

Sudan University of Science and Technology

College of Graduate Studies



**Prevalence Rate of *Trichomonas vaginalis* Infection in
Khartoum State**

معدل إنتشار عدوى المشعرية المهبلية في ولاية الخرطوم

A dissertation submitted in partial fulfillment of the requirements of the
.M.Sc. degree in Medical Laboratory Science (Parasitology and Medical
Entomology)

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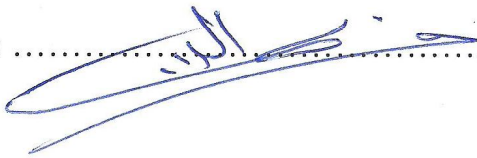
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الآية

بسم الله الرحمن الرحيم

وَقَالَ لَهُمْ نَبِيُّهُمْ إِنَّ آيَةَ مُلْكِهِ أَنْ يَأْتِيَكُمُ
التَّابُوتُ فِيهِ سَكِينَةٌ مِّن رَّبِّكُمْ وَبَقِيَّةٌ مِّمَّا
تَرَكَ آلُ مُوسَىٰ وَآلُ هَارُونَ تَحْمِلُهُ الْمَلَائِكَةُ
إِنَّ فِي ذَلِكَ لَآيَةً لِّكُمْ إِن كُنتُمْ مُّؤْمِنِينَ ﴿٢٤٨﴾

صدق الله العظيم

(سورة البقرة الآية 248)

Dedication

To my parents who supported me with love, encouragement and patience.

To the rest of my family and friends.

To all those who provided me with moral support and also to those who gave me a helping hand in any day of my life.

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Abstract

The study was conducted in Khartoum State during the period from March to September 2016.

The main aim of the study was to determine the prevalence of *T. vaginalis* among females in Khartoum State. The study employed 250 females of different age groups. This was done through the examination of females' urine using wet preparation.

The study showed the prevalence rate of *T.vaginalis* was 6% among the studied females. The high prevalence (14.5%) was reported among the single females, while the lowest prevalence rate (4.9%) was reported among the married females. The highest infection rate (12%) was found in the age group 14-24 years while the lowest infection rate (0%) was found among the age group over 54 years.

The study showed that the high prevalence (8.7%) was reported among the high secondary school students, while the lowest prevalence (0%) was reported among university students.

مستخلص الدراسة

أجريت هذه الدراسة في ولاية الخرطوم في الفترة من شهر مارس حتى شهر سبتمبر 2016. الهدف من هذه الدراسة هو تحديد إنتشار المشعرية المهبلية وسط الإناث في ولاية الخرطوم. أجريت هذه الدراسة على عدد 250 من الإناث من فئات عمرية مختلفة. وذلك عن طريق فحص البول للإناث وذلك بإستعمال تقنية التحضير الرطب.

أظهرت الدراسة أن معدل إنتشار المشعرية المهبلية هو 6 % وسط الإناث من موضوع الدراسة. أعلى إنتشار (14.5 %) سجل وسط الإناث غير المتزوجات في حين أن أقل معدل إنتشار (4.9%) سجل وسط المتزوجات.

أعلى معدل إصابة (12 %) وجد وسط الفئة العمرية 14-24 سنة في حين أن أقل معدل إصابة (0%) وجد وسط الفئة العمرية فوق ال54 سنة. أظهرت الدراسة أن أعلى إنتشار (8.7 %) سجل وسط طالبات المرحلة الثانوية في حين أن أقل إنتشار (0%) سجل وسط طالبات المرحلة الجامعية.

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Chapter one

Introduction and literature Review

Chapter one

Introduction and literature review

1.1 Introduction

Trichomonads are widely distributed and infect nearly every mammal associated with humans. There are three human species, *T.vaginalis* of the vagina, *T.hominis* of the intestine and *T.tenax* of the mouth. They may be differentiated by site of origin, morphology, cultural characteristics and failure to cross infect (Neva and Brown, 1994). In women, the infection can be found in the vagina and the urethra [tube where urine comes out]. In men, it can be found in the urethra. The infection is easily passed from one person to another through sexual contact. Anyone who is sexually active can get it and pass it on. The infection can be spread through unprotected vaginal sex and possibly through sharing sex toys if they are not washed or covered with a new condom each time they are used. We don't know if the infection can be spread between women by rubbing vulvas [female genitals] together or by transferring discharge from one vagina to another on the fingers (Quon *et al*, 1992)

In Iran, Chalechale and Karimi (2010) reported that among the 33, 690 women, 300 were infected with *T.vaginalis* [a prevalence rate of 0.9%]. The 30-39 year old age group had a significantly higher prevalence of infection [33.0%; $p < 0.050$] than the 20-29 year old [29.0%] and 40-49 year old of age groups [21.0%; $p > 0.05$]. The lowest rates of infection were observed in those <20 years of age (5.6%) and >50 years of age (11.4%; $p > 0.05$).

A study of *Trichomonas vaginalis* infection among women of Onitsha community in onitsha north local government area anambra state, Nigeria was carried out between January to June, 2013 (Iweuze *et.al*, 2014), A total

of 200 women less than 20 years and above, who attended hospital/health centers during the study were involved in the study. Two hundred samples of vaginal swabs were carefully and aseptically collected from the vaginal area using well-labeled, sterile, non-abrasive high vaginal swab sticks. All samples were centrifuged and examined microscopically within 2 hours of collection. The presence of *Trichomonas vaginalis* was detected by its characteristic jerking movement in a wet preparation. An overall prevalence of 17.5% *T.vaginalis* infection was obtained. The highest prevalence of *T.vaginalis* infection (27.5%) was obtained from general hospital Onitsha. However, none (0%) was obtained in safety medical laboratory. The prevalence (47.2%) was obtained from age group 30-39 years age old. Non-pregnant women were more infected (17.8%) than pregnant ones (16.7%) but the difference was not statistically significant ($p>0.05$). Adequate personal hygiene, avoidance of promiscuity, faithfulness to one sexual partner, and health education were advocated for improvement of their health. In India, Deivam *et.al*, (2014) examined a total of 434 married women within the age range of 26-55 years. The vaginal swabs were taken and smears were prepared for all the subjects for the wet mount examinations to detect trichomoniasis and candidiasis, and Gram stain for clue cells. Among the subjects, 212 are confirmed for bacterial vaginitis, thereby total *Trichomonas vaginalis* (tv) infection found among 35 cases. The tv vaginosis (tvv) alone, tvv with bacterial vaginosis, tvv with candidiasis and tvv with bacterial vaginosis and vaginal candidiasis was found among 10,20,4, and 1 cases, respectively. In the investigation, the marital and socioeconomic status was also well determined.

In Sudan, Dahab *et.al*, (2012) made a survey on the prevalence of tv in 2 different hospitals representing rural and urban areas (Ibrahim Malik and

Umbada hospitals respectively. Urine samples were collected weekly every month and examined using wet mount preparation method. 297 women were found infected with *Trichomonas vaginalis* of a total of 2473 examined making an overall prevalence rate of 12%. Prevalence of infection was slightly higher among women in Umbada hospital than those in Ibrahim Malik hospital but the difference is not statistically significant. However, difference in infection is statistically significant regarding areas of residence ($p < 0.05$). The highest (15.6%) and the lowest (4.8%) prevalence rate were recorded in Alsalam locality (Umbada hospital) and Khartoum locality (Ibrahim Malik hospital) respectively suggesting a difference in awareness between rural and urban areas. Significant differences related to age were recorded. The highest prevalence rates were among women in the age groups 15 to 19 and 20 to 24 years.

1.2 Classification of *Trichomonas vaginalis*:

According to Schmidt and Roberts (1989), *Trichomonas vaginalis* is classified as follows:

- Phylum: sarcomastigophora
- sub phylum: mastigophora
- Class: zoomastigophora
- Order: trichomonadida
- Family: trichomoadidae
- Genus: *Trichomonas*
- Species: *Trichomonas vaginalis*

1.3 Historical Background:

Donne was the first who discovered and named *T.vaginalis* in 1836 after finding the organism in genital secretion of both women and men.

T.vaginalis pathogenicity was initially thought to be non pathogenic because a majority of infected patients were asymptomatic.

The development of culture medium in the 1940 allowed more detailed study of the organism and its pathogenicity (Thomason and Gelbart, 1989). In 1942, Hesseltine inoculated 70 pregnant volunteers with unadulterated *T.vaginalis* culture (10,000 to 120,000 Trichomonads per inoculum). Fifty-three patients were tested for trichomoniasis using vaginal smear and 17 received clinical observation. Seven of the 53 patients (13%) developed trichomoniasis shortly after inoculation. Two additional patients who tested negative for trichomoniasis prior to giving birth tested positive after giving birth. In 1953, Lanceley and McEntegart inoculated five male volunteers with 2ml of *T.vaginalis* culture (2 million protozoa per ml) and another five volunteers inoculated with culture developed urethritis from which *T.vaginalis* organisms were removed.

1.4 Morphology:

T.vaginalis is colorless pyriform flagellate. It is smaller when fixed than fresh preparation. There is no cyst in the life cycle, so transmission is via the trophozoite stage (Neva and Brown, 1994). Although there were reported cases, that under unfavorable growth conditions, *T.vaginalis* can round up and internalize the flagella, in which it is believed that these forms are pseudo cysts, because they have not been reported to give rise to normal motile forms. This urinogenital pathogen varies in size and shape, the average length and width being 10 and 7 μm respectively. *T.vaginalis* has four anterior flagella and a recurrent flagellum attached to the body by an undulating membrane. The fifth recurrent flagellum not trailing beyond the undulating membrane reaches up to the middle of the body. The flagella and the undulating membrane give the parasite a characteristic quivering

motility. A round nucleus is located at its anterior portion (Ichhpujani and Bhatia, 1998). A slender hyaline rod-like structure called axostyle, commences at the nucleus and bisects the protozoan longitudinally. It protrudes through the posterior end of the parasite terminating in a sharp point. This structure is thought to anchor the parasite to the vaginal epithelial cells. The parabolas body is single and v-shape and has a filament associated with it. The order trichomoandida to which *T.vaginalis* belongs lack mitochondrion. Granules can be seen associated with the costa and axostyle. There are two sets of granules: paracoastal and paraxostylar. The latter set is arranged along the axostyle in three parallel rows which is a distinguishing feature of *T.vaginalis* (Petrin *et.al*, 1998) (figure1).

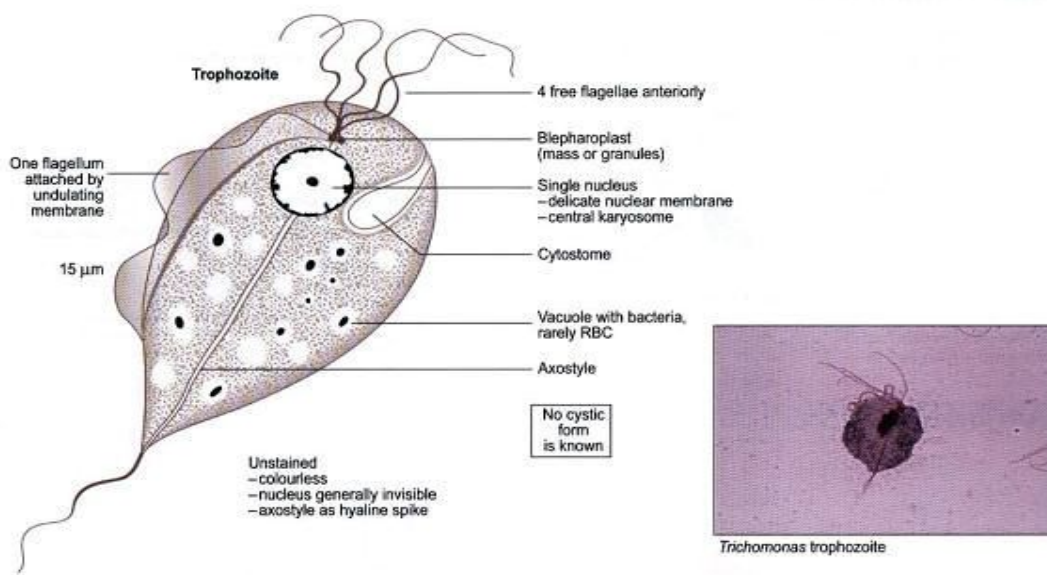


Figure 1: Morphology of *Trichomonas vaginalis* (WWW.CDC. com)

1.5 Life cycles:

T.vaginalis reproduces asexually through longitudinal fission, with mitotic division of the nucleus. The trophozoite is one of the most resistant of the parasitic protozoa (Neva and Brown, 1994), also the Trophozoite (adults) then live in the urinary or reproductive tract, until they are passed onto their next human host via unprotected sexual contact, where the whole process starts over again. Unlike many other protists, *T.vaginalis* does not have a cyst stage as part of reproduction (Stary *et.al*, 2000) (figure 2).

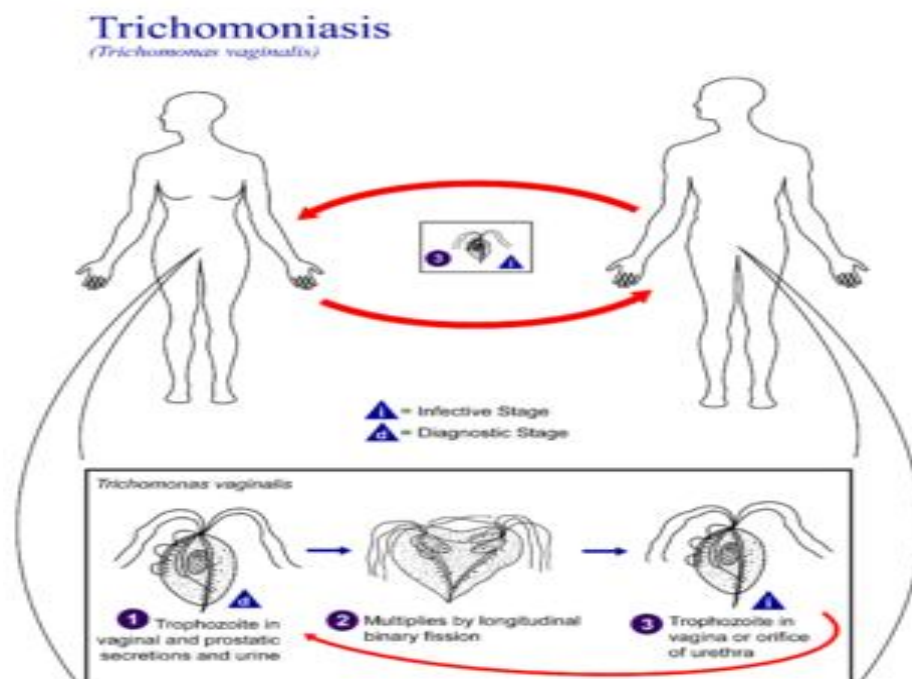


Figure 2: life cycle of *Trichomonas vaginalis* (WWW.CDC. com).

1.6 Mode of transmission:

The parasite that causes trichomoniasis can survive for a little while in a moist environment. In theory, a person could get the infection by having direct contact with surfaces that are contaminated with the parasite, like a Wet towel, a bathing suit, or a toilet seat. But it is unusual to get *T.vaginalis*

without some sort of sexual contact (Petrin *et.al*, 1998). However, since young virgins are found infected, it appears that direct contact with infected females, contaminated toilet articles, and toilet seats transmit the infection. Infections acquired while passing through the birth canal probably account for some infection in babies (Neva and Brown, 1994).

1.7 Global epidemiology and distribution of *Trichomonas vaginalis*:

T.vaginalis is the most common curable sexually transmitted infection (STD). Despite a number of serious health consequences, the infection is common among sexually active males and females especially during the child bearing age (Abdulazeez and Alo, 2007). The incidence of infection is about 10% to 15% in women (Neva and Brown, 1994). *T. vaginalis* is known to be responsible for an estimated 180 million new infections per year, making it the most prevalent non viral sexually transmitted pathogen worldwide (Petrin *et.al*, 1998). Only about one seventh of the women with the Trichomonads infection complain of symptoms, although the vaginal secretion is invariably altered. The detected infection rate in husbands of infected wives is surprisingly low, but this may be due to technical difficulties in securing adequate specimens for diagnosis (Neva and Brown, 1994). It is estimated that in the United States, 7.4 million new cases of trichomoniasis appear annually. In Kenya, a study reported a prevalence of 34.4% of urino-genital trichomoniasis in the country (Mirza *et.al*, 1983). In Nigeria, a research conducted at Lagos university teaching hospital showed trichomoniasis prevalence rate of 8.2% among the women sampled. At university hospital, Ibadan, prevalence rate of 20.5% out of 151 women attending infertility clinic was recorded. In Jos city, Adabayo (1988) recorded 12.3% prevalence rate of *T.vaginalis* from pregnant women

attending the gynecology clinic, while (24.1% was recorded in a similar work done among pregnant women in the same city by Ogbonna, (1998). Infection of the lower reproductive tract is common in Indian women of reproductive age. Hospital based cytological screening was under taken on 63.265 women. The smears were examined for the presence of specific infection, such as Candida, herpes simplex virus, human papillomavirus, *T.vaginalis* infection inflammatory cervical smears among the various infections detected, the rate of *T.vaginalis* infection was the highest (5.1%). On further analysis, the rate of *T.vaginalis* infection showed an increasing trend up to the age of 49 years, an inverse association was observed with the educational status of the women. The prevalence was high in women with clinical signs (vaginitis, 6.9%) and low in those with a prolapsed uterus (1.2%) as compared to a normal cervix (Sardana *et.al.* 1994). The world health organization estimates that in the Australian region, the estimate is of approximately 610.000 cases per annum. These estimates may be low as they are based on an assumed sensitivity of wet mount microscopy of 60-80% (Drummond, 2009).

1.8 *Trichomonas vaginalis* in Sudan:

In Sudan, little work was published on the epidemiology .The first paper to outline the concept of trichomoniasis was published by Omer (1987). He investigated 631 women presented with vaginal discharge and attending sexual transmitted disease clinic. 65 women (10.3%) were found to harbor *T.vaginalis*. 64 of the positive patient, 98.5% were in the child bearing period 14-45 years. The highest frequency of trichomoniasis (12.5%) was detected among women after menopause 47 years and over. Seven specimens (10.8%) identified by microscopy alone, 19 specimens (29.2%) by culture alone, 39 specimens (60%) by both microscopy and culture

methods. All patients examined were married and many of them have children. Taha *et.al*, (1979) in study in Khartoum using direct examination of wet preparation among males and females, *T.vaginalis* was not identified in males. From the women examined, 23 (32.4%) were found infected and most cases were in the age group 20-29 years (10%), however, the number of women investigated was comparatively low. Omer *et.al*, (1980) studied the prevalence of trichomoniasis among Sudanese women attending the outpatient clinic of health center in Khartoum, using the wet mount technique. 114 women were investigated and *T.vaginalis* was identified in 23 (20.2%) of them. Seven patients were found to be infected with both *Candida albicans* and *T.vaginalis*. Only one patient was simultaneously infected with *T.vaginalis* and *Nisseria gonorrhoea*. The frequency of trichomoniasis was highest among women in the age group 20-29 years. They also studied the effect of contraceptive on the frequency of trichomoniasis in Sudanese women. They observed that most infections were detected among women with tube ligation (25%). Trichomoniasis was also found to be more common among non pregnant women (20%) than pregnant women (4%). Keleida *et.al*, (1979) investigated 136 women at Khartoum teaching hospital with vaginal discharged and observed that 13 patients (9.6%) were infected. Mixed infection of *Candida* and *T.vaginalis* were found in 3.7% of the patient. Hag Ali *et.al*, (1979) studied the incidence of trichomoniasis in female patient attending an outpatient clinic in Khartoum. They investigated 51 patients. *T.vaginalis* was identified in 17.6% of them. The occurrence of trichomoniasis was observed among pregnant women (40%) and 25% infections were found among widowed or divorced women. Omer *et.al*, (1984) Investigated 978 individuals at high risk of sexually transmitted diseases for *T.vaginalis* by both microscopy and

culture of urine sample. Group covered were soldier, seamen, boarding-house student, teenagers, female prisoners and individuals living under poor socioeconomic conditions. Both males and females of different age groups were investigated. The overall frequency of *T.vaginalis* infection was 3.1%. The rate of the infection was highest (18.9%) among female prisoners. The concept to infection was discussed according to the incidence of age and marital status.

At sexually transmitted disease clinic at Khartoum, 613 Sudanese women presented with vaginal discharge were investigated by Omer *et.al*, (1985). Specimens were examined by microscopy and culture. *T.vaginalis* infection was found in 123 patients (20.1%), predominantly in the age group 16-19 years (27.1%) and 46-65 years (27%). Frequency of *Trichomonas vaginalis* was highest (35.9%) among divorced women. Of the pregnant women investigated, 16.3% were found to harbor the parasite.

1.9 Pathology and pathogenesis:

T.vaginalis is the causative agent of a persistent vaginitis. The flagellate is responsible for a low grade inflammation, hence, it is frequently found in the urine (Neva and Brown, 1994), although trichomoniasis is the most common non viral sexually transmitted disease, the pathogenicity of *T.vaginalis* is not thoroughly understood. Trichomonads participate in a host parasite relationship, causing them to adhere to epithelial cells. The ability of Trichomonads to adhere is affected by time, temperature, and ph level. *T.vaginalis* grows best in an anaerobic environment with >6 (Diamond, 1986). Binding of *T.vaginalis* to vaginal epithelial cells for colonization and infection is dependent upon specific parasite surface proteins. Parasites treated with Tinidazole, Metronidazole, or other Nitroimidazoles lose their ability to adhere, making them ineffective disease agents. In Trichomonads

vaginitis, the vaginal walls are injected and tender, in some instances showing hyperemia and petechial hemorrhages and in advanced cases granular areas (Neva and Brown, 1994). Trichomoniasis has been seen to increase in severity during or slightly after menstruation (Graves and Gardner, 1993). The relationship between *T.vaginalis* growth and protective lactobacilli is a complex one. It is currently unknown whether *T.vaginalis* infection alters the vaginal environment by creating an anaerobic situation, or if anaerobes in the vaginal glycogen levels are elevated in women of reproductive age. Glycogen is broken down into glucose, a nutrient *T.vaginalis* requires for growth (Petrin *et.al*, 1998). Several studies have suggested that pregnant women infected with *T.vaginalis* may be at increased risk of an adverse outcome. Pre mature rupture of membranes, pre mature labor, low birth weight and post-abortion or post-hysterectomy infection are complications of *Trichomonas vaginalis* that have been reported (Coth *et.al*, 1997).

1.10 Clinical pictures:

T.vaginalis infection in women is frequently symptomatic (Nada *et.al*, 2006: Sckwebke and Burgess, 2004). Symptoms can include:-

T.vaginalis with a purulent discharges ranging in color from gray to green to yellow, with a watery to milky consistency and foul odour are the prominent symptoms, and can be accompanied by vulvar and cervical lesions and abdominal pain, itching and tenderness in or around the vagina.

- Pain during sex.
- Bleeding after sex.
- Pain during urination, dysuria and dyspareunia.
- Soreness or itching of the labia and inner thighs.

- Swollen labia.

Males who have trichomoniasis often don't show any symptoms .but if they do, symptoms can include (Wolner-hanssen *et.al*, 1989: Petrin *et.al*, 2006).

- Mild urethral itching.
- Mild burning after urination or ejaculation.
- Painfull or difficult urination.
- Inflammation of the prostate gland.
- Pain and inflammation of the scrotum.
- Intermittent frothy or pus-like discharge from the urethra.
- The incubation period of the disease is 5 to 28 days.

1.11 Diagnoses of *Trichomonas vaginalis*:

Clinical diagnosis is based on symptoms (Neva and Brown, 1994). To diagnose trichomoniasis in a woman. First we have to look at her vagina and vaginal discharges, normal discharge is usually clear, but in trichomoniasis it may appear yellow or greenish in color. The discharge may be tested for abnormal or foul odor using a potassium hydroxide (koh)"whiff test" also for acidity. If trichomoniasis is suspected, further tests will be conducted. Diagnostic methods range from simple visual detection under a microscope to polymerase chain reaction (DNA analysis). Each method has its advantages, but no single method is ideal (Perl, 1972).

1.11.1 Microscopy:

1.11.1.1 Wet preparations

The diagnosis of trichomoniasis is traditionally depending on the microscopic observation of motile protozoa from sedimented urine, vaginal or cervical samples, and urethral or prostatic secretion (Paniker, 2002). This

technique was first described in 1836 by (Donne, 1836). Specimen collected on cotton swabs through a vaginal speculum and left for some time in tube containing 5 percent glucose saline show better shape and motility (Krieger *et al.*, 1988). The slide is then visually examined for trichomonads. Wet mount is the most common method used to diagnose trichomoniasis. *T.vaginalis* can be differentiated on the basis of its characteristic jerky movements. On occasion, flagellar movement can also be noted. The sensitivity of the test varies from 38% to 82% and is dependent on the inoculum size because fewer than 10⁴ organisms/ml will not be seen. As well, the need for the specimen to remain moist and the experience of the observer are important variables. The size of the Trichomonads is approximately the same as that of a lymphocyte (10 µm to 20 µm) or a small neutrophil: when not motile. Trichomonads can be difficult to differentiate from the nucleus of a vaginal epithelial cell. Motility is very dependent on the temperature of the specimen. At room temperature in phosphate-buffered saline, the organism will remain alive for more than 6 hours: however, the motility of the organism becomes significantly attenuated. This wet mount examination is clearly the most cost-effective diagnosis test, but the lack of sensitivity contributes to the under diagnosis of the disease, because viable organism is required, delay in transport and evaporation of moisture from the specimen reduces motility and consequently, diagnostic sensitivity (Wiese *et al.*, 2000).

1.11.1.2 Staining methods:

Staining methods include Giemsa, Papanicolaou and Schiff stains

1.11.1.2.1 Giemsa stain test:

A smear of the secretion is also made on a slide, air dried and fixed in absolute methanol for 1 minute. Diluted Gimesa stain is poured on the smear

and allowed to stain for 10 minutes after which, it is washed, air dried and examined under the microscope (Rein, 1995).

An example of *T.vaginalis* obtained from culture stained with Giemsa is shown in figure 3.

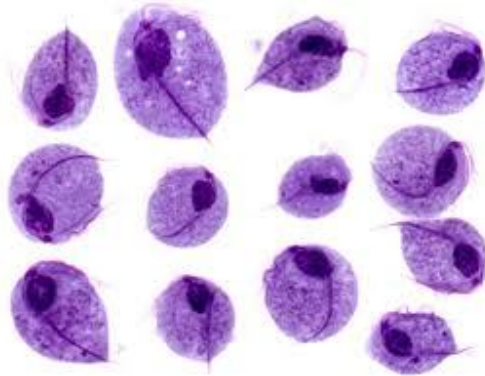


Figure 3: *Trichomonas vaginalis* stained with Giemsa (WWW.CDC. com).

1.11.1.2 Papanicolaou test (Pap smear):

The Papanicolaou test is a microscopic examination of a stained specimen. It is mainly used as a diagnostic test for the screening of various cervical abnormalities and genital infections. The difficulties with staining techniques include the elimination of motility due to the fixative and fact that *T.vaginalis* does not always have its characteristic pear shape form. Thus, staining in most cases is best used in conjunction with direct wet mount motility observation (Purja and Shurbaj, 2001).

1.11.1.3 Culture:

The trophozoite in culture loses its vitality below PH4.9 hence; it cannot live in the normally acid vaginal secretion (PH 3.8 to 4.4) of healthy adult. In culture, it has been observed to ingest bacteria, starch, and even erythrocytes, dextrose, maltose, and other carbohydrates stimulate growth (Neva and Brown, 1994).

1.11.1.3.1 Broth culture:

Broth culture technique has been the gold standard for *T.vaginalis* for the past 40 years. The inoculum size required is only in the ranges of 10² organisms/ml and growth of the organism is easy to interpret. The standard broth is diamond's tyi medium in glass tubes. For this method, a specimen is placed in a culture medium for 2-7 days before it is examined.

If trichomonads are present in the original specimen, they will multiply while in culture and be easier to detect. Culture is considered the gold standard for the diagnosis of trichomoniasis. It is both highly sensitive and highly specific. Its disadvantages include cost and prolonged time before diagnosis (Garber *et.al*, 1987).

1.11.1.3.2 Culture/in pouch™ TV (biomed diagnostics):

Biomed diagnostics' in pouch tv system is a two-c chambered bag that allows one to perform a wet mount using the upper chamber and a culture uses the lower chamber. The wet mount's fast results allow some patients to begin treatment without having to wait, untreated, for the results of the more sensitive culture (Sobel, 1997).

1.11.1.4 Affirm™vpiii microbial identification test (bd):

BD's Affirm VPIII test is a moderately complex DNA probe for vaginitis. Identification to *Candida* (yeast) Species, *Gardnerella vaginalis*, and *Trichomonas vaginalis* is possible from a single vaginal sample. The test's sensitivity for detecting *T.vaginalis* is high and it can provide results in as little as 45 minutes (Fouts and Kraus, 1980).

1.11.1.5 OSOM®Trichomonas rapid test (genzyme diagnostics):

Genzyme diagnostic's OSOM Trichomonas rapid test is a new point of care, antigen-detecting diagnostic test for Trichomoniasis. By inserting a vaginal swab into a test tube with 0.5ml of a special buffer, mixing the solution vigorously by hand, removing the swab, and then inserting a test strip.

Physicians and staff can read results in 10 minutes. The OSOM test is more sensitive than the wet mount (Pugh. 1982).

1.11.1.6 Polymerase chain reaction (PCR):

In polymerase chain reaction (PCR), sample is treated with enzymes that amplify specific regions of *T.vaginalis*' DNA .After amplification; the numbers of DNA fragments are quantified. PCR has proven to be the most accurate diagnostic method in recent studies. PCR is currently only used in research, not clinical settings (Madico *et al.*, 1998).

1.11.1.7 Potassium hydroxide (koh) “whiff test:

The ‘whiff’ test is a rudimentary technique that may be used as part of a clinical diagnosis .The test is conducted by mixing a swab of vaginal fluid with a10% potassium hydroxide solution, then smelling it. A strong amine (fishy) smell could be an indication of trichomoniasis or bacterial vaginalis (Blake *et al.*, 1999).

1.11.1.8 Vaginal ph test:

Trichomonads grow best in less acidic environments, and elevated vaginal ph may be an indication of Trichomoniasis. A health care provider performs the test by touching ph paper to the vaginal wall or to a vaginal swab specimen, then comparing it to color scale to determine the ph (Diamond, 1986).

Rationale

Trichomonas vaginalis, is a protozoan parasite which can be transmitted sexually (sexually transmitted infection (STI)).The infection is common among sexually active male and females especially during the child bearing age. The detected infection rate in husbands of infected wives is surprisingly low, but this may be due to technical difficulties in securing adequate specimens for diagnosis.

The study aims to determine the prevalence of *Trichomonas vaginalis* among women urine sample using wet preparation method.

Objectives

General objective:

- To investigate the prevalence of *T.vaginalis* in the urine of females in Khartoum state.

Specific objectives:

- To study the prevalence of *Trichomonas vaginalis* among females in study area according to age.
- To study prevalence of *Trichomonas vaginalis* among females in study area according to education level.
- To study prevalence of *Trichomonas vaginalis* among females in study area according to marital status.

Chapter Two

Materials and Methods

Chapter two

Materials and methods

2.1 Study design:

The study was across sectional study.

2.2 Study area:

The study was conducted in study areas.

2.3 Study population:

The study areas are frequented by education level and marital status. The samples were taken from 250 females representing the above mentioned education level and marital status. The samples were categorized according to the following age groups:

Group a: age between 14-24 years, Group b: age between 25-34 years,

Group c: age between 35-44 years, Group d: age between 45-54 years,

Group e: age above 54 years.

2.4 Data collection:

A questionnaire was designed to include data on age, education level and marital status (appendix).

2.5 Sampling:

Urine samples group were randomly collected in containers with tight lid covers to prevent contamination.

2.6 Techniques used:

2.6.1 Wet smear:

The collected urine samples were centrifuged {at 12.000 rpm for 3 min} and supernatant fluid was decanted and a drop of sediment was placed on a clean slide and covered with a clean cover glass and was examined microscopically under the low power (10x) and high power (40x).

2.6.2 Giemsa stained smear:

Drop of sedimented urine was placed on a clean slide. The preparation was allowed to air dry.

2.6.2.1 Preparation of Giemsa stain:

1ml was taken from stock Giemsa stain, diluted by 9ml buffered distilled water (10% Giemsa stain).

2.6.2.2 Staining procedure:

The smear was fixed with absolute methanol for one minute, then air-dried and stained with Giemsa stain. The stained smear was examined microscopically under 100X.

2.7 Data analysis:

Data were analyzed using Statistical Package for the Social Sciences (SPSS) program. Then data were presented in tables.

2.8 Ethical Consideration:

Ethical consideration was taken from the College of Medical Laboratory Science- Sudan University of Science and Technology. A verbal consent was taken from each patient.

Chapter Three

Results

Chapter three

Results

The study was conducted in Khartoum State, on the prevalence of *T.vaginalis* among females. Revealed that out of the 250 females examined, 15 were found positive for *T.vaginalis* infection. This constituted an overall prevalence rate of 6% (table 1).

Among the 15 positive cases to *T.vaginalis*, the highest rate of infection (12%) was found to be among the 14-24 age group, while the lowest rate (0%) was found to be among the above 54 age group (table 2). This difference in rates was found to be statistically insignificant (p.value = 0.178).

The infection of *T.vaginalis* was higher in singles than in married females (14.5% and 4.9% respectively) (table 3). The difference was found to be statistically significant (p.value = 0.041).

The highest prevalence rate (8.7%) was found among the high secondary school students, while the lowest prevalence rate (0%) was found among both university graduates and Post University graduates (table 4). These differences in rates were found to be statistically insignificant (p.value=0.331).

Table 1: The overall prevalence rate of *T.vaginalis* among females:

Number examined	Number positive	Prevalence
250	15	6%

Table 2: Prevalence of *T.vaginalis* among females according to age groups:

Age groups	Number examined	Number positive	Prevalence
14-24	25	3	12%
25-34	65	6	9.2%
35-44	65	4	6.2%
45-54	42	2	4.8%
Over 54	53	0	0%
Total	250	15	6%

p.value=0.178

Table 3: Prevalence of *T.vaginalis* among females according to social status:

Social status	Number examined	Number positive	Prevalence
Married	223	11	4.9%
Single	27	4	14.5%

p.value=0.041

Table 4: The prevalence of *T.vaginalis* among females according to education level:

Education level	Number examined	Number positive	Prevalence
Illiterate	61	2	3.3%
Primary school	30	2	6.7%
High secondary school	126	11	8.7%
University graduate	32	0	0%
Post university graduate	1	0	0%

p.value=0.331

Chapter Four

Discussion

Chapter four

Discussion

A study which was conducted to determine the prevalence of *T.vaginalis* in Khartoum state among females revealed that 15 out of the 250 females examined were infected with *T.vaginalis* constituting an overall prevalence rate of 6%. This rate, in our opinion is exceptionally high for a sexually transmitted disease. This rate was found to be almost similar the rates reported by Omer (1987) and Kleida *et al.* (1979) (10.3% and 9.6% respectively). However, our rate was lesser than the rate reported by Mirza *et al.*(1983) (34.4) and was also lesser than the rate reported by Adabayo (1988) and Ogbonna (1998) in Nigeria (12.3%and 24.1% respectively).

In Sudan, other workers (Omer *et al.*1980; Taha *et al.*1979) reported higher prevalence rates (20.2% and 32.4% respectively).

From this investigation, the high prevalence rate of *T.vaginalis* was reported among the 25-34 and 35-44 age groups. This finding was in line with the finding of Omer *et al.* (1985) who reported higher rates among the 24- 33 years age group. The result was also in agreement with Abdelgadir (2005) who found the highest prevalence rate among the 14-34 age groups. High prevalence rate among the above mentioned age groups might probably be attributed to the high sexual activities among them. From the study, it was shown that the prevalence rate among the married women reached up to 4.9% while among the singles, it was 14.5%. For married women, the infection in our opinion might has occurred from their infected husbands, while the infection among single females has only one justification that they were practicing illegitimate sex .The highest prevalence rate (8.7%) was reported among the high secondary school, graduates while the lowest

prevalence rate (0%) was among both university graduates and Post University graduates. Surprisingly and despite the reported figures, the difference was found to be statistically in significant.

Chapter Five

Conclusion and Recommendations

Chapter five

Conclusion and Recommendations

5.1 Conclusion:

The study concluded that *T.vaginalis* is by now prevalent in different areas of Khartoum state. And illegitimate sex practices especially by males play very important role in the increasing occurrence of infection in the area of study.

5.2 Recommendations:

- Establishment of specialized clinic for diagnosis and treatment of trichomoniasis.
- Intensive health education to the public in the area to enlighten them to avoid illegal sexual intercourse.
- Advise females with vaginal discharge and irritation to visit the specialist to seek proper treatment.
- A proper survey on males in the area is highly requested to exclude carriers and put them under treatment.

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Appendix

Appendix

Questionnaire

Patient number ()

1 .Name.....

2. Age.....

3. Education level.....

4. Marital status.....