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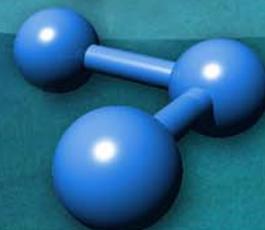
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Research Paper

## COMPARISON OF METHODS OF DIAGNOSIS OF TRICHOMONIASIS IN PREGNANCY AMONG ANTE NATAL PATIENTS IN SOME PARTS OF OYO STATE, NIGERIA

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This study on evaluation of Saline Wet Preparation, Staining and Cultural techniques of *Trichomonas vaginalis* among pregnant women in three selected hospitals covering rural and urban area in Oyo state. 150 samples of High Vagina Swab collected from both symptomatic and non-symptomatic were analyzed. Wet preparation, staining and culture were carried out. 38 (25.3%) of the women were found to harbor *Trichomonas vaginalis*. It was found that this parasite is present predominantly in female between the age of 29-31 years and are more common within the third trimester period. Also there were cases of multiple infection especially *Candida* spp. Results obtained in this study showed that the geographical distribution had no effect on the occurrence of Trichomoniasis as the difference was statistically not significant ( $p = 0.6051$ ). Results obtained from this study also showed that the best method of diagnosis is the cultural technique ( $p = 0.001$ ) among the three methods used.

**Keywords:** Trichomoniasis, Diagnostic Methods, Pregnant Women, Oyo State, Nigeria

### INTRODUCTION

Trichomoniasis is an infection caused by a single-celled protozoan called *Trichomonas vaginalis*. This is an extremely common cause of vaginal infections and the most pathogenic of all the three species infecting human, other includes *Trichomonas tenax* found in the mouth and *Trichomonas hominis* usually found in the small intestine Dinno *et al.* (1998). Trichomoniasis is

also known as *Trichomonas vaginitis* or sometimes trich (pronounced “trick”). Infection rates between men and women are the same with women showing symptoms while infections in men are usually asymptomatic (STD, 2004). Trichomoniasis is a sexually transmitted infection which can occur in females if the normal acidity of the vagina is shifted from a healthy, semi-acidic pH (3.8-4.2) to a much more basic or alkaline

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one that is conducive to *T. vaginalis* growth. Approximately 180 million women worldwide may be infected with *T. vaginalis*. Prevalence estimates vary between populations studied, but range from 5-74% in women and 5-29% in men, with the highest rates reported in either sex from STI clinics and in other high risk populations Anorlu *et al.* (2001).

*T. vaginalis*, is the most common Sexually Transmitted Infection (STI) today, with an annual incidence of more than 170 million cases worldwide. Over eight million women are infected with *T. vaginalis* in North America alone. Although once considered a nuisance infection, *T. vaginalis* in women has since been associated with an increased incidence of prolonged postpartum fever and endometritis, premature rupture of membranes and cytological changes in cervical cell morphology (Schwebke *et al.*, 2004). Trichomoniasis is a sexually transmitted disease with important public health ramifications; it has been associated with vaginitis, cervicitis, urethritis, and Pelvic Inflammatory Disease (PID). Trichomoniasis also have impact on birth outcomes and is a co-factor in Human Immunodeficiency Virus (HIV) transmission and acquisition, the vagina is the most common site of infection in women and the urethra (urinary canal) is the most common site of infection in men Laga *et al.* (1993).

The organism has now been associated with a significantly higher risk of HIV transmission (OR 2 to 2.5) Laga *et al.* (1993), and it is suggested that this parasite may increase maternal-to-infant transmission in HIV. This increased transmission in females is believed to be due to the denudation of the cervicovaginal epithelium along with the accumulation of CD4 lymphocytes and macrophages at the site of infection, which could

provide pools of HIV-susceptible or HIV-infected cells. The parasite is sexually transmitted through penis-to-vagina intercourse or vulva-vulva (the genital area outside the vaginal) contact with an infected partner. Women can acquire the disease from infected men or women, but men usually contact it from infected women, however the parasite is harder to detect in men than in women STD (2004). Though confined to the vagina, this organism also can invade the urinary tract and cause cystitis (inflammation of the bladder). *Trichomonas vaginalis* can also be severe enough to cause an abnormal Pap smear from which the organism also can be detected. Although *T. vaginalis* can cause small red lesions on the cervix, it does not invade the uterus or fallopian tubes, nor does it affect fertility Cotch *et al.* (1991a). Trichomoniasis is associated with significant morbidity for women with vaginitis symptoms ranging from mild to severe inflammation, foul-smelling discharge and severe irritation and discomfort, these symptoms may take several days to several weeks to develop; however, treatment is easy with prescribed antibiotics CDC (2007). *Trichomonas vaginalis* prevalence depends on many factors such as age, sexual activity and the number of sex partners, it also vary between population studied, but range from 5-74% in women and 5-29% in men with the highest rates reported in either sex from STI clinics and in other high risk populations. In men, the prevalence is less well described because many men are asymptomatic and may not seek evaluation. Furthermore, the diagnosis is less commonly sought in men who could contribute to a long duration of the disease and its continued spread. Krieger and Alderete (1999).

*T. vaginalis* infection is more common in pregnant women than in non-pregnant women

due to the fact that the infection reaches its height during pregnancy because of the exuberance production of oestrogen hormones which produces glycogen in the vaginal mucosa in which *Trichomonas vaginalis* utilizes and thrive in. Some of the complications of *T. vaginalis* in women include: preterm delivery, low birth weight, and increased mortality as well as predisposing to HIV infection, AIDS, and cervical cancer Schwebke *et al.* (2004). Because of the impact on premature rupture of membranes and low birth rate, *T. vaginalis* is considered to be a significant cause of neonatal morbidity in USA. Cotch *et al.* (1991b). It appears to be subject to underdiagnosis and misdiagnosis in clinical practice because the symptom complex can overlap with other causes of vaginitis. As well, conventional diagnostic tests are often not readily available.

Historically, detection of the parasite is made possible by examination of urine deposit and microscopically examination of High Vaginal Swab (HVS) in a drop of saline or trichomonas diluents for the characteristic wobbling and rotating motion of *Trichomonas vaginalis* Weise *et al.* (2000). Although highly specific and rapid, direct microscopy examination offers poor sensitivity, detecting only half of all culture-positive cases). Dried swears may be stained with haematoxylin or one of the Romanowsky stains and the parasite can as well be isolated using cultural method Levi *et al.* (1997). Despite all findings on the subject, inadequate attention is been paid by laboratory personnel in proper diagnosis of this parasite in Nigeria as in other developing countries especially to its usual association with sexually transmitted diseases and other related infection. It appears to be subject to under diagnosis and misdiagnosis in clinical practice because the

symptom complex can overlap with other causes of vaginitis. As well, conventional diagnostic tests are often not readily available.

Thus the purpose of this study is to obtain HVS from both symptomatic and asymptomatic pregnant women attending ante-natal clinic at three different Health centers representing rural and urban around Ibadan and its environs in view of isolating *Trichomonas vaginalis* from them and comparing different diagnostic methods.

## **MATERIALS AND METHODS**

### **Study Population**

A total of 150 pregnant women were considered for this study. Among these were both symptomatic and asymptomatic patients, those with symptoms ranges from vaginal itching with or without discharge and dysuria. They were drawn from Eruwa Local Government Health Center, Adeoyo State Hospital and Mission Hospital all within the age 20 and 40 years within the period of June and July 2011. The study received ethical approval from concerned hospitals and informed consent of the subjects.

### **Samples Collection**

Two samples of HVS were collected from each patient. The first swab was used for wet preparation (microscopy) and smear was made from it for staining technique. The second swab was used for culture. 50 samples were collected from patients in each of the centers considered.

### **WET MICROSCOPY**

Vaginal secretions were collected and the secretions were suspended in 0.85% normal saline, mixed gently but thoroughly and a drop was placed on a 22 × 40 glass slide and covered with a cover slip. The preparation was examined

for the wobbling motility of the trophozoites of *Trichomonas vaginalis* under the microscope using  $\times 10$  and  $\times 40$  objectives within 1 h of sample collection.

## STAINING METHOD (PAPANICOLAOU STAINING)

Suspension of the vaginal discharge was made in 0.5 mL of saline. Smear was made on a grease-free slide with the swab stick. Smear was fixed by dipping in absolute methanol (This does not enhance staining activity but preserves the cellular integrity of the flagella) Slide was then flooded with working solution of stain (Alcoholic ferric chloride complex) for 5 min and rinsed with water. It was flooded with crystal violet solution for 3 min and then rinsed with tap water. It was allowed to dry in upright position and slide was examined using  $\times 100$  oil immersion objective. The oval body of the organism stains purple, the nucleus and axostyle dark purple to black. The flagella are purple to blue-black and surrounding epithelial cells appeared purple with darker diffuse nuclei material.

## CULTURE

Many media are available for the cultural diagnosis of *Trichomonas vaginalis* but commercially available Oxoid Trichomonas medium was used for this study. Trichomonas Medium No. 2 Formula (per liter): Liver digest (Oxoid L27) 25.0 g, Dextrose 5.0 g, Sodium chloride 6.5 g, 'Ionagar' No. 2 1.0 g, PH 6.4 (apprx.) Preparation: Suspend 37.5 g in 1 L of distilled water, Sterilize by autoclaving at 121 °C for 15 min. Cool to 50 °C; add 8 mL of serum, 1000 units of penicillin and 50 mcg of Streptomycin/mL of media. Dispense 10 mL into culture tubes with tight caps tubes. Cultures were inoculated directly from the same

site as those for microscopy. Culture tubes with tight cap were incubated in an aerobic atmosphere at 37 °C for the first 24 h after which wet preparation was done by taking a drop of the sample of medium from the bottom of each tube with the use of a pasture pipette and examined for trophozoites of *T. vaginalis* and change in color to yellow. This is repeated at two days and continued up to the seventh day before negative culture was discarded (Cheesbrough, 1987).

## RESULTS AND DISCUSSION

The result showed that 38 of the patients out of the 150 studied had *T. vaginalis* giving overall prevalence of 25.3%. *T. vaginalis* infection in this study found to be highest among pregnant women in the age range 29-31 years with 16 (10.6%), this is followed by those in the age range 26-28 years with 13.0 (8.6%) The lowest percentage positive was in the age of 20-22 years while none of the women in the age group 35-40 years had the infection (Table 1). Table 2 shows the frequencies of infection with *T. vaginalis* in different trimester periods with third trimester period having the highest 27 (32.1%), followed by second trimester 11 (18.0%). No *T. vaginalis* infection among subjects in first trimester. Table 3 shows the result obtained from the three different techniques (Saline wet preparation, Staining and Culture). Culture had the highest prevalence of 53.3% compared to wet preparation and staining technique 25.3% and the difference was statistically significant ( $p = 0.001$ ). The geographical distribution of the occurrence of the parasite is shown in Table 4. The Missionary Hospital, Ibadan (Urban/Private) had the highest prevalence of 36%, followed by State Hospital, Eruwa (Rural) having 28% while Adeoyo State Hospital (urban) had the lowest 12%. The

difference was not statistically significant ( $p = 0.6051$ ). Table 5 showed that 12(8.0%) out of the sample collected had multiple infection of *T. vaginalis* with yeast cells, 17(11.3%) had *T. vaginalis* associated epithelia cells and 9(6.0%) had *T. vaginalis* with pus cells which probably signifies secondary bacterial infection. Also, 24 (16.0%) of the sample had predominantly yeast cells, and 13 (8.6%) had predominantly pus cells. Table 6 showed that 54 (36.0%) of the pregnant women studied had vagina discharge with or

without itching while 17 (11.3%) of the women had dysuria as their symptoms giving the total of symptomatic women to be 71, the remaining 79 (52.7%) women were asymptomatic out of which 5 (13.1%) was diagnosed with *T. vaginalis* infection.

Trichomoniasis is estimated by the WHO to account for almost half of all curable sexually transmitted infection worldwide and it's said to be the most prevalent non-viral STI in the world. Schwebke et al. (2004).

**Table 1: Age Distribution of *Trichomonas vaginalis* Among Antenatal Subjects by Saline Mount (Convectional Method) in Parts of Oyo State**

Age (Year)	No of Subject	No Positive for <i>T. vaginalis</i>	% Positive
20 - 22	14	01	0.6%
23 - 25	23	06	4%
26 - 28	25	13	8.6%
29 - 31	44	16	10.6%
32 - 34	28	02	1.3%
35 - 37	11	0	-
38 - 40	05	0	-
TOTAL	150	38	25.3

**Table 2: Distribution of *T. vaginalis* Among Antenatal Subjects in Oyo State by Trimester Period**

Weeks	Trimester Period	No of Subjects	No positive for <i>T. vaginalis</i> (%)
1 to 12	First Trimester	5	0 (0)
13 to 24	Second Trimester	61	11 (18.0)
25 to 36	Third Trimester	84	27 (32.1)

**Table 3: Distribution of *T. vaginalis* Among Antenatal Subjects in Parts of Oyo State by Diagnostic Methods**

Diagnostic Methods	No Examined	No Positive (%)	P value
Wet Preparation and Staining	150	38 (25.3)	0.001
Culture	150	80 (53.3)	

**Table 4: Geographical Distribution of Antenatal Subjects Positive for *T. vaginalis* in Parts of Oyo State**

Location	No. of Subject	No positive for <i>T. vaginalis</i> (%)	P value
Adeoyo State Hospital, Ibadan (urban)	50	06 (12)	0.6051
State Hospital Eruwa (rural)	50	14 (28)	
Mission Hospital(urban/private)	50	18 (36)	

**Table 5: Multiple and Other Infection with *T. vaginalis* Distribution Among Antenatal Subjects in Parts Of Oyo State (N= 150)**

Other Infections	No. of Subjects	% of Subjects
<i>Trichomonas vaginalis</i> + <i>Candida</i> spp.	12	8.0%
<i>Trichomonas vaginalis</i> + epithelial cells	17	11.3%
<i>Trichomonas vaginalis</i> + pus cells	09	6.0%
<i>Candida</i> spp. only	24	16.0%
Pus cells only	13	8.7%
Epithelia cells only	75	50.0%

**Table 6: Distribution of *T. vaginalis* in Symptomatic and Asymptomatic Antenatal Subject in Parts of Oyo State**

Symptoms	No. of Subjects	No of <i>T. vaginalis</i> infection/(%)
Dysuria	17	10(26.3)
Vaginal discharge	54	23(60.5)
No symptom	79	05(13.1)
<b>Total</b>	<b>150</b>	<b>38(25.3)</b>

It is evident in this study where 150 HVS samples of both symptomatic and asymptomatic pregnant women were collected and 38 (25.3%) were found to have *Trichomonas vaginalis*. This finding agrees with other reports which indicate frequency of 20.0% among pregnant women in Abeokuta, Nigeria (Ojurongbe *et al.* 2010); it is however higher than what is obtained in the study carried out among women in Iraq with frequency of 5.3% (Al-saeed, 2011). There were also cases of multiple infection in this study with 12 (8%) of

the pregnant women having *Candida* spp. and 9 (6%) having pus cells which probably signifies secondary bacterial infection in conjunction with *Trichomonas vaginalis*. It is thus now established that a great majority of women with trichomoniasis have some other infections.

The young women (20-30) years were believed to be predominantly infected (Al-saeed, 2011). And this study supports this data with the percentages of 4.0%, 8.6% and 10.6% found among pregnant women within age range 23-25 years, 26-28

years and 29-31 years, respectively. *Trichomonas vaginalis* in pregnancy is still a case for concern and a pointer to the imminent danger to our pregnant women as the frequency is found to be higher within the third trimester period 27 (32.1 %) , considering the morbidity and the threatening mortality disease state being associated with the protozoan infection. Also from this study , it can be concluded that pregnant women with *T. vaginalis* infection had vaginal discharge as the highest symptoms (60.5%) followed by Dysuria (26.3%) and can concluded that pregnant women with Trichomoniasis can be asymptomatic as shown in the study where we had 5 (13.1%) with *T. vaginalis* infection from the 79 (52%) asymptomatic pregnant women.

Diagnosis of trichomoniasis has relied mostly on wet mount demonstration and staining of the parasite in the laboratory, various success ranging from 20-80% of this method is documented, Fouts and Kraus (1980). However a combination of cultural method with microscopic wet mount demonstration is now the acceptable procedure for effective diagnosis of this protozoa infection Arora and Arora (2010). Based on this study result, it was concluded that for the laboratory diagnosis of *T. vaginalis*, papanicolaou staining technique does not have any superiority over direct microscopy. Although direct microscopy is easily performed and economical method, it has low sensitivity, so all of the suspected samples which are found negative by this method, should be cultivated for a definite diagnosis.

Nevertheless, cultural method further isolate 42 out of the 112 samples negative for both wet prep and staining method thus cultural method still remains the "GOLD STANDARD" for the diagnosis of *Trichomonas vaginalis* among three methods used.

## CONCLUSION AND RECOMMENDATION

Examination for *T vaginalis* is most often done as part of the investigation of vaginal discharge. It is recommended that the examination should be included as part of any routine examination for vaginitis. PH evaluation with pH paper can help to rapidly differentiate *T. vaginalis* from yeast at the bedside. The vaginal pH, which is normally pH 4.5, is not altered by yeast infection but is elevated in bacterial diagnosis and often rises above pH 6 in florid trichomoniasis. Quality concerns include the need to have the microscopic examination done immediately after specimen collection.

As the majority of the assessed populations in this study were in a fertile age range, this findings support the need for improved disease control activities to reduce adverse trichomoniasis, associated reproductive health outcomes such as ectopic pregnancy, low infant birth weight.

The use of condom is recommended for those sexually active women with multiple sex partners to avoid spreading of the infection and treatment should be given to the sex partners of infected women. Also, awareness program to sensitize the populace on the health challenges that are associated with the presence of *Trichomonas vaginalis* should be given a priority.

A routine vaginal swab for pregnant women in the third trimester is also recommended as found in this study, where 29 out of the 38 women positive for *T. vaginalis* were in the third trimester so that adequate treatment will be given before delivery to ensure the safety and good health condition of the baby.

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