Assessing the Effectiveness of Wet Mount and PCR Methods in Diagnosing *Trichomonas vaginalis* Infections in Endocervical and Urine samples

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KEYWORDS

Trichomonas vaginalis, wet mount preparation, PCR. STD and vaginal inflammation.

ABSTRACT

Trichomonas vaginalis is the cause of trichomonas's infection, which is referred to be the global sexually transmitted disease (STD) worldwide. This parasite primarily spreads through sexual activity, though it can also spread through intimate. Vaginal inflammation in females causes a variety of clinical symptoms, such as a profusion of greenish-yellow, frothy, foul-smelling discharge, valvar enlargement, itching, and punctate erythematous lesions of the cervix, commonly referred to as "strawberry cervix." There are numerous scenarios in which women may be sick but not exhibit any symptoms. The purpose of the current study is to compare the diagnostic efficacy of polymerase chain reaction (PCR) and wet mount preparation for detecting *T. vaginalis* in various sample types, including endocervical discharge, endocervical swabs, and urine, of 300 female patients (married and single), ages ranging from 15 to 62, who were referred to Dohuk Maternity Hospital, Shariya Camp, either inside or outside of Dohuk city. Based on the clinical symptoms of the patients, the diagnosis of parasite infection was validated by microscopically confirming the infection in the laboratory using two methods: PCR and direct wet mount preparation. The study findings demonstrated, out of 240 samples, 61 (25.4%) at Dohuk Maternity Hospital and 15 (25%) out of 60 samples in Shariya Camp were contaminated with *T. vaginalis*. Of the 300 samples, 74 (24.6%) had wet mount preparation infection, and 75 (25.0%) had PCR infection. In the current study, 42 single women had 10 (23.8%) and 258 married women had 66 (25.5%) infected. The age range of 15 to 25 had a higher infection rate (32.8%).

1. Introduction

T. vaginalis was first encountered by Donne inatör protozoan parasite in 1836, concerning the existence of this condition in the male urinary tract, Kunstler already reported it in 1883 only (Liston, 1940; Hoffmann *et al*., 1961). Trichomonas's infection is the universal sexually transmitted disease (STD) which is primarily caused by the protozoan anaerobic parasite *T. vaginalis*, means of transmission is sexual contact although it can also be through contact with objects infected with the parasite known as fomites (Hussein and Shaker, 2023). Symptomatic trichomoniasis in women leads to vaginitis which manifests with lot of foul smelling, greenish-yellow, foamy discharges, valval swelling, uncomfortable feeling in lower part abdomen area, itchiness and punctate, erythematous lesions over the cervix as a (Gashi, 2017; Amadi and Nwagbo, 2013). Influence of transmission of HIV from the mother to the child *T. vaginalis* is said to cause inflammation and erodes the vaginal mucosa which gives HIV virus easy access to transmit the virus to the child either while being born or through breast milk (Garber, 2005; Rada *et al*., 2022). Laboratory diagnosis of *T. vaginalis* used to depend on a few key methods: saline wet preparation in female patient and nucleic acid detection (NAD) (Der Pol *et al*., 2021). *T. vaginalis* typically has five flagella and single nucleus: the first three free anterior flagella and the 4th one is present in the base of the undulating membrane that runs longitudinally along the parasite and hence the parasite moves in a rather 'twitchy' manner (Coceres *et al*., 2021). Trophozoite stage: *T. vaginalis* has two forms of cycles, namely trophozoite and pseudocyst forms the former is pathogenic, flagellated which is formed from binary and multiple fission; multiple division form is a multiple division form that occurs in between 3 to 8 divisions (Dias-Lopes *et al*., 2018). In the unfavorable environment of the liver the trophozoites are larger developed that holds the encystation cyst-like structures (CLS) and transforms into pseudocysts not responding to chemical or physical aside from plasmodium, Trophozoites of CLS can revert back to

the excystation stage in favor if host cell is available (Kusdian *et al*., 2013; Beri *et al*., 2020). In carrying out treatment the treponemal disease is treated using antiprotozoal of metronidazole or tinidazole, effective treatment should be provided with a view of eradicating the parasite and avoiding spread to the sexual partner (Petrin *et al*., 1998). According to the current history of invention the compound microscope was invented by Hans Lippershey and Zacharias Janssen in the year 1590 in the Netherlands (Sushmasusik and Hayath, 2015; Hajdu, 2002). Actually, it is possible to mention that PCR for the detection of *T. vaginalis* in clinical samples was first applied by Riley in 1992 (Shaio *et al*., 1997).

2. Methodology

The From the beginning of November 2023 to the end of April 2024, an epidemiological study involving 300 married and single women aged 15 to 62 was carried out in several sites, including Dohuk Maternity Hospital and Shariya Camp.

Sample collection: Patients with symptoms provided endocervical swabs, urine, and endocervical discharge samples. The cervical discharge were appropriately collected aseptically by taking a swab from the posterior fornix of the vagina using a vaginal speculum. When examining the swab for motile trophozoites of *T. vaginalis* under a light microscope, add 1 ml of sterile normal saline and mix thoroughly. Place the swab on a slide and cover it with a concave slip. Some of the trophozoites were stored at -20°C for PCR. Endocervical discharge is collected with a small, single-use syringe, and then 1 ml of normal saline (NS) is added. The endocervical discharge is then placed on a slide, covered with a concave slip, viewed under a light microscope, and stored at -20°C to serve as endocervical swab samples for PCR. Urine samples collected midstream were placed in sterile containers. Urine can be centrifuged at 3000 rpm for 10 minutes. The urine sediment can then be placed on a slide, and 1 milliliter of phosphate buffer saline (PBS), a solution made of salt and water, can be added. The urine sediment can then be kept at -20°C until needed.

Laboratory examinations: The pathogen that causes trichomoniasis, *T. vaginalis*, can be diagnosed using a variety of techniques, each with advantages and disadvantages. Two common diagnostic techniques are PCR and wet mount microscopy.

1. **Microscopy Test:** Using a clean, round, grease-free glass microscope slide, a droplet of the mixture was placed on it. It was then covered with a concave slip and examined under a light microscope using a 10X and 40X objective lens for motile Trichomonas (Rahi and Jaleel *et al*., 2022; Adjei *et al*., 2019). This technique is known as the wet mount method. Urine samples collected midstream were placed in sterile containers, mixed thoroughly once more, and centrifuged for five minutes at 1000 rpm. The supernatant was then disposed of, and a drop of the sediment was placed on a grease-free, clean microscopy slide. The cover slip was then placed, and the slide was viewed at 40X (Barbara *et al*., 2021). After mixing endo cervical discharge with saline on a sterile slide, the sample is spread out and covered, then examined under a 40x microscope to check the flagella's direction of movement, examine its morphology, and count the number of *T. vaginalis* (Fule *et al*., 2012).

2. **Molecular Methods for PCR Detection of *T. vaginalis* DNA extraction:**

DNA Extraction from endocervical discharge, endocervical swab and urine Samples

· **Sample Preparation:**

- **Centrifuge:** 1 ml sample at 14,000–16,000 x g for 5 minutes. Discard supernatant.
- **Resuspend Pellet:** Add 200 µl GT Buffer + 20 µl Proteinase K, vortex.
- **Incubate:** 60ºC for 10 minutes, flip tube every 3 minutes.

· **Lysis:**

- **Add GB Buffer:** Add 200 µl, vortex 10 seconds.

- **Incubate:** 70ºC for 10 minutes, flip tube every 3 minutes.
- **(Optional)**: Add 5 µl RNase A for RNA removal, incubate 5 minutes at room temperature.

· **DNA Binding:**

- **Add Ethanol:** Mix 200 µl 100% ethanol immediately.
- **Transfer to GD Column:** Centrifuge for 2 minutes at 14,000–16,000 x g.

· **Wash:**

- **W1 Buffer:** Add 400 µl, centrifuge 30 seconds.
- **Wash Buffer:** Add 600 µl (with ethanol), centrifuge 30 seconds.
- **Dry Column:** Centrifuge for 3 minutes.

· **Elution**

- **Transfer GD Column to New Tube.**
- **Add Elution Buffer (100 µl):** Incubate 3 minutes.
- **Centrifuge:** 30 seconds to elute pure DNA.

➢ **Polymerase Chain Reaction (PCR) Protocol:**

T. vaginalis was found in endocervical discharge, endocervical swab, and urine samples using a polymerase chain reaction (PCR) method that targets the TV-E650-1 region of the DNA gene. The amplification process was carried out in a single step, beginning with a PCR using the TVP1 and TVP2 primers. (Table 3.1).

Table (3.1): The list of primers was employed used in the molecular analysis study (Ryu *et al.*, 1999).

No.	Primer Name	Sequence	Fragment Size
1.	Forward Primer (TVP1)	5' GAGTTAGGGTATAATGTTTGATGTG 3'	330bp
2.	Reverse Primer (TVP2)	5' AGAAGTGTATAGCGAAATGGG 3'	330bp

➢ **TVP1 and TVP2 Primers for PCR Amplification**

The first PCR used the TVP1 5' GAGTTAGGGTATAATGTTTGATGTG 3' and TVP2 5' AGAAGTGTATAGCGAAATGGG 3' primers to target a 330 bp fragment of the *T. vaginalis* TV-E650-1 region. One milliliter of reaction volume was used for the reaction.

(Table3.2) Conditions for TVP1 and TVP2 expected size 330bp

Segment	Cycle	Temperature	Time
Initial Denaturation	**1**	**95**	**3 min**
Denaturation	**35**	**95**	**45 sec**
Annealing		**48**	**45 sec**
Extension		**72**	**45 sec**
Final extension	**1**	**72**	**3min**

- **Electrophoresis Procedure**

The electrophoresis chamber was prepared by filling it with 1X TAE buffer following gel polymerization. To prepare DNA samples for loading on the gel, 1µl of loading dye was diluted with 5µl of the DNA sample. After that, these samples were carefully pipetted onto the appropriate agarose gel wells that had been prepared as previously mentioned. For 45 minutes, the electrophoresis was run at 50 volts per centimeter. After that, a UV trans-illuminator was used to take pictures of the DNA bands.

3. Results and Discussion

300 specimens were subjected to direct wet mount and PCR: *T. vaginalis* trophozoites were observed in 76 (25.3%).

- **The occurrence of *T. vaginalis* based on geographical location.**

The epidemiological study was conducted in multiple locations, including Dohuk Maternity Hospital and Shariya Camp. The population comprised individuals from various demographics and socioeconomic statuses among 60 cases in Shariya camp 15 (25 *T. vaginalis*.0%) were infected and among 240 cases 61 (25.4%) were infected by. These is due to of differences in study populations, geographic areas, environmental factors, hygiene practices, and the availability and utilization of water.

Table 1: Number of samples that were tested for the presence of *T. vaginalis* based on geographical location.

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

- **The occurrence of *T. vaginalis* based on marital state:**

66 (25%) samples of the 258 samples that were gathered and tested were found to be *T. vaginalis* trophozoite positive. These samples were taken from married females. Out of the 42 samples that were analyzed from the single female subjects, only 10 samples (23.8%) tested positive for *T. vaginalis*. Table 2 presents the findings, which indicate that there was no significant (P=0. 957), (χ^2:

0.003) comparability of the patient's marital status.

Table 2: *T. vaginalis* infection among both married and unmarried state.

Marital state	No. of Cases	No. of Infected Individuals	%
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, then there might be other factors like:

1. **Increased Sexual Activity.**
2. **Absence of Barrier Protection.**
3. **Partner's Infection.**

- **The occurrence of T. vaginalis based on types of sample:**

Of the 64 patients who had an endocervical discharge, 25 patients (39%), had a *T. vaginalis* infection. Of the 37 endocervical swab samples, 11 patients (29.7%) had an infection. Of the 60 urine samples, 15 proved to be contaminated, accounting for 25% of the samples. Analyzed data from 100 patients' urine and endocervical swab samples, of which 9 (9%) had the infection. Endocervical discharge, endocervical swab, and urine samples were obtained from 39 study participants; 41% of the endocervical discharge samples tested positive for infection. Only 3 (7.69%) of the 39 patients had positive results for *T. vaginalis* in their endocervical swabs and urine samples. As show in (table 3).

Distribution of sample types among patients infected with *T. vaginalis* is shown in (Table 3).

Sample types	No. Of cases	No. Of infected individuals	**%**
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 urine become positive)**	41% for Endo cervical discharge, for endo cervical swab 25.6% and for urine is 7.69%
Total	**300**	**76**	25%

Given that the endocervical discharge sample type had the greatest infection incidence in this dataset, it is a significant predictor of T. vaginalis, particularly in general samples and multiple sample types. The following are some of the factors that increase the sensitivity of endocervical discharge in detecting *T. vaginalis*:

1. **Greater Parasite Load.**

2. **Direct Presence.**
3. **Simpler Detectio**n.
4. **Improved Conditions.**

➢ **The occurrence of *T. vaginalis* based on Age:**

T. vaginalis was found to be more common in 32.8% of people aged 15 to 25, 22.7% of teenagers aged 26 to 35, 23.4% of middle-aged adults aged 36 to 45, and 26.3% of older persons aged 46 and above, with an overall frequency of 25.3%.

Table 4: The relationship between *T. vaginalis* infection and age.

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

For the following reasons, the age group (15–25 years old) had the highest infection rates (32.8%) compared to other age groups:

1. **Increased Sexual Activity.**
2. **Risky Behaviors.**
3. **Peer Pressure.**
4. **Educational Gaps.**

➢ **The occurrence of T. vaginalis based on laboratories method**

According to the findings, out of 300 samples, 74 samples (24.6%) of wet mount preparation use were positive and 75 samples were positive *for T. vaginalis* by using PCR. It was discovered that there aren't many differences between the various laboratory techniques for differentiating *T. vaginalis*.

Distribution of laboratory methods among *T. vaginalis* patients is shown in (Table 5).

Lab. Method	No. of Cases	No. of Infected Individuals	%
Wet mount	**300**	**74**	**24.6**
PCR	**300**	**75**	**25.0**
Total	**300**	**76**	**25.3**

The following reasons make PCR more sensitive than the wet mount method:

1. **Increased Sensitivity.**
2. **Detects Non-Visible Organisms.**
3. **Effective with Degraded Samples.**

4. **Reduced Human Error.**

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