

## PREVALENCE, SOCIODEMOGRAPHIC FACTORS AND CLINICAL CRITERIA OF TRICHOMONAS VAGINALIS INFECTION AMONG SYMPTOMATIC WOMEN IN BENI-SUEF GOVERNORATE, EGYPT

By

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### Abstract

One hundred female patients (age 18-50yrs) were enrolled in this study. Females were non-menstruating and non-pregnant with no intercourse or douching for the last 2 days, no use of anti-protozoa, antibiotics and steroids for the past 2 weeks and complaining mainly of vaginal discharge, itching or both. Vaginal swabs were obtained from all patients and examined for *Trichomonas vaginalis* by direct wet mount, Giemsa staining, modified Diamond's culture medium and Latex agglutination test (Kalon). *Results*: Out of 100 patients, *T. vaginalis* was found in 8 (8%) patients by any of the used diagnostic methods. Latex agglutination test detected 8 positive cases, 6 of which were also positive by Diamond's culture, 3 cases were detected by Giemsa staining and only one case was detected by wet mount. The wet mount, Giemsa staining and Kalon latex test had sensitivities of 16.7%, 50% and 100% respectively and accuracy rates 95.2%, 97.1% and 98% respectively as compared to the modified Diamond's culture as the gold standard. Meanwhile all of them had specificities 100% except for latex agglutination test, it was 97.9%. There was no significant association between potentially probable risk factors and confirmed trichomoniasis detected by Diamond's culture (the gold standard test) except in patients being from urban areas and complaining of either dysuria or dyspareunia or both.

**Keywords:** *Trichomonas vaginalis*, Wet mount, Diamond's culture, Latex test.

### Introduction

*Trichomonas vaginalis* is a flagellated anaerobic protozoan parasite that inhabits the urogenital tract of humans (Kaul *et al*, 2004). Although trichomoniasis is one of the most important sexually transmitted infections (STIs) all over the world that has significant adverse health outcomes, it is the most common curable one (Mabey *et al*, 2006). The estimated global incidence of *T. vaginalis* infection was 276.4 million new cases per year (WHO, 2012).

Female infected patients with *T. vaginalis* infection have a wide range of clinical symptoms, ranging from an asymptomatic to an acute or chronic inflammatory disease (Sherrard *et al*, 2011). This disease plays an important role in causing tubal factor of infertility, ectopic pregnancy and adverse outcomes of pregnancy in infected patients such as premature rupture of membranes, preterm labor and delivery of low-birth weight infants (Cotch *et al*, 1997; Chesson *et al*, 2004; Pararas *et al*, 2006). Trichomoniasis is

related to increased risk of HIV-type1 acquisition and transmission (Wang *et al*, 2001; McClelland *et al*, 2007). Also, *T. vaginalis* has been incriminated in vaginitis, urethritis, cervicitis, pelvic inflammatory disease and prostatitis in patients infected with HIV (Moodley *et al*, 2002). The prostate cancer was associated with male trichomoniasis (Sutcliffe *et al*, 2006; Stark *et al*, 2009; Twu *et al*, 2014).

Control programs for *T. vaginalis* infection had become back behind efforts to control other STIs, that's why *T. vaginalis* was classified as a neglected parasitic infection (Secor *et al*, 2014). Many risk factors are associated with *T. vaginalis* infection such as age, race, residence, number of sex partners, marital status, education, intrauterine device (IUD) /condom usage or other used contraceptives, any used drug, presence of vaginal discharge and history of sexually transmitted diseases (Madhivanan *et al*, 2009; Dahab *et al*, 2012; Eshete *et al*, 2013; Miranda *et al*, 2014; Ton Nu *et al*, 2015).

Conventional diagnosis of *T. vaginalis* infection depends on microscopic wet mount examination, in which trophozoite "corkscrew" motility is obvious (Huppert *et al*, 2007) and Modified Diamond culture which has been used as the gold standard for diagnosis of *T. vaginalis* infection (Harp and Chowdhury, 2011). Both methods suffer from poor sensitivity and rely on collection of fresh viable trichomonads. Other diagnostic used methods include enzyme-linked immuno-sorbent assay (Sibau *et al*, 1987), staining, immunochromatography and nucleic acid amplification tests (Chalamilla *et al*, 2006), latex agglutination test (Darani *et al*, 2010). Ideal diagnosis should depend on high sensitive and specific, available, simple, and inexpensive tests (Patel *et al*, 2000).

This study aimed to determine prevalence of *T. vaginalis* and risk factors among suspected women attending the Gynecology and Obstetrics Outpatient Clinic at Beni-Suef General Hospital using conventional diagnostic methods as wet mount examination, stain, culture and Latex agglutination test. Also, accuracy of direct wet smear, Giemsa stain and Latex agglutination test were evaluated as compared to the gold standard; Diamond's media culture.

### **Materials and Methods**

**Study population:** This cross-sectional study was carried out from April 2016 to February 2017 on 100 non-menstruating and non-pregnant female patients attending the Gynecology and Obstetrics Outpatient Clinic at Ben-Suef General Hospital, Egypt. The patients were 18 to 50 years old with no intercourse or douching for the last 2 days, no use of antiprotozoal, antibiotics, or steroids for the past 2 weeks and complaining of variable gynecological complaints including mainly vaginal discharge, itching, or both.

Relative sociodemographic and clinical data were collected from all enrolled patients using standardized questionnaire sheet that fulfill the following data about residence, age, marital status, parity, educational level, menstrual and contraceptive history.

**Sample collection:** Each patient was subjected to PV examination and non-lubricated sterile speculum examination. Three vaginal swabs were collected from the posterior vaginal fornix using a sterile vaginal swab (a cotton wool swab-sticks with wooden shafts) to be examined as follows: 1- The first swab was kept in tube containing 3ml preworned sterile Phosphate Buffered Saline (PBS), pH: 7.2, and squeezed into a tube wall to be used, through one hour, for wet mount microscopy and Giemsa staining. 2- The second swab was kept in one of the Kalon TV latex tubes provided by the kits containing sterile PBS, pH 7.2, to perform the latex agglutination. 3. The third swab was immersed immediately into the culture tube containing modified Diamond's medium and squeezed for cultivation. Samples were labeled and rapidly transported to the Medical Parasitology Laboratory for processing.

**Wet mount smear:** within 10-30 min, one drop of the first swab tube was put into a microscopic clean glass slide then covered by a cover slip and examined microscopically for motile *T. vaginalis* trophozoite under low and high powers.

**Giemsa-stained smear:** one drop of the vaginal swab-PBS suspension was applied on a microscopic glass slide, air dried, fixed in methanol for 1 minute, stained with 20% Giemsa for 20 minutes. The slide was rinsed by water to remove excess stain, drained in a vertical position and left to dry and examined microscopically by X100 to detect trophozoites (Radonjic *et al*, 2006).

**Kalon Latex agglutination test (Kalon Biological Ltd):** the latex is mixed on a slide with the elute from the vaginal swab. Presence of *T. vaginalis* antigen in the sample led to agglutination of the sensitized latex. The agglutination of positive samples started to appear after the 2<sup>nd</sup> minute and established at the 3<sup>rd</sup> minute. Agglutination of the beads was an indicator for *T. vaginalis*. The degree of agglutination was recorded following the manufacturer's instructions to distinguish between highly positive, weak positive

and negative results.

In vitro cultivation of *T. vaginalis*: Directly, the swab was inserted into the culture tubes containing the modified Diamond's medium and after mixing, expressed on the tube side and discarded. The tubes of inoculated medium were incubated vertically at 37°C in anaerobic condition. It was examined daily or every other day for the trophozoites. If the parasite was not seen until the 7<sup>th</sup> day, specimen was considered negative and discarded.

Statistical analysis: Data were tabulated and analyzed using SPSS-23 (IBM, Somers, NY, USA) software. Numerical data were presented as mean  $\pm$  SD while categorical data were expressed as number and percentage. The qualitative variables were studied by chi-square (X<sup>2</sup>) test. *P*-value was significant at  $\leq 0.05$ . Receiver operating characteristic curve (ROC) was used to assess the diagnostic performances of the different techniques used in diagnosis.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All the participants gave informed consent to do vaginal swabbing after explaining the study purpose.

## Results

Out of 100 female patients with symptoms suggestive of vaginal trichomoniasis using the mentioned methods (Tab. 1), positive *T. vaginalis* infection was detected in 8 (8%) of patients by any of the used diagnostic tests. Kalon Latex agglutination test detected trichomoniasis in 8 cases (100% of positive cases) of which 6 cases (75%) were positive by Diamond's culture. While 3 cases and only one case (37.5% and 12.5% respectively) were positive by Giemsa staining and wet mount. These results were highly statistically significant ( $P < 0.001$ ).

Receiver operating characteristic curve (ROC) with analysis of sensitivity and speci-

ficity of the used different diagnostic techniques was assessed. A perfect discrimination test has a ROC plot that directed more to the upper left corner (100% sensitivity, 100% specificity). In this study, culture method was used as a gold standard. Females were grouped according to their ages into 4 groups with an age range between 18 & 50 years and a mean of 32.3 ( $\pm 9.7$ ) years. The highest positivity was in age group 31-40.

No positive cases were reported after the age of 40 years. No statistical significant difference detected in distribution of trichomoniasis in different age groups ( $P = 0.2$ ).

Obtained results clearly showed that *T. vaginalis* infection was more common among women living in urban areas and that was statistically significant ( $P = 0.006$ ). Vaginal discharge, dyspareunia and lower abdominal pain associated with backache were the most frequent symptoms (100%) among 6 positive detected cases by Diamond's culture method, followed by itching (83.3%), and then the least frequent symptom was dysuria (66.7%).

In this study, 2/8 (25%) out of the positive cases detected by Kalon latex test were represented by vaginal discharge only. While there wasn't any positive case found among women complaining of infertility at all. Among all positive cases, a malodorous frothy purulent yellowish green vaginal discharge was detected in 6 cases (75%). Two cases were represented by a non-purulent yellowish discharge. Meanwhile yellowish white, bloody and watery vaginal discharge was not related to *T. vaginalis* infection. Association between the nature of the vaginal discharge and trichomoniasis revealed a highly significant difference ( $P < 0.001$ ).

Of 8 positive cases, 4 cases were using IUD (50%), 2 cases were using hormonal contraception (25%) and 2 cases used no contraception (25%). This showed insignificant correlation between infection and contraceptives usage. There was insignificant association between sociodemographic risk factors and trichomoniasis by Diamond's

culture except those living in urban areas ( $P=0.006$ ) with dysuria ( $P=0.003$ ), or dyspareunia ( $P<0.001$ ) or both. Details were given in tables (1, 2 & 3) and figures (1 & 2).

Table 1: Accuracy of direct wet mount, Giemsa staining technique and Kalon Latex agglutination test versus Diamond's culture, gold standard test for diagnosis of vaginal trichomoniasis.

	Culture			Accuracy measure			
	Positive	Negative	Total	Variable	%	95% CI	P value
Wet mount				Sensitivity	16.7	89.2-98.4	<0.001*
Positive	1	0	1	Specificity	100		
Negative	5	94	99	PPV	100		
Total	6	94	100	NPV	95.2		
				Accuracy	95.2		
Giemsa stain				Sensitivity	50	91.7-99.4	<0.001*
Positive	3	0	3	Specificity	100		
Negative	3	94	97	PPV	100		
Total	6	94	100	NPV	97		
				Accuracy	97.1		
Kalon test				Sensitivity	100	93.1-99.8	<0.001*
Positive	6	2	8	Specificity	97.9		
Negative	0	92	92	PPV	80		
Total	6	94	100	NPV	100		
				Accuracy	98		

\* = Significant P-value

Table 2: Sociodemographic profile and clinical criteria of female patients versus Diamond's culture, gold standard test.

Characteristics	Total cases=100	Culture positive No. %	Culture negative No. %	P-value
Age groups:18-20y	16	0(0)	16(17)	0.2
21-30y	28	2(33.3)	26 (27.7)	
31-40y	34	4 (66.7)	30 (31.9)	
41-50y	22	0(0)	22(23.4)	
Residence Rural	78	2 (33.3)	76 (80.9)	0.006*
Urban	22	4 (66.7)	18 (19.1)	
Education; Illiterate	29	2(33.3)	27 (28.7)	0.8
Mid Education	43	1(16.7)	40 (42.6)	
Higher Education	28	3 (50)	27 (28.7)	
Marital status:Married	70	6 (100)	64(68.1)	0.4
Unmarried (divorced- widow)	30	0 (0)	30 (31.9)	
Vaginal discharge: Yes	70	6 (100)	64(68.1)	0.1
No	30	0 (0)	30 (31.9)	
Dysuria :Yes	20	4 (66.7)	16(17)	0.003*
No	80	2 (33.3)	78(83)	
Dyspareunia :Yes	11	6 (100)	5 (5.3)	<0.001*
No	89	0(0)	89 (94.7)	
Lower abdominal pain +backache: Yes	31	6 (100)	25 (26.6%)	0.3
No	69	0 (0)	69 (73.4%)	
Itching: Yes	43	5 (83.3)	38 (40.4)	0.4
No	57	1(16.7)	56 (59.6)	
Infertility: Yes	8	0 (0)	8 (8.5)	0.5
No	92	6 (100)	86 (91.5)	
Contraception :IUD	44	4 (66.7)	40 (42.6)	0.2
Hormonal	12	1(16.7)	11(11.7)	
No contraception	44	1(16.7)	43 (45.7)	

Table 3 Corrected as followed by its complete cells:

Characteristics	Total no. of cases	Positive cases (%)	P-value
Yellowish greenish	14	6 (75)	<0.001*
Yellowish	26	2 (25)	
Yellowish white/watery/bloody	30	0 (0)	
Total	70	8 (100)	

## Discussion

In the present study, prevalence of *T. vaginalis* infection among suspected women was 8% by all methods. This result of some reports in Egypt, which recorded that the prevalence rates among symptomatic women from Cairo, Benha and El-Minia were 10.16%, 11% and 12.7%, respectively (Zaki *et al*, 2011; Hussein *et al*, 2015; Gabr *et al*, 2006). Higher prevalence rates of infection were reported from the cities of Cairo, Mansoura and Alexandria with prevalence 23%, 36%, 79.16% and 91.3 (Elsherif and Youssef, 2013; Aboulghar *et al*, 2009; Hegazy *et al*, 2009; Negm and Abd El-Haleem, 2004) respectively. Lower prevalence rate of 5% was reported from Cairo and Ismailia by both Mahmoud *et al*. (2015) and El-Moamly and Rashad, (2008) respectively. The global prevalence estimates among suspected patients ranged from 0.9% to 80% (Valadkhani *et al*, 2008; Javanbakht *et al*, 2013).

The epidemiology of trichomoniasis and disparity between different studies was changeable and may be attributed to socio-cultural factors in different variable communities. Many other factors may affect accurate diagnosis and prevalence variations including variations in the selected study population, age, menstrual cycle phase, the number of sexual partners, sexual activity, other STIs, specimen type, collection and processing, available diagnostic and laboratory techniques and test interpretation (Fernando *et al*, 2012; Hussein *et al*, 2015).

In this study, 4 (50%) of positive cases were patients represented by manifestations of typical vaginal trichomoniasis (vaginal discharge, itching, dyspareunia, lower abdominal pain associated with backache and dysuria). Meanwhile, in symptomatic patients with partial clinical picture, the rate of detected positive infection falls down to 25% in patients who suffering from vaginal discharge only, on the other hand another two cases (25%) of positive cases were found inpatients complaining of both vaginal discharge and itching. There weren't any

positive detected cases in patients suffering from itching alone. This result comes in accordance with results of Hussein *et al*. (2015) and Valadkhani *et al*. (2008). They had declared that combined vaginal discharge, itching and dysuria were the main complaints in 50% and 52% of *T. vaginalis* infected patients, respectively.

In this work, the highest rate of positivity (75% of positive cases) was detected among women complaining of malodorous frothy purulent yellowish greenish vaginal discharge, however (25%) of them were complaining of non-purulent yellowish discharge. These results are higher than but in agreement with results of Fernando *et al*. (2012) and Hussein *et al*. (2015) who detected that a characteristic frothy yellowish, and malodorous discharge in 47% and 41% of women respectively.

The results showed that except for patients living in urban areas with suggestive dysuria or dyspareunia or both there isn't any association between *T.vaginalis* infection and age, educational level, marital status and contraceptives use. This is partially agreed with others (Madhivanan *et al*, 2009; Fernando *et al*, 2012; Silva *et al*, 2013, Hussein *et al*, 2015). The Six out of eight positive cases were in the age group 31-40 years, the group of high sexual activity. This agreed with others (Dragsted *et al*, 2001; Aboulghar *et al*, 2009, Mahmoud *et al*, 2015; Hussein *et al*, 2015).

The culture results showed that infection was significantly common among women in urban areas ( $P=0.006$ ) that agreed with Ton Nu *et al*. (2015). But, Dahab *et al*. (2012) reported that trichomoniasis was more in rural areas than urban ones with a significance difference.

In the present study, the sociodemographic data, behavioral data and clinical manifestations were debatable. Some authors related between trichomoniasis and the race/ ethnicity (Hollman *et al*, 2010), age (Krashin *et al*, 2010), marital status (Dahab *et al*, 2012), education, presence of vaginal discharge,

use of condom/IUD, (Eshete *et al.*, 2013), and history of STIs and drug use (Miranda *et al.*, 2014).

In the present study, 8/100 suspected patients (8%) was positive for trichomoniasis. Kalon Latex agglutination test detected all positive, but only 6 cases (75%) were positive by Diamond's culture. Three cases and one case (37.5% & 12.5%) were positive by Giemsa staining and wet mount respectively.

The direct wet smear is a rapid, cheap, practical and simple technique but with lowest sensitivity. This might be due to trophozoite deterioration and loss of its jerky movement, retraction of its flagella making it more rounded. Thus, became difficult to detect in vaginal with epithelial cells and/or leucocytes. In this study, one case was positive by direct wet mount. This result agreed with others who showed that culture method was more sensitive than wet mount (Gavvani *et al.*, 2008; Aboulghar *et al.*, 2009; Zaki *et al.*, 2011; Patil *et al.*, 2013; Mahmoud *et al.*, 2015; Hussein *et al.*, 2015).

Giemsa staining is more accurate method than direct wet mount but still less sensitive than culture method. Radonjic *et al.* (2006) and Ojuromi *et al.* (2007) reported the same result. In the present study, it was noticed that Giemsa staining detected 3 cases only. This is may be attributed to the usual damage and loss of most trophozoites from the slide during processing and this is accepted by other studies (Aboulghar *et al.*, 2009; Mahmoud *et al.*, 2015). On the other side, Hussein *et al.* (2015) reported that Giemsa staining could give false positive results being its diagnosis relies only on shape and size not on motility of the trophozoite as in cases of culture and wet mount techniques.

In this study, culture is considered to be the reference test and the "gold standard" to which findings of other tests performed were compared. Radonjic *et al.* (2006) had confirmed that culture still remains the most sensitive and reliable method in the diagnosis of *T. vaginalis* infection. The modified Diamond culture was considered the gold

standard test (Ojurom *et al.*, 2007; Domeika *et al.*, 2010; Hussein *et al.*, 2015; Mahmoud *et al.*, 2015). Meanwhile other researchers depended on diagnostic PCR as the gold standard reference test (Saleh *et al.*, 2014). Nevertheless, Diamond culture might unavailable in most laboratories as it is expensive, and requires daily follow up for a week to detect growth, and time consuming. In many of the developing countries, patients might not visit the health facility for their culture results due to substantial cost, thus prolongation of the infection led more complications (Patel *et al.*, 2000).

In this study, wet mount, Giemsa stain and Kalon latex agglutination test gave sensitivities of 16.7%, 50% & 100% and the specificities of 100%, 100% & 97.9% respectively. Mahmoud *et al.* (2015) reported that the sensitivity and specificity of latex test versus culture as standard golden test were 100% and 97.9% respectively.

The Kalon Latex test detected 8 positive cases. But, Diamond culture gave 6 positive cases. However, the confirmatory tests that might be more sensitive than culture should be performed as PCR to evaluate if the extra 2 cases detected by the Kalon test are truly false positive results or not as done previously by Saleh *et al.* (2014) who confirmed that latex agglutination test gave false positive results as compared with Diamond's media and PCR positive. The Kalon Latex test gave a high sensitivity when compared to culture but its positive result was weak to confirm infection (PPV = 80%), meanwhile its negative result excluded the infection (NPV = 100%).

### Conclusion

Trichomoniasis should be included in the control programs of sexually transmitted diseases and screening tests should be done routinely to detect such infection. Socio-demographic predictors do not have a great conflict on the prevalence among different Egyptian women. Laboratory investigations are essential for accurate diagnosis. Although wet mount examination is not a sensi-

tive technique yet, it is a good positive one, sonegative samples should be reexamined by more sensitive methods. Staining may be helpful in cases of heavy infection. Latex agglutination is a highly sensitive, simple and rapid screening test.

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### Explanation of figures

Fig. 1: Comparison of Receiver operating characteristic curve (ROC) for diagnostic performance of wet mount, Giemsa staining technique and Kalon latex agglutination test as referred to in-vitro culture in diagnosis of vaginal trichomoniasis.

Fig. 2: *Trichomonas vaginalis* "pear" shaped flagellated trophozoite after staining with Giemsa stain by  $\times 100$ .

