0.05).

Table 5: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by marital status

Marital Status	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Singles	468	0	91 (19.4)	0
Married	234	6 (2.6)	55 (23.5)	4 (1.7)
Divorced	6	0	1 (16.7)	0
Widowed	26	1 (3.8)	3 (11.5)	0
Total	734	7 (0.95)	150 (20.4)	4 (0.5)

X^2 = 8.594, df = 3 P<0.05

The highest co-infection of 1(14.3%) was observed among the non-formal educational group and the least was among secondary educational group, 1(1.7%) (Table 6)**.** The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates among the educational groups showed a significant difference (P<0.05).

Table 6: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by educational status

Educational Status	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Non-formal	7	1 (14.3)	4 (57.1)	1 (14.3)
Primary	42	3 (7.1)	22 (52.4)	2 (4.8)
Secondary	60	3 (5.1)	25 (41.7)	1 (1.7)
Tetiary	625	0	99 (15.8)	0
Total	734	7 (0.95)	150 (20.4)	4 (0.5)

X^2 = 42.983, df = 3, P<0.05

The women from polygamous families had higher co-infection rate 1(3.6%) of *T. vaginalis* and *C. albicans* than the women from monogamous families 3(1.5%). The prevalence of *T. vaginalis* and co-infection rates were significantly difference (P<0.05) while the prevalence in *C. albicans* had no significant difference in marital structure(P>0.05) (Table 7).

Table 7: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by marital structure

Marital Structure	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Monogamy	206	5 (2.4)	44 (21.4)	3 (1.5)
Polygamy	28	1 (3.6)	11 (39.3)	1 (3.6)

X^2 = 656, df = 1, P<0.05

The pregnant women had higher infection of *T. vaginalis* 1(2.3%) than the non-pregnant women 6(0.9%). There was no co-infection of *T. vaginalis and C. albicans* among the pregnant women. The prevalence of *T. vaginalis* and co-infection rates showed no significant difference (P>0.05) while the prevalence in *C. albicans* showed significant difference (P<0.05) (Table 8).

Table 8: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by pregnancy status

Pregnancy Status	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Pregnant	44	1 (2.3)	19 (43.2)	0
Non-Pregnant	690	6 (0.9)	131 (18.98)	4 (0.5)
Total	**734**	**7 (0.95)**	**150 (20.4)**	**4 (0.5)**

X^2 = 256, df = 3, P<0.05

Trichomonas vaginalis infection was highest among primigravida, 2(2.9%) and least 1(0.2%) among the nulligravida. *Candida albicans* infection was highest among the multigravida, 67(32.5%) and the least 9(12.9%) among the primigravida (Table 9). The multigravida had a higher rate of co-infection 3(1.5%) than the primigravida 1(1.4%). There was no significant difference in the prevalence of *T. vaginalis, C. albicans* and their co-infection rates among the study participants (P<0.05).

Table 9: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by gravidity

Gravidity No. examined	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C. albicans* (%)
Nulligravidae	458	1 (0.2)	4 (57.1)	0
Primigravidae	70	2 (2.9)	9 (12.9)	1 (1.4)
Multigravidae	206	4 (1.9)	67 (32.5)	3 (1.5)
Total	734	7 (0.95)	150 (20.4)	4 (0.5)

X^2 = 6.675, df = 3, P<0.05

Asymptomatic women 5 (1.0%) had higher prevalence of *T. vaginalis* than the symptomatic women (Table 10). The co-infection occurred more among the symptomatic women 2(0.8) than asymptomatic women 2(0.4). The prevalence of *C. albicans* showed significant difference (P>0.05) while the prevalence in *T. vaginalis* and co-infection rates showed no significant difference (P<0.05).

Table 10: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by clinical manifestation among the women participants.

Clinical Manifestation	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Symptomatic	248 (33.8)	2 (0.8)	81 (32.7)	2 (0.8)
Asymptomatic	486 (66.2)	5 (1.0)	69 (14.2)	2 (0.4)
Total	734 (100)	7 (0.95)	150 (20.4)	4 (0.5)

X^2 =0.473, df=2, P<0.05

Women using Metronidazole always, had the highest prevalence of *C. albicans* infection, 122(23.5%) while those who do not take Metonidazole always, had the least, 28(13.1%). There was no statistical difference between them (P>0.05).

Table 11: **Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates according to treatment habit of the women participants**.

Treatment Habit	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Use of Metronidazole	Yes 520	1 (0.2)	122 (23.5)	0
	No 214	6 (2.8)	28 (13.1)	4 (1.9)
Self-medication of antibiotics	Yes 534	6 (1.1)	138 (25.80)	3 (0.6)
	No 200	1 (0.5)	12 (6.0)	1 (0.5)
Use of Contraceptives	Yes 186	5 (2.7)	122 (65.6)	2 (1.1)
	No 548	2 (0.4)	28 (5.1)	2 (0.4)

X^2 = 4.800, df = 3, P<0.05

4. DISCUSSION

The high prevalence of *C. albicans* (20.4%) reported in this study could be due to the ability of the pathogen as a frequent colonizer and responsible for most cases of vulvovaginitis [27,28]. The result is higher than some studies [29,30,31,32,33] who reported 2.0%, 2.20%, 12.0%, 2.6%, and 17.8% respectively. However, this is lower than other studies [16,34,35] who reported the prevalence rate of 28.0%, 52.5% and 33.6% respectively. It has previously been observed that high incidence of *C. albicans* could be associated with severe immunosuppression or illness, and sexual activities of a woman [36]. Some studies noted that *Candida* species are part of the lower genital tract flora in 20-50% of healthy asymptomatic women and may spread due to sexual activities or immunosuppression [37,38].

High prevalence of *C. albicans* was recorded among age groups 20-29 years (17.0%), 30-39 years (28.6%) and 40-49 years (55.6%). This disagrees with the findings of a previous study [30] who observed that the infections were

almost uniformly distributed in all age groups studied. But it agrees with [39] who reported the prevalence of *C. albicans* to be significantly higher in cohort of 30 years Old Dutch women and lower in the cohorts of 45-50, 55 and 60 years. The age range of between 20-49 years constitute the sexually active period of most women. Although C. albicans infection is not a conventional sexually transmitted disease, it is known to be spread in some cases through sexual intercourse, particularly among sexually active people and sex workers, according to prior studies [19]. It was also noted that the high prevalence rate discovered in the younger age group was ascribed to the group being the most sexually active age range, bolstering the assumption that sexual activity may play a big role in the disease's transmission [40].

The low prevalence rate of trichomoniasis (1.0%) recorded in this study is in tandem with low prevalence of (2.3%) and (3.3%) reported in some studies [41,42]. Also, other investigations reported prevalence rate of 0.2% [43] and 1.2% [44]. However, some studies have reported higher prevalence rates of 24.1% [45] and 24% [46]. Low prevalence of *T. vaginalis* in this study could be attributed to multiple factors such as awareness of people towards unsafe sex. The use of protection during sex help in reducing transmission of pathogenic organisms especially *T. vaginalis* that affects the vaginal mucosa in women [47].

The co-infection rate of trichomoniasis and candidiasis in this study was 0.5%. This compares favorably with a previous observation [35] who reported co-infection rate of 0.6% of *T. vaginalis* and *C. albicans* in Lagos metropolis. Also, another study [48] reported co-infection rate of 3.2% which is slightly higher. However, higher rate of co-infection has been reported in other studies including 22% observed in Abakaliki, Ebonyi State, Nigeria [46]. Low co-infection rate of the parasites observed in this study could be attributed to improved life style, especially in personal hygiene and literacy in reproductive health, as well as the use of Metronidazole in treatment of other protozoa infections.

5. CONCLUSION

Infections caused by T. vaginalis and C. albicans, as well as their co-infections, are found among women of reproductive age in Ihiala, according to the study. The information gathered will serve as a foundation for developing policies and control mechanisms to combat the illnesses. It will also be useful in training reproductive-aged women to be more aware of their reproductive health. As a result, it is advised that widespread identification and treatment, as well as health education, will aid in the eradication of illnesses in the study region.

Consent

Informed written consent was obtained from each woman before enrolment.

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