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Comparative Analysis of Wet Mount and Diamond Media for Detecting *Trichomonas vaginalis* Among Women in Duhok City/ Iraq

Zhiman N. Mosa **Senaa A. Al-Jarjari**

Department of Biology/College of Science/ University of Mosul

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Corresponding Author:
Zhiman N. Mosa
Jimannuri96@gmail.com
Senaa Abdullah Al-Jarjari
Sensbio@uomosul.edu.iq

ABSTRACT

Trichomoniasis infection can be described as the world's universal sexually transmitted disease (STD), caused by T. vaginalis. This parasite transmits mainly through sexual practices; however, it can also be transmitted through fomites. Women produce vaginitis, and the clinical manifestations include abundant greenish-yellow foamy, foul-smelling discharge, valvar swelling, itching, and punctate erythematous lesions of the cervix (also known as 'strawberry cervix'). There are many possible cases whereby women may not have symptoms but are infected. The current study is aimed to compare the diagnostic effectiveness of wet mount preparation and diamond media for detecting T. vaginalis infection in different sample types in the endocervical discharge, endocervical swab, and urine of 300 female patient's female (married and unmarried), whose ages ranged between 15 and 62 years old, of the total 300 patients referred to Dohuk Maternity Hospital, Shariya Camp, inside or outside Dohuk city. The diagnosis of parasitic infection was confirmed on the clinical symptoms of patients by confirming the infection microscopically in the laboratory by two methods: direct wet mount preparation and culture by diamond media. The results of this study showed. In Dohuk Maternity Hospital, among 240 samples, 61 (25.4%) were infected, and in Shariya Camp, out of 60 samples, 15 (25%) were infected by T. vaginalis. Among 300 samples, 74 (24.6%) were found to be infected using wet mount preparation and 76 (25.3%) using culture diamond media. The present study on 258 married women found that 66 (25.5%) had trichomoniasis infection and among 42 unmarried women, 10 (23.8%) had the infection. The highest infection rate was observed in the (15-25) age group women (32.8%).

Keywords: *T. vaginalis*, wet mount preparation, trichomoniasis, STD and vaginal inflammation, Diamond Media.

INTRODUCTION

The *Tricumonas vaginalis* is a protozoan human flagellate that was first found in the male preputial sac and the female vaginal discharge, according to Donne (1836) (Nicoletti, 1961). As for the existence of this condition in the male urinary tract, Kunstler was already referring to the parasite in 1883 (Hoffmann *et al*., 1961). Trichomoniasis infection can be described as the world's universal sexually transmitted disease (STD). Caused by *T. vaginalis.* This parasite transmits mainly through sexual practices; however, it can also be transmitted through fomites (Hussein and Shaker, 2023). Trichomoniasis infection in women may produces vaginitis, in which they may induce abundant greenish-yellow foamy foul-smelling discharge, valvar swelling, itching, and punctate erythematous lesions of the cervix due to Burtonian papillae (Amadi and Nwagbo, 2013). *T. vaginalis* is believed to increase the likelihood of Human Immunodeficiency Virus (HIV) transmission from mother to child and as a result of infections and changes to the vaginal mucosa that give HIV a direct opportunity to infect the child during birth or through breast feeding (Garber, 2005; Rada et al., 2022). *T. vaginalis* typically has five flagella and a single nucleus: the first three free anterior flagella, and the fourth one is found at the base of the undulating membrane that runs longitudinally along the parasite, which is the reason why it moves in a rather 'twitchy' manner (Coceres *et al*., 2021). In the unfavorable environment, the trophozoites turn into cyst-like structures (CLS) and become pseudocysts not reacting to chemical or physical effectors. Trophozoites of CLS can change back to the excystation stage in favor if host cell is available (Kusdian *et al*., 2013; Beri *et al*., 2020). The diseases are curative by using the antiprotozoal drug like metronidazole or tinidazole; efficient treatment should be done applied to eliminate the parasite and prevent transmission to the sexual partner (Petrin *et al*., 1998). Current history of invention believes that Hans Lippershey and Zacharias Janssen, in the year 1590, invented compound microscopes in the Netherlands (Sushmasusik and Hayath, 2015; Hajdu, 2002). *T. vaginalis* diagnosis is most commonly carried out using wet mount preparation because it is easy, quick, and cheap, but the issue here is that its sensitivity is much lower than that of Diamond's media (Saleh *et al*., 2014; Asmah *et al*., 2018). The parasite under study thrives optimally in Diamond, lessening chances of its detection in the specimens since the inoculum may contain a few of these microorganisms (Machado *et al*., 2023).

MATERIALS AND METHODS

In the present study (300) woman were examined for trichomoniasis infection. The chosen women either married or single, their ages were ranged between 15 and 62 years old, the epidemiological study included Dohuk Maternity Hospital and Shariya Camp from November (2023) until the end of April.(2024)

Sample collection: Urine, endocervical swab, and endocervical discharge samples were obtained from symptomatic patients; endocervical discharge was properly obtained aseptically by using a vaginal speculum to get the swab from the posterior fornix of the vagina. For endocervical discharge using a narrow 1 ml disposable syringe to collect endocervical discharge, Midstream urine samples were obtained in a sterile container .

Laboratory examinations: Two methods were performed to investigate trichomoniasis infections in the given samples: wet preparation method and Diamond cultivation method as following:

1. Wet amount method: To make direct wet preparation for the endocervical swab and discharche samples , add 1 ml of sterile normal saline to the swab and mix well, then put on a slide, covered with a concave slip, and examined under a light microscope using 10X and 40X objective lenses for motile Trichomonas, and some of the samples were added to Diamond culture medium and incubated at 37 °C for 4 days. A droplet of the samples those were prepared previously was put on dry clean slide. examined microscopically under high power lense (40X) look for motile Trichomonas, (Rahi and Jaleel *et al*., 2022; Adjei *et al*., 2019). As for urine sample exam, Midstream urine samples were

collected, mixed thoroughly again, and centrifuged for five minutes at 1000 rpm in sterile containers. One drop of the sediment was then placed on a clean, microscope slide, The cover slip was then applied, the preparation examined under 40X microscopic lense (Barbara *et al*., 2021). The sample was spread out and covered after being mixed with normal saline to observed under 40x microscopic lense (high power lense) to determine the twisty movement of the flagellated organism, look at their morphology, and count the numbers of numbered *T. vaginalis* trophozoites (Fule *et al*., 2012). Add 1 ml of normal saline (NS), then put on a slide, covered with a concave slip, and examined under a light microscope and added to Diamond culture medium as endocervical swab samples.

2. Detecting trichomoniasis infection using Diamond culture: This medium is commonly described as the 'gold standard' in the detection of trichomoniasis because of its great sensitivity and specificity (Farouk *et al*., 2021). The preparation procedure begins with dissolving the commercial powdered media in distilled water, then adding 1 gram of agar to make the medium semi-solid. To eliminate any possible impurities, the medium is then autoclaved and cooled to room temperature (Ryu *et al*., 2001). The medium is subsequently dispensed into sterile plain tubes. It is crucial to use fresh, properly prepared Diamond's medium for optimal results, the sensitivity of diagnostic tests can vary depending on the quality and freshness of the medium. Urine sediment, endocervical swab, and endocervical discharge were added to Diamond culture medium and incubated at 37 °C for 4 days (Clay *et al.*, 1988; Salh *et al*., 2023). The chosen cases were included Patients who had symptoms of trichomoniasis, including redness, valval swelling, discharge, itching, and abdominal pain. In this investigation, 300 specimens were grown on diamond media and analyzed examined microscopically after four days of incubation to make shore whether contain trichomonad or not by making direct preparation for droplet of culture. under a microscope using a direct wet mount. *T. vaginalis* trophozoites were found in 25.3% (76/300) of the cultured specimens, while 24.6% (74/300) of the wet mount specimens were infected.

RESULTS AND DISCUSSION

The rate of vaginalis infections by geographical location:

300 specimens were subjected to direct wet mounting and cultured by diamond media; *T. vaginalis* trophozoites were observed in 76 (25.3%). In this study, Chi-square (χ^2) test was used to analyze the association between infection rates and different variables. The level of significance was set at $p < 0.05$. Results with p-values greater than 0.05 were considered not statistically significant, while p-values less than 0.05 indicated significant differences between groups. There is no statistically significant difference in infection rates between Shariya Camp and Dohuk Maternity Hospital, according to the p-value of 1.0. As summarized in (Table 1).

Table (1): Distribution of trichomoniasis infections according to the study areas.

Location	No. of Cases	No. of Infected women	Infection %
Shariya Camp	60	15	25.0
Dohuk Maternity Hospital	240	61	25.4
Total	300	76	25.3

The rate of vaginalis infections according to marital state:

Out of the 258 total samples collected and tested, 66 samples were positive for *T. vaginalis* infection. One the other hands, the samples those were collected from married females, which is only 25% of the total samples. In the unmarried female participants, 42 samples were analyzed, and among them, only 10 samples were recorded positivity for *T. vaginalis,* which was 23.8%. The p-value, which is roughly 0.96, shows that the rates of infection in married and single people women did not do not differ statistically significantly. It can be seen from in (Table 2).

Table (2): Distribution of trichomoniasis infections according to marital state.

Marital state	No. of Cases	No. of Infected women	Infection %
Married	258	66	25.5
Unmarried	42	10	23.8
Total	300	76	25.3

Married females normally record slightly higher infection rates than unmarried females, but since the differences are not significantly high at (x_2=0.003, p=0.957), then there might be other factors like increased sexual activity, lack of barrier protection. This study's outcomes are in agreement with the results obtained in a study conducted by (Jarallah, 2013), it was observed that married female exhibited higher infection rate (61.58%) compared to unmarried females (31.81%). This study's outcomes are in disagreement with the results of (Arámbulo *et al*., 1977) who noted that the prevalence was significantly higher among single women compared to married women.

The rate of vaginalis infections according to types of samples:

Out of 64 women with endocervical copies discharge, 25 cases were found to be positivity infected with *T. vaginalis* (39%). Among the 37 endocervical swab samples, 11 (29.7%) of the patients were positively infected with trichomoniasis. There were 15 out of the 60 collected urine samples that turned out to be infected; all these make up 25% of the samples. Data from urine and endocervical swab samples were analyzed from 100 patients in total, of whom only 9 (9%) were exhibit the infection. Among 39 study studied patients, for whom endocervical discharge, endocervical swab, and urine samples were collected, the rate of positivity for infection was 41% in endocervical discharge samples. However, out of these 39 patients, *T. vaginalis found* in endocervical swabs and urine samples were positive in only 3 (7.69%) patients. The p-value is far lower than the usual significance level of 0.05, at about $3.25\times10-5$. This suggests that the infection rates for the various sample groups differ statistically significantly. As shown in (Table 3).

Table 3: Distribution of detected trichomoniasis infections according to sample types:

Sample types	No. Of cases	No. of infected women	Infection %
Endo cervical discharge	64	25	39
Endo cervical swab	37	11	29.7
Urine	60	15	25
Urine and endo cervical swab	100	9	9%
Urine, endo cervical swab and endo cervical discharge	39	16 endo cervical discharge (Among 39 just 10 swabs and 3 urines become positive)	41% for Endo cervical discharge, for endo cervical swab 25.6% and for urine is 7.69%
Total	300	76	25%

Using endocervical discharge sample the highest infection rate was detected, and is thus the significant predictor when it comes to *T. vaginalis* in this dataset, more so in general samples as well as the multiple sample type. Some of the reasons that make endocervical discharge to be more sensitive in identifying *T. vaginalis* include higher parasite concentration, direct presence, easier detection, and optimal conditions (Testardini *et al*., 2016).

The rate of vaginalis infections according to age:

The prevalence of *T. vaginalis* infection was observed to vary across different age groups: 32.8% in group aged 15-25 years, 22.7% in adolescents aged 26-35 years, 23.4% in middle-aged adults aged 36-45 years, and 26.3% in older adults aged 46 and above years, with an overall prevalence of 25.3%. The age group differences were not statistically significant ($\chi^2 = 2.52$, $p = 0.54$) in spite of these variances. As shown in (Table 4).

Table (4): Distribution of *T. vaginalis* infections according to age groups:

Ages	No. of Cases	No. of Infected women	infection%
15-25 years	64	21	32.8
26-35 years	136	31	22.7
36-45 years	81	19	23.4
46--Above years	19	5	26.3
Total	300	76	25.3

15 -20 age group had the highest infection rates The high prevalence in trichomoniasis infection among this age group may explain the role of: Higher sexual activity, risky sexual behaviors and Social and peer pressure Among women of 15-25 age group compared with other groups, these results came agree with that of (Stemmer *et al.*, 2012; Momeni *et al.* 2016) who indicated that women of age group have more verniability toword trichomoniasis infection.

Positivity rate of trichomoniasis infection according to type of diagnostic method:

The results the present study indicate that among 300 examined samples 74 samples were positive for trichomoniasis infection (24.6%) when wet mount preparation used. and 76 samples were positive using Diamond's medium cultivation 25%. Statistical analysis (p=0.84) shows no significant difference between the two lab methods in identifying *T. vaginalis*. It can be seen from in (Table 5).

Table (5): Number and percentage of detected trichomoniasis infections according to type of diagnostic method:

Lab. Diagnostic Method	No. of Cases	No. of Infected women	infection%
Wet mount	300	74	24.6
Culture	300	76	25.3
Total	300	76	25.3

Using diamond media was more sensitive in detecting trichomoniasis infection than the wet mount method, since that cultivation method will increase number of the trichomonads, make it easier to make shore whether the examined specimen include *Trichumonu*s parasite or not, extended incubation time, viability of organisms, and a lower detection threshold (Al-Saeed 2011; Testardini *et al*. 2016; Dadwal *et al*. 2023)

CONCLUSIONS

We conclude that Diamond's culture method proved to be the most sensitive and reliable technique for accurate detection of *T. vaginalis*. Among the various sample types tested, endocervical discharge demonstrated the highest sensitivity and diagnostic accuracy. Therefore, ensuring proper sampling techniques is essential to achieve trustworthy and precise identification of *T. vaginalis* infections.

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مقارنة بين تقنية المسحة الرطبة ووسيط دايموند في الكشف عن طفيلي داء المشعرات المهبلية بين النساء في مدينة دهوك/ العراق

زيمان نوري موسى **سيناء عبد الله الجرجري**

قسم علوم الحياة/ كلية العلوم/ جامعة الموصل

الملخص

يمكن وصف عدوى داء المشعرات بأنها من الأمراض المنقولة جنسياً الأكثر انتشاراً في العالم، والتي تسببها طفيلية المشعرة المهبلية. ينتقل هذا الطفيل بشكل رئيسي من خلال الممارسات الجنسية، ولكنه يمكن أن ينتقل أيضًا عبر الأجسام غير الحية. في النساء، تسبب داء التهاب المهبل وتتمثل الأعراض السريرية في إفرازات غزيرة ذات لون أصفر–أخضر رغوي برائحة كريهة، تورم الفرج، الحكة، وظهور آفات حمراء دقيقة على عنق الرحم تُعرف أيضًا باسم "عنق الرحم الفراولي". وهناك العديد من الحالات المحتملة التي قد تكون فيها النساء مصابات دون ظهور أعراض. تهدف الدراسة الحالية إلى مقارنة فعالية التشخيص بين تحضير العينة المبللة ووسيط وسط دياموند للكشف عن المشعرة المهبلية في أنواع مختلفة من العينات، بما في ذلك إفرازات عنق الرحم، ومسحات عنق الرحم، والبول لدى 300 مريضة (متزوجات وغير متزوجات) تراوحت أعمارهن بين (15–62) سنة. تم إحالة جميع المرضى إلى مستشفى الولادة في دهوك ومخيم شاريا داخل أو خارج مدينة دهوك.

تم تأكيد تشخيص العدوى الطفيلية بناءً على الأعراض السريرية للمرضى من خلال تأكيد العدوى مجهرياً في المختبر باستخدام طريقتين: التحضير المباشر للعينة المبللة والزراعة باستخدام وسط دياموند.

أظهرت نتائج هذه الدراسة أنه في مستشفى الولادة في دهوك، من بين 240 عينة، تم إصابة كشفت 61 حالة (25.4%) اصابة بطفيلي المشعرات المهبلية، وفي مخيم شاريا، من بين 60 عينة، تم إصابة كشفت 15 حالة اصابة بالطفيلي (25%) بالطفيلي المشعرة المهبلية. من بين 300 عينة، تم إصابة الكشف عن 74 حالة اصابة (24.6%) بواسطة التحضير المبلل، و76 حالة اصابة (25.3%) بواسطة الزراعة باستخدام وسط دياموند. تبين في الدراسة الحالية التي اجريت على 258 امرأة متزوجة، كانت ان عدد الاصابات بالطفيلي كانت نسبة الإصابة 66 حالة (25.5%)، بينما كانت عدد حالات الاصابة في نسبة الإصابة بين 42 امرأة غير متزوجة 10 حالات (23.8%). كانت نسبة الإصابة سجلت أعلى نسبة اصابة بطفيلي المشعرات في الفئة العمرية (15–25) حيث بلغت (32.8%).

الكلمات الدالة: المشعرة المهبلية، التحضير بالمسحة الرطبة، داء المشعرات، الأمراض المنقولة جنسيا، وسط دايموند.