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Prevalence of *Trichomonas vaginalis* in patients with vaginal discharge in Mosul, Iraq

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ABSTRACT.

Objectives: 1-To detect *T.vaginalis* in women with vaginal discharge and to study it's relation to contraception and its duration. 2-To evaluate the effect of vaginal PH, menstrual cycle, pregnancy on the survival of *T.vaginalis*. 3 -To evaluate the diagnostic validity of ELISA test to detect *T.vaginalis* antigen from a vaginal swab and to compare this test with wet mount and culture preparation.

Subjects and methods: This study was conducted during the period from Nov.2007 to June 2008.The subjects enrolled in the study were 180 females attending out patients clinics of Al – Battol and Al – Salaam teaching hospitals in Mosul city. Two vaginal swabs from posterior vaginal fornix were taken from each subject. The first swab after mixed with normal saline was used for wet mount preparation and examined immediately. The second swab was placed in Bijou bottle containing 5 ml of Oxoid *Trichomonas* medium (CM161), and incubated at 37 ºC and checked for the presence of *T.vaginalis* after 24.48 hours until 7 days of incubation. The remaining swab with normal saline was stored at -20 ºC for subsequent evaluation by ELISA (kalon Biological Ltd for the detection of *T.vaginalis* antigen). ELISA test was done according to the instructions of the manufacturers.

Results: From a total of 180 women. Twenty one cases (11.67%) were found positive for *T.vaginalis*. The highest incidence of infection was seen among (21 - 30) years age group comprising 47.6%; ELISA for antigen detection was found to be superior 18 (19.35%) in comparison to wet mount 12 (12.9%) and culture method 13 (13.98%).

Conclusions: *T.vaginalis* is still prevalent in women with vaginal discharge. ELISA for antigen detection is superior than wet mount and culture methods. These findings confirm the advantage of using a sensitive screening test for the diagnosis of *T.vaginalis*.

Key words: *Trichomonas vaginitis*, diagnosis, culture, ELISA.

INTRODUCTION

Trichomonad are protozoan parasite that infect human and animals. There are over 15 species of Trichomonads. *Trichomonas vaginalis* affects humans and cause condition called trichomoniasis in both men and women **[1].** The parasite is a common cause of infection in the female genital tract and its clinical presentation ranges from totally asymptomatic infection to a severe vaginitis **[2].** Trichmoniasis the most common non viral STD infects 250-350 million people worldwide every year **[3]**. It's account approximately one fourth of vaginitis cases **[4]** .

Approximately 180 million women worldwide may be infected with *T. vaginalis* prevalence vary between population studies, but range from 5%-74% in women and 5%- 29% in men **[5].** The infection can occur in women at any age[6**]** but was reported to be more prevalent during the child bearing age **[7,8]** . Infection produce immunity that at best is only partial protective **[9]**. In women the symptoms are usually appear within 5 to 28 days of exposure **[10]** . They include a heavy, yellow-green or grey vaginal discharge, discomfort during intercourse, unpleasant vaginal odor and painful urination. Irritation and itching of the female genital area and on rare occasion, lower abdominal pain can be present **[11, 37]**. In about two-third of infected female, there is edema, inflammation, cell hypertrophy and metaplasia **[11]**. *Trichomonas vaginalis* is highly contagious and easily transmitted, the incubation of the infection varies from 4-28 days and in women may persist for many years **[12 ,13]** . Trichomoniasis caused by *T. vaginalis* is considered as number one, non viral STD **[14**] . Trichomoniasis can cause serious discomfort to women with associated problems of adverse pregnancy outcome, preterm delivery, Low birth weight infant **[15**]. Diagnosis of trichmoniasis in the female is

usually accomplished via direct microscopic examination of the vaginal fluid, however even with skilled diagnosticians ,the sensitivity of this test is only 60 % and may be less in asymptomatic women **[16**]. *Trichmonas vaginalis* can often be identified easily because of its motility **[17]**.Wet mount microscopy is most commonly used in clinical practice, for it is easy to perform, inexpensive and available at different laboratories , however a negative wet mount result dose not rule out the infection with *T.vaginalis* **[16]**.Different culture media are commercially available and are currently considered the gold standard for the diagnosis **[18]**. It has sensitivity that may reach up to 95, however it requires a week to produce accurate results [**19**]. A commercially available kits for immunodetection of *T. vaginalis* antigen in clinical specimens include ELISA test is also available **[20**]. Many workers study the prevalence of trichiomoniasis and found that it may vary from 3.9-19.16% **[6, 21]** by using both wet preparation and culture technique. The aims of the present study are **to:** 1. Determine the frequency of *Trichomonas vaginalis* in women with vaginal discharge, with different factors influence its frequency. 2.Find out the possible factors (as contraception, menstrual cycle, pregnancy, vaginal pH, educational level) which may play an important role in *T.vaginalis* infection. 3.Evaluate the importance of ELISA in the diagnosis of *T. vaginalis* antigen in comparison to other diagnostic methods as wet mount and culture.

MATERIALS AND METHODS

Subjects : This study was carried out during the period from November 2007 to June 2008 at Al- Batool and Al - Salaam Teaching Hospitals in Mosul City. Full informational data were recorded from 180 female attending Out Patient Clinics of these hospitals complaining from vaginal discharge Physical examination was done by inspection of external genitalia for any inflammation, lesion, masses, and by speculum to detect erythema, or lesions, The pooled vaginal discharge was assessed for various parameters according to the procedure described by **[**22].

Materials and methods

Swab :A Sterile cotton tipped swabs were used to take vaginal discharge.

Stains used for tissues were : Hematoxylin and Eosin (H& E) stain.

ELISA Kit : Kalon Biological Ltd, Unit G Perram works, Merrow Lane, Guildford, Surrey GU4 7BN U.K was used according to the instructions of the manufactures.

Methods
Vaginal swabs examination

Two vaginal swabs were taken from each patient. The patient was put in lithotomy position and a non-lubricated sterile bivalve speculum was used to expose the cervix and vagina, and vaginal discharge was collected using two sterile cotton tipped applicator and simultaneously introduced into the posterior vaginal fornix 23.The first swab was placed in tube containing 2 ml of normal saline for wet mount examination, [23]. The wet mount were examined immediately after taking the swab. It was examined under X40 light microscopy.

The second swab (about 5 drops) was placed under a sterile condition on Bijou bottle containing 5 ml of (CM 161) culture **[**24], then incubated at 37 °C and checked for the presence of *T. vaginalis* after 24hr, 48 hr, until 7 days. This was done by taking a drop from the bottom of the culture using a sterile micropipette, spreading it on a clean slide covered by a cover slip and then examined under high power of the microscope **[**21]. The vaginal discharge material stored at (-20°C) for subsequent evaluation by ELISA.

Examination of vaginal discharge pH:

The pH level was determined by placing litmus paper to the pooled vaginal discharge secretion, the color of paper is then compared to the color corresponding pH values on standard chart **[**22, 23] .

Method of staining:

The positive slides for *Trichomonas* were fixed with alcohol for 2-3 minutes, then it was placed in hematoxylin and left for 3-5 minutes then washed with water, and placed in Eosin stain for 1-2 minutes, washed with water,left to dry and examined microscopically under X40 andX100.

Oxoid Trichomonas Medium (CM161)

The medium prepared by dissolving 37.5 gm of powder of the mentioned substances in a sterile flask, then the mixture brought to boiling in order to dissolve the powder completely. The pH was adjusted to 6.4 by using pH meter, then the medium was sterilized by an autoclave at 121 °C for about 15 minutes then it was left to cool. Eighty ml of inactivated horse serum was added to the medium. The inactivation of the serum was made by putting container of horse serum in

a water bath at 56 °C for 30 minutes.After that and under a septic technique the addition of the antibiotics was done, these antibiotics were penicillin G (1000 000 I.U/L) and setreptomycin (500 000 I.U/L) in addition to antifungal agent as nystatin (0.1 gm/L).The medium then poured in Bijou bottle under a septic condition (5 ml in each of these sterile bottles) and stored at 4 °C in the refrigerator till used **[21 , 24]** .

An enzyme immunoassay for the detection of *Trichomonas vaginalis*
Principle of the assay

Polystyrene microtitre plate wells were supplied pre-coated with affinity purified polyclonal antibodies to *Trichomonas vaginalis*. Swab elution buffer was incubated in these wells, during which *T. vaginalis* antigen was captured. After a wash step the surface was probed for antigen by incubation with an enzyme conjugated anti-TV antigen tracer. Following a second wash step, enzyme substrate (containing a chromogenic reagent) was added to the wells.The enzyme incubation was halted by the addition of acid, which also has the effect of both changing and enhancing the amount of color produced. This was measured in a photometer. The optical density is proportional to the amount of *T. vaginalis* antigen present in the original sample which can be compared with a cut-off calibrator that has been designed to distinguish between non-specific binding and a true positive reaction. The score results with an optical density greater than cut-off x 1.1 was considered reactive, Score results with an optical density less than Cut-off x 0.9 was considered non-reactive.

Statistical analysis:

Statistical formulas as (SPSS program) were used to detect variances among parameters in the study at probability 0.05 and 0.01 according to **[25].**

Results
A total of 180 women with vaginal discharge were examined, there age ranged between 20 to 41 years.

The frequency of *T. vaginalis* infection according to the age

The highest frequency of infection was most commonly seen in the 21-30 years age group (47.6%) ,while the lowest incidence of infection (4.8%) was found among ≥ 41 years age group (Table 1). There was no statistical difference between the positive and negative cases at different age groups.

Table 1: The frequency of *T. vaginalis* infection according to the age

Age	+ ve cases		- ve cases		Total
	No.	%	No.	%	No.
≤ 20	3	14.3	22	13.8	25
21-30	10	47.6	56	35.2	66
31-40	7	33.3	48	30.2	55
≥ 41	1	4.8	33	20.8	34
Total	21	100	159	100	180

Calc χ^2 = 3.35 (Not significant) Tab χ^2 0.05, 3 = 7.815 Probability = 0.340

The frequency of *T. vaginalis* infection according to the educational level
Females with primary education 10(47.6%) showed the highest rate of positive results of *T. vaginalis*, followed by intermediate educational level where only 5(23.8%), showed positive result(Table 2). The detection of *T. vaginalis* infection was very low among females with university education level; only one (4.8%) was found positive. Illiterate women showed also a lower rate of *T. vaginalis* 2(9.5%). There was no statistical difference between the positive and negative cases.

Table 2: The frequency of *T. vaginalis* infection according to the educational level

Educational Level	+ ve cases		- ve cases		Total.
	No.	%	No.	%	
Illiterate	2	9.5	6	3.8	8
primary	10	47.6	62	39	72
Intermediate	5	23.8	55	34.6	60
secondary	3	14.3	15	9.4	18
university	1	4.8	21	13.2	22
Total	21	100	159	100	180

Calc χ^2 = 3.89 (Not significant) Tab χ^2 0.05, 4 = 9.488 Probability = 0.421

The frequency of *T. vaginalis* in correlation to fertility

Fertile women showed higher 18(85.7%) *T. vaginalis* infection (Table 3) than non-fertile women 3(14.3%). Similar pattern was observed among 159 women without *T. vaginalis* infection who showed 91.8% and 8.2% respectively. There was no statistical difference between the positive and negative cases concerning fertitity.

Table 3: The frequency of *T. vaginalis* in correlation to fertility

fertility	+ cases ve		- ve cases		Total
	No.	%	No.	%	
fertile	18	85.7	146	91.8	164
infertile	3	14.3	13	8.2	16
Total	21	100	159	100	180

Calc χ^2 = 0.855 (Not significant) Tab χ^2 0.05, 1 = 3.84 Probability = 0.355

Frequency of *T. vaginalis* according to the use of contraceptive methods

T. vaginalis infection was more common among women who did not use contraception (43.8%) than among those who used any kind of contraceptive methods. There were no positive cases of *T. vaginalis* diagnosed among condom user. Women used intra uterine contraceptive device (IUCD) showed a 25% of *T. vaginalis* infection in comparison to 10.5% among women without *T. vaginalis* infection (Table 4). The difference between user and non user of contraception was statistically not significant .

Table 4: Frequency of *T. vaginalis* according to the use of contraception

Contraception	+ ve cases		- ve cases		Total No.
	No.	%	No.	%	
Negative	7	43.8	66	57.9	73
IUCD	4	25	12	10.5	16
OCP	2	12.5	12	10.5	14
Medroxy progesteron injection	3	18.7	6	5.3	9
Condom	-	-	18	15.8	18
Total	16	100	114	100	130*

Calc χ^2 = 9.15 (Not significant) Tab χ^2 0.05, 4 = 9.488 Probability = 0.057

IUCD: intrauterine contraceptive device . OCP: oral contraceptive pills
*The sample consist only of (130) patients because the rest (50) were pregnant.

Frequency of *T. vaginalis* according to the color of vaginal discharge

Out of 21 women with positive *T. vaginalis* the color of discharge was white in 2(9.5%) patients, 6(28.6%) patients had white-yellow discharge, 8(38.1%) patients had yellow discharge, 3 (14.3%) patients had yellow-green discharge and only 2 (9.5%) patients showed green discharge (Table 5). Twenty five women showed a clear discharge with no positive cases of *T. vaginalis*. A highly statistical significant difference was observed between positive of *T. vaginalis* and negative cases according to color of the vaginal discharge.

Table 5: Frequency of *T. vaginalis* according to the color of discharge

Color of discharge	+ ve cases		- ve cases		Total
	No.	%	No.	%	
clear	-	-	25	15.7	25
White	2	9.5	84	52.8	86
White – Yellow	6	28.6	12	7.6	18
Yellow	8	38.1	23	14.5	31
Yellow – Green	3	14.3	10	9.3	13
Green	2	9.5	5	3.1	7
Total	21	100	159	100	180

Calc χ^2= 28.4 **(** Highly significant) Tab χ^2 0.01,5 = 20.517 Probability = 0.000

The relation between *T. vaginalis* infection and pH of discharge

Out of 21 females with *T. vaginalis* infection, the pH of vaginal discharge was 5-6 in 11(52.4%) cases and 4-5 in 8(38.1%) cases. Only 2(9.5%) cases had pH of 3-4. A statistical significant difference was detected between positive and negative groups (Table 6).

Table 6: The relation between *T. vaginalis* infection and pH of vaginal discharge

pH	+ ve cases		- ve cases		Total
	No.	%	No.	%	
3 - 4	2	9.5	73	45.9	75
4 - 5	8	38.1	64	40.3	72
5 - 6	11	52.4	22	13.8	33
Total	21	100	159	100	180

Calc χ^2 = 20.94785 **(**Highly significant) Tab χ^2 0.01,2 = 13.815 Probability = 0.000

Detection of *T. vaginalis* by different diagnostic methods

ELISA for the detection of *T. vaginalis* antigen showed the highest infection rate 18(19.35%) followed by culture 13(13.98%) and wet mount 12(12.90%), (Table 7 , Fig. 1)

Table 7: Results obtained from different diagnostic methods for *T.vaginalis*

Test	No. of positive cases	%	No. of negative cases	Total
Wet mount	12	12.90	81	93*
Culture	13	13.98	80	93*
ELISA	18	19.35	75	93*

***The number (93) was taken for each test (wet mount, culture) because ELISA kit contain 93 tests only.**

Evaluation of different diagnostic methods for the detection of *T. vaginalis*

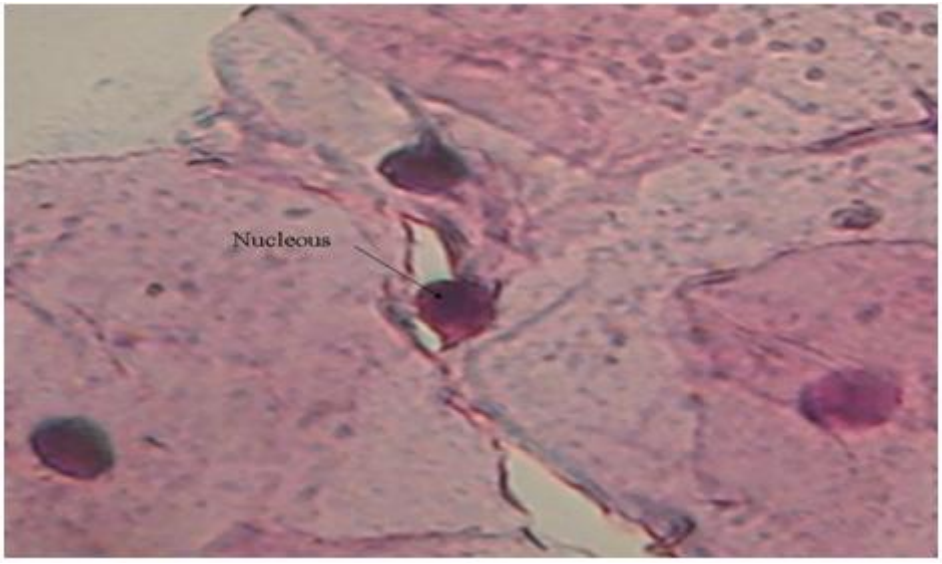
ELISA was more sensitive than wet mount (100% versus 84.61%) but slightly less specific (93.75% versus 98.75%), (Table 1). However the strength of positive results obtained by ELISA was represented by positive likelihood ratio(PLR) which indicate that the likelihood of each positive case to be positive is 16 times and to be negative is zero times as indicated by negative likelihood ratio(NLR).

Table 8: Comparisons between wet mount and ELISA

	Sensitivity %	Specificity %	NLR %	PLR %	NPV %	PPV %
Wet mount	84.61	98.75	91.67	97.53	6.77	13.57
ELISA	100	93.75	72.22	100	16	Zero

***T. vaginalis* form wet mount and culture stained by Hematoxylin and Eosin (H & E) stain**

Positive slides of wet mount stained by (H & E) showed clear *T. vaginalis* trophozoite with it's nucleus (Figure 1).



DISCUSSION

The peak age having *T. vaginalis* infection in this study was 21-30 years (47.6%), followed by age group 31-40 (33.3%) This is in agreement with the results obtained by other investigators [**21, 26**] . During the reproductive period there is an increase in the sexual activity which may increase the chance of having *T. vaginalis* infection, however, the present results are not in agreement with [**28,35,37]** who reported the highest rate of *T. vaginalis* infection among 14-19 years old. The lowest rate of infection (4.8%) was detected among age group more than 41 years. This may be due to the lack of proper vaginal environmental condition necessary for the growth of *T. vaginalis* mainly estrogen and glycogen level in the vaginal epithelial cells and neutral vaginal pH. In the present study high level of infection (47.6%) was detected among females with primary education (Table 2). This result is in agreement with [**21]** who found that the incidence of trichomoniasis was high (67.6%) among women with primary education level. The detection of *T. vaginalis* infection was very low (4.8%) among females with university education level. This is probably a reflection of their personal hygiene and high socio-economic status of this group. Fertile women showed higher 18(85.7%)*T.vaginalis* infection than non-fertile women 3(14.3%). Similar pattern was shown among 159 women without *T.vaginalis* infection who showed 91.8% and 8.2% respectively (Table 3). Recent reports indicated that trichomoniasis can cause infertility[**27,28]**. Other studies have demonstrated *T.vaginalis* as a risk factor for tubal infertility [**29, 30].**

It was reported that *T.vaginalis* is higher in infertile women as compared with pregnant control [**31].** A higher rate of infection (43.8%) was seen among women who did not use any type of contraception in comparisons to those who used any type of contraception. This is in agreement with the results obtained by others **[21, 33].** The results showed that the higher rate of infection was found between those using IUCD (25%) followed by (12.5%) oral contraceptive pills (OCP) user. However, among condom user, no positive cases of *T. vaginalis* have been detected (Table 4). Condom proved to be the most effective mechanical barrier and preventing method to various microorganisms [**34].** The present data showed that the difference between user and non user of contraceptive methods was statistically significant. On speculum examination, the vaginal discharge may be of any color or characteristics and although a frothy yellow-green discharge has classically been associated with trichomoniasis [**25**]. In the present study this was observed in only 3(14.3%) women with discharge caused by trichomoniasis. It was reported that among 149 Nigerian women with discharge of *T. vaginalis*, green discharge has not been observed [**5].** In the present study white vaginal discharge was detected from only 2 (9.5%) of *T. vaginalis* cases.

The yellow discharge occurred in 38.1% of cases followed by white-yellowish discharge which was seen among 28.6% of infected cases, where a statistically significant difference was observed between positive *T. vaginalis* cases and negative cases (Table 5). *T. vaginalis* appeared mostly in a discharge characterized physically by yellowish color in 40-47% of cases [26] . Clear discharge has not been detected in the present study. However, clear discharge was found only in 2.38% of the cases studied by others [**26]**. The vaginal discharge color may occasionally suggested the possible diagnosis of trichomoniasis, although the present result agree with those who found that *T. vaginalis* may be found in any type of vaginal discharge **[36]**. No precise explanation can be given for these results and the circumstances that determine whether trichomoniasis will produce a discharge with varying color are not completely understood **[38]**. A high vaginal pH is like to be associated with the presence of trichomoniasis **[39**]. It has been reported that a high vaginal pH> 4.5 indicates menopausal patients, trichomoniasis and bacterial vaginosis **[38].** Since *T. vaginalis* infection is associated with more alkaline vaginal pH, it has been recommended to use acid for vaginal acidification in the treatment of *T. vaginalis* infection **[40]**. Diagnosis of trichomoniasis has traditionally depended on the microscopic observation of motile protozoa in vaginal and cervical secretion **[32**].

Trichomonals can be differentiated on the basis of their motility. In this study, the data that showed a lower detection 12(12.9%) of *T.vaginalis* by wet mount method in comparison to culture method (13.98%) and ELISA antigen method 18(19.35%), The sensitivity of wet mount technique was 84.61%, although other studies reported variation from 38% to as high as 82% **[32]**. Other reports also indicated that the sensitivity of this test varies from 40% to 60% and the specificity may be elevated to 100%, if the smear examined immediately **[1,11]**. Other reports indicated that that wet mount was less efficient in the detection of *T.vaginalis* than culture method [**21,26].** This is in accordance with the result of wet mount presented in this study. The sensitivity of wet mount may be related to skill, experience and the time spend for specimen examination. The reduced motility may influence the results. Wet preparation is rapid and economical**.** By using culture method, the positive cases were increased 13(13.98%) in comparison to wet preparation 12(12.90%). It was found that culture method can increase the number of positive cases of *T. vaginalis*. This may be due to high sensitivity of culture which was reported to be 88%–95% **[12]** . Culture media for *T.vaginalis* remain a gold standard for the diagnosis of trichomoniasis [**1]**. In the present study, Oxoid Trichomonas medium (CM 161) was used to cultivate *T. vaginalis*, It is worthy to mention that six different culturing media were tested to cultivate Iraqi strain of *T. vaginalis* and (CM 161) was the best for cultivation **[24**]. The disadvantages of cultures are that they are laborious and require several days (2-5) days to complete leading to delay the treatment [**1,13]**. Furthermore cultivation should also be conducted in special laboratories with trained personnel; in addition the organism should be viable before they can be detected **[1]**. Detection of *T. vaginalis* antigen by ELISA in this study showed the highest rate of infection 18(19.35%) followed by culture 13(13.98%) and wet preparation 12(12.90%). ELISA was found more sensitive 100%,

than wet mount (84.61%) but slightly less specific 93.75%. However, the strength of the positive results obtained by ELISA was represented by positive likely hood ratio (PLR) which indicates that the like hood of each positive case to be positive is 16 times and to be negative is zero time as indicated by negative likely hook ratio . This is in agreement with others who reported a high sensitivity and specificity of ELISA to detect *T. vaginalis* antigen [**41]**. The limitation of culture and microscopy methods for the detection of *T. vaginalis* prompted the advance of the more sophisticated methods which can detect antigen, antibody or nucleic acid in urethral or vaginal exudates[**32]**. Despite the continued high number of cases all over the world and the potential complication of infection, trichomoniasis remains ignored as a public health issue. The only setting in which this infection is routinely screened for it in public health STD clinics and the screening tests, which is the vaginal discharge examination by wet preparation, which has limited sensitivity.

CONCLUSIONS

T.vaginalis is still prevalent in women with vaginal discharge. ELISA for antigen detection is superior than wet mount and culture methods. These findings confirm the advantage of using a sensitive screening test for the diagnosis of *T.vaginalis*.

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