World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-3, October-2021
ISSN: 2749-3644

DETECTION AND COMPARISON OF SIZE OF TRICHOMONAS VAGINALIS IN DIRECT SMEAR AND CULTURE MEDIA

Muhsin E. Sulyman
MSc, K1 Hospital, North oil company, Kirkuk-Iraq[1].
Mohammed A. Kadir
Ph.D, Imam Ja'afar AL- Sadiq University, Kirkuk branch – Iraq[2].
Corresponding author:
mohammdsalam@yahoo.com
muhsin.ama60@gmail.com

Article history:

Received: August 7th 2021
Accepted: September 10th 2021
Published: October 12th 2021

Abstract:

Objectives: *Trichomonas vaginalis* is one of the common human parasites among women in Iraq. It is transmitted mainly via sexual intercourse. Our aim is to compare the size of infection in direct smear and culture media.
Materials & Methods: A study was carried on 250 women attending gynecology clinic in K1 hospital in North Oil Company, Kirkuk Iraq. Laboratory investigation included vaginal swabs for direct examination and culture of swabs.
Results: The infection rate among women with vaginal discharge was 7 (2.8 %). The length and width of flagella and oxystyle oval and longitudinal parasite in culture media was significantly greater than in direct stained smears. The movement of parasite in culture media become sluggish after 72 hours, very weak after 96 hours and complete death after 120 hours.
Conclusion: The culture media was more efficient than stained smears for detection of *Trichomonas vaginalis*. The size of parasite in culture media was greater than direct stained smears.

Keywords: Trichomonas vaginalis, women, K1 hospital Kirkuk-Iraq.

INTRODUCTION:

Trichomoniasis is a sexually transmitted parasitic disease caused by trichomonas *vaginalis* which has worldwide distribution and responsible for 276 million new infections annually (1). in women it may cause vaginitis, urethritis and cervicitis (2). One of the most common symptoms of the disease is vaginal discharge (3). The requires an optimum acidic pH about 6, while the vaginal pH is about 4 so the parasite cannot survive in normal acidic vagina; and abnormality alkaline vaginal environment favors the consistence of the disease (4).

The morphology of trophozoites of *T. vaginalis* resembles other trichomonads. The usual size of the parasite includes; body length about 8 – 13 micrometers and flagella length about 8 - 15 micrometer with 5 – 8 micrometer widths, with uniformly distributed nuclear chromatin, while there are a large number of hydrogenosomes that are particularly evident around the axostyle, it has no cyst form and is recognized by its characteristic jerky movement. Structurally the parasite lacks mitochondria that is replaced by hydrogenosomes for metabolic and energy production (5).

The parasite is diagnosed by the common method which *is* the wet mount examination showing motility and morphology (6). This method has low sensitivity despite being simple with low cost, as it requires viability of parasites and well-trained microscopists, so the gold standard method for the diagnosis is culture method while is currently replaced by the nucleic acid amplification methods (NAATs) (7). It has been reported that culture of the parasite is specialized media will improve the sensitivity from (60%) of wet mount preparation to (85 – 95 %) using culture medias (8, 9, 10, 11), with the best and most sensitive media being modified Diamond medium (9,10,11).

In astudy done by Dawood et al. in Kirkuk, they reported (2.4 %) of the women had the disease using direct microscopic method while culture method the percentage increased to (2.8 %) (12). While in Duhok it was shown that (2.4%) of vaginal swabs detected *T. vaginalis* using wet mount; (3.5 %) using haematoxylin-eosin-stained smear while the rate improved to (5.4 %) using Diamond modified culture (13).

Many contradictory researches have been published showing comparison between the results of culture and direct microscopy regarding the sensitivity of both tests (14).

The present study was carried out to compared the size of *Trichomonas vaginalis* in direct wet mount technique and modified Diamond medium, among

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-3, October-2021
ISSN: 2749-3644

women attending Gynecology Clinic in K1 hospital in North Oil Company, in Kirkuk city in Iraq.

MATERIALS AND METHODS:

This study was carried out on 250 women patients attended Gynecology Clinic, K1 hospital, in North Oil Company, Kirkuk, K1 hospital for the period from beginning of 2018 until end of 2019 to compare the size of *Trichomonas vaginalis* in direct smears stained with safranin, gram stain and leishman stain and in culture media, in addition to find the difference between direct smear and culture media in detection of parasite.

Two vaginal swabs were obtained from each patient. One of them was kept in sterile physiological normal saline and was sent to the laboratory within one hour. Vaginal exudates were prepared as smears on clean glass slides and examined by direct microscopic examination and the other swab was cultured in a culture bottle containing the modified Diamond media.

Following preparation of smear, the swabs were incubated at 36°C, then the specimens were withdrawn from the medium at 48, 72, 96, 120 hours post inoculation and examined for motile trichomonas. Identification of *Trichomonas vaginalis* was done by using wet smear preparation under the microscope at (X40) and in modified culture media.

Examination of stained smears were done by making smears from vaginal discharge and stained by giemsa stains, leishman stain, gram stain and safranin.

Culture of *Trichomonas vaginalis* in artificial media was done by immersing the vaginal swab in Diamond modified broth.

The size of 100 parasites were measured from direct stained smears and 50 parasites in the culture medium using the inserted slide and included ophthalmic lens using magnification of X40 and an oily lens with magnification force of 100. The length and width of the parasite and the standard deviation were estimated.

Statistical analysis: Chi-square test was used to show significant difference between groups and student t-test was used to show the difference between any two groups (15).

RESULTS:

A total of 250 women attending K1 hospital, were examined using wet smear and culture techniques, the rate of infection by wet mount technique and culture media were (1.2%) and (1.6%) respectively.

The activity and mean number of 500.000 parasites in culture media (5 mililiters) were indicated in table 1, it was found the activity of parasite become sluggish after 72 hours, and became very weak after 96 hours and complete death of parasite occur after 120 hours. In the mean time the number of parasites were decreasing with duration of incubation.

Table 1. Movement and mean number of *T. vaginalis* in culture media within different period of times (hours).

No. parasites (1000)

Concentration	24 hours	No.	48 hours	No.	72 hours	No.	96 hours	No.	120 hours	No.
100%	Active	$600x10^3$	normal	$480x 10^3$	weak	$350x 10^3$	very weak	$150x10^3$	complete death	$15x10^3$

Comparison was made between morphology of *Trichomonas vaginalis* in direct smear stained with safranin and gram stain and in culture media, the safranin stain was more efficient to stain the parasite than smears stained with leishman's and giemsa's stains, as shown in figs (1, 2 &3).

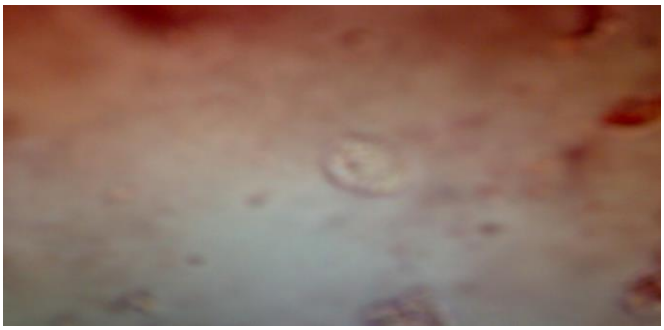

Fig 1. Morphology of *T. vaginalis* stained by safranine stain (X100)

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-3, October-2021
ISSN: 2749-3644

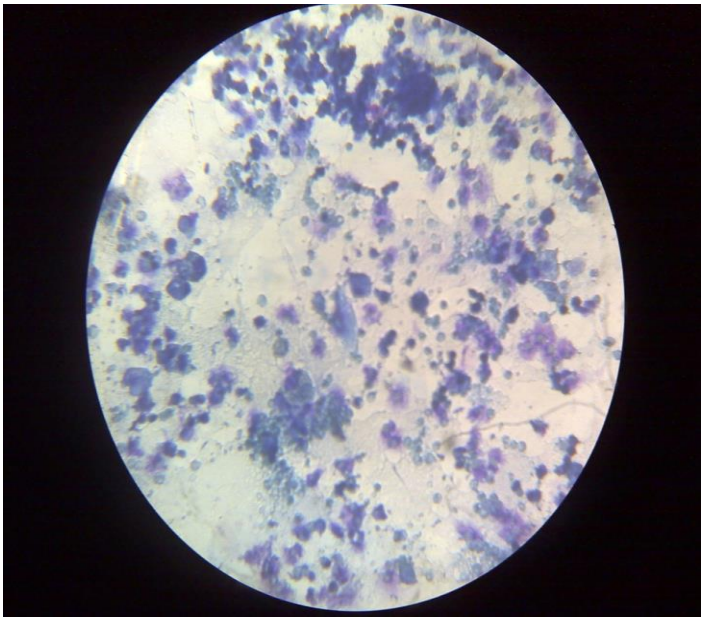

Fig 2.Morphology of *T. vaginalis* stained by Leishman stain (X100)

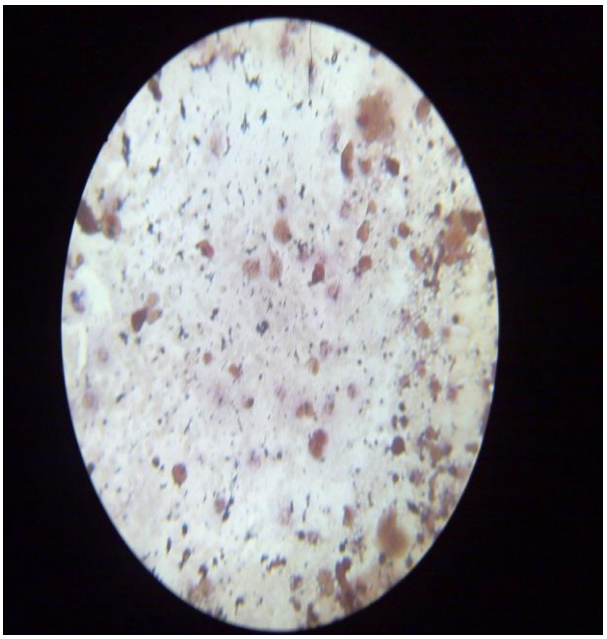

Fig.3. Morphology of *T. vaginalis* stained by Giemsa stain (X100)

The size of 100 parasites were measured in stained smears and size of 50 parasites were from culture media were measured by slide micrometer and ocular micrometer using lens X40 and oil immersion lens X100. The mean value and standard deviation of length and width of parasite was estimate. It was found that the size of *Trichomonas vaginalis* in culture media 32 X 21.1 micron was greater than those in stained direct smear 19.95 X 11.75 micron. Statistically there was significant difference in size of parasite in culture media and direct smears as indicated in table 2.

Table 2. Comparison between size of *T. vaginalis* in direct smear stained and in culture media.

Type of Specimen	Number of parasites measured	Mean length of parasite (µ) *	Mean Width of parasite (µ) **	Mean size of parasite Length X Size (µ)
Direct stained Smear	100	19.95 ± 7.3	11.75 ± 5.8	19.95X 11.75
Culture Medias	50	32 ± 8.2	21.1 ± 7.3	32X21.1

*t=19.558 d.f.=148 (P<0.05)
**t=11.462 d.f.=148 (P<0.05)

Table (3) comparison between length of flagellum in stained smear and culture media. It was found that the length of flagellum in culture media was significantly greater than direct stained smears.

Table (3) Comparison between lengths of flagellum in stained smear and culture media.

Type of sample	No. sample examined	Average length of flagellum
Direct stained smear	50	20.1 ± 6.5
Culture media	25	21.6 ± 1.6

t=19.566 d.f.=73 (P< 0.05)

Table (4) shows that the size of long flagella of *T. vaginalis* was significantly (P< 0.05) greater than short flagella, and the size of long axostyle of *T. vaginalis* was also significantly (P< 0.05) greater than short axostyle.

Table (4) The sizes of flagellates and axostyle of *Trichomonas vaginalis*.

Parameters	Size
1-Flagella	
Long	a 23.14± 5.01
Short	b 13.00±3.16
2-Axostyle	
Long	a 22.86±3.38
Short	b 13.00±0.00

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-3, October-2021
ISSN: 2749-3644

DISCUSSION:

Trichomoniasis is one of the parasitic diseases that is sexually transmitted with similarity to other STI in various aspects as; rate of infection, existence of other (STIs), failure to use contraceptive barrier and existence of multisexual partners (16).

Generarlly the estimated rate of women infected with *Trichomonas vaginalis* is almost (5.4 %); in Kirkuk aprevios study has shown that the rate of infection was (7.5 %), which was fortounatly lower than other areas, this may be related to sample size; and women education programme, regarding STI and the sequale of the disease in maternal care office with increased standard of living in the community. While in Iraq it was recorded to be (10%) in Erbil and (14%) in Mosul (17, 18, 19).

In Sulaimani Governorate, Kadir and Fattah found the prevalence of *T. vaginalis* in direct wet mount was (1.3%) and in culture method was (5%), using Trichomonas selective medium product of Oxoid-Factory (20).

Trichomoniasis in women has been diagnosed traditionally depending on the microscopic examination of their motility and jerky movement leading to movement of adjacent cells; by direct microscopic observation, but the sensitivity is variable and ranges between (38% - 82%), as the accuracy requires two essential factors; the first being that moist sample is needed to retain viability and motility and the second factor is a well – trained personal should examine the sample within 30 minutes. To keep Trichomonad vital it needs phosphate buffer saline at room temperature to remain alive for 6 hours, as the motility depends on temperature . An important point is that the size of trichomonad is almost the same of lymphocyte , but in non motile form it is not easy to differentiate them from other vaginal epithelial cells nucleus; the lack of this test sensitivity may be the cause of under estimation (21).

Comparing the size of *Trichomonas vaginalis* indirect stained smears and culture media, it was clearly seen the size of organism was significantly greater in culture media than stained smears. This might be due to contraction of parasite during fixation of slides and the affect of some chemicals present in stains on the size of parasites and its parts.

The length and shape of trophozoites are affected by environmental factors ie. In vivo and in vitro, as it has been reported that the length of axostyle and flagella of freshly isolated trophozoites were longer than these cultivated in trypticase yeast extract-maltose (TYM) (22).

Although *T. vaginalis* is an anaerobic organism, it may be grown slowly under aerobic conditions (21). for which using incubation of CO2 may improve recovery. In a previous study it has been shown that immediate wet mount examination and rapid incubation may be effective as Diamond medium in glass vials (23).

Niether Giemsa nor gram staining were appropriate to visualize the morphology of the organism (6).

In many East European Countries, studies has shown that using gram, Giemsa and methylene blue stain in examining vaginal exudates has many advantages in comparison to wet preparation so they are not recommended for use, as the procedure is more time consuming, needs expert technician and has low sensitivity (24).

It has been reported that staining techniques eliminate the motility of the parasite due to fixation and it is difficult to have the characteristic pear shape form, always, so this method may be used a supplementary test with direct wet mount techiques (25).

The sensitivity of wet mount smears in asymptomatic women is low; in arrange of (60 – 80 %) in spite of being fast and inexpensive while the highest rate of infection was detected by modified Diamond culture(5.4 %) (16).

It has been reported that Diamond modified medium is the most sensitive medium for culture of *T. vaginalis*, which may be due to existence of the starch as a constituent in the media resembling vaginal environment (26).

It is concluded from the result of this study that culture media was more efficient than stained smears for detection of parasite; safranin stain can detect the morphology of parasite better than leishman and giemsa stains and the size of infection was greater in modified culture media than direct smear examination.
It is recommended to carry on further study using more advanced sensitive techniques such as papanicolaou smear and polymerase chain reaction (PCR) in association with wet mount and culture techniques to improve the sensitivity in the diagnosis of trichomoniasis..

REFERENCES:

1. World Health Organization. Global incidence and prevalence of selected curable sexually transmitted infections-2008. Geneva, 2012.
2. Sood S. and Kapil A. An update on *Trichomonas vaginalis*. Indian J. Sex Transm Dis, 2008, 29(2): 7-14.
3. Egan MF. and Lipsky MS. Diagnosis of vaginitis. J. Am Fam Phys, 2000, 62(5): 1095-1104.
4. Harvey RA, Chame PC and Fisher BD. Microbiology. 2nd edit. Lippincott Williams and Wilkins, Philadelphia, 2007.

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-3, October-2021
ISSN: 2749-3644

5. Muller M. The hydrogenosome. In the Eukaryotic cell, Gooday G, Trance AP, Liuogd DC edss. Symposia of the society for General Microbiology, 1980, 30, 127-142.
6. Menzes CB, Mello Mdos S. and Tasca T. Comparison of permanent staining methods for the laboratory diagnosis of trichomoniasis. Revista do Instituto de Medicina Tropical de Sao Paulo, 2016, 58: 1/5-5/5.
7. Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines,2015. MMWR Recomm Rep. 2015, 64: 1-137.
8. Petrin D. et al. Clinical and microbiological aspects of *Trichomonas vaginalis*. Clinical Microbiology Reviews, 1998, 11: 300-317.
9. Gelbart SM, Thomason JL, Osypowski PJ et al. Growth of *Trichomonas vaginalis* in commercial culture media. J. Clinical Microbiology, 1990, 28: 962-964.
10. Levi MH, Torres J, Pina C et al. Comparison of the InPouch TV culture system and Diamond 's modified medium for detection of *Trichomonas vaginalis.* J. Clin. Microbiology, 1997, 35: 3308-3310.
11. Wiese WS, Patel SR, Patel Sc et al. A meta-analysis of the papanicolaou smear and wet mount for the diagnosis of vaginal trichomoniasis. American J. Medicine, 2000, 108: 301-308.
12. Dawood IS, Kadir MA. and Sulyman MA. Epidemiological study of infection with *Trichomonas vaginalis* in Kirkuk city. Tikrit J Pure Science, 2013, 18(1): 48-55.
13. Al-Saeed WM. Detection of *Trichomonas vaginalis* by different methods in women from Dohok province, Iraq. Easter Mediterranean Health J., 2011, 17(9): 1/8-8/8.
14. Mason PR, Super H. and Fripp PJ. Comparison of four techniques for the routine diagnosis of *Trichomonas vaginalis* infection. J. Clin. Path., 1976, 29: 154-157.
15. Linton JC. Chi square Tutorial. Georgetown linguistics. WWW. Georgetown linguistics.edu.
16. Heine P, McGregor R. (wisam 12). *Trichomonas vaginalis* a reemerging pathogen. Clin. Obstet. and Gynecology, 1993, 36: 137-144.
17. Kadir MA, Jerjis BJ. Incidence of trichomoniasis in Kirkuk city. J. Faculty Medicine, Baghdad, 1999, 28(2): 75-79.
18. Kadir MA, Salehy A., Hamed EE. Studies on *Trichomonas vaginalis* in Erbil teaching hospital. J. Faculty Medicine, Baghdad, 1996, 23(1): 83-88.
19. Kharofa MA. An epidemiological study and cultivation of *Trichomonas vaginalis* in Mosul city. MSc thesis, Department of Microbiology, College of Medicine, Mosul University, Mosul, Iraq, 1999.
20. Kadir MA. and Fattah COD. Prevalence of *Trichomonas vaginalis* among women in Sulaimani Governorate. Tikrit J. Pharmac. Sci., 2010, 6(1): 1-9.
21. Domeika M, Zhurauskaya L, Savicheva A. et al. Guidelines for the laboratory diagnosis of trichomoniasis in East European countries. J. European Academy of Dermatology and Venerology, 2010, 24: 1125-1134.
22. Jae-Sook RYU and Duk-Young MIN (2006). *Trichomonas vaginalis* and trichomoniasis in the Republic of Korea. Korean J. Parasitol., 44(2): 101-116.
23. Beal C, Goldsmith R, Kotby M et al. The plastic envelop method, a simplified technique for culture diagnosis of trichomoniasis. J. Clin. Microbiology, 1992, 30: 2265-8.
24. Fripp PJ, Mason PR, Super H. A method for the diagnosis of *Trichomonas vaginalis* using acridine orange. J. Parasitol, 1975, 61: 966-967.
25. Peri G. Errors in the diagnosis of *Trichomonas vaginalis* infections as observed among 1199 patients. Obstet Gynecol, 1972, 39: 7-9.
26. Lawing LF, Hedges SR, Schwebke JR. Detection of trichomoniasis in vaginal and urine specimens from women in culture and PCR. J. Clin. Microbiology, 2000, 38: 2585-2588.