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EVALUATION OF STAINING METHODS FOR DIAGNOSIS OF TRICHOMONIASIS IN CLINICALLY SUSPECTED WOMEN IN JAZAN, KSA.

By

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Abstract

Forty female patients experiencing symptoms suggestive of trichomoniasis were included into the present investigation. Patients` self-obtained vaginal swabs were gathered and examined for *T. vaginalis* by wet mount, Giemsa stain, Gram stain and culture techniques. *T. vaginalis* was recognized in 11 out of 40 specimens by culture (27.5 %), 10 by wet mount (25 %), 8 by Gram stain (20 %) and 7 by Giemsa stain (17.5 %). Contrasted with culture as the standard technique, Giemsa staining method indicated 63.6 % sensitivity compared with 54.5% for wet mount or Gram staining. Giemsa staining specificity was 100 % contrasted with 86.2 % for wet mount and 93.1 % for Gram stain. In addition, a considerable agreement was found amongst culture and Giemsa staining (Kappa= 0.717) while moderate agreement of Gram stain (Kappa= 0.52) and wet mount (Kappa = 0.419) with culture was found.

Key words: Kingdom of Saudi Arabia, Trichomoniasis, Females, Diagnosis

Introduction

Trichomonas vaginalis (T. vaginlis) is the most prevalent sexually transmitted pathogen accounting for 180 million infections annually (Weger *et al,* 2018). It is one of the most important cofactors increasing HIV transmission and giving adverse birth outcomes. In Saudi Arabia, Trichomonaisis is one of the most commonly STIs reported to the ministry of health during 2005 to 2012 with overall incidence of 18.4% of all STIs and average annual incidence of 9.1% (Memish *et al,* 2016). *T. vaginalis* infection is not only restricted to the vagina but the parasite may be found and isolated from all genitourinary structures. The classical symptoms associated with trichomoniasis include a yellowish-green odorous vaginal discharge; pruritus, dysuria, and "strawberry" cervix which is characterized by punctuate hemorrhagic lesions (Gary, 2005). *T. vaginalis* infect men as well as women with a comparable frequency, but in men symptoms are normally mild and infections are cleared by the host's immune system within weeks. In women, trichomonaisis could persist for many years, and symptoms can attain a severity which is debilitating (Swygard *et al,* 2004). But, asymptomatic disease exists in both men and women (Leitsch, 2016).

Accurate diagnosis for trichomonasis is a fundamental issue for successful treatment that helps controlling its spread. Diagnosis cannot be made entirely on the basis of symptoms and clinical approach as it may mimic those of other STDs. So, laboratory based diagnosis has to be considered necessary for early and accurate diagnosis (Petrin *et al,* 1998; Garber, 2005). Detection of *T. vaginalis* was performed by several laboratory methods that varied in sensitivity and specificity (Radonjic *et al,* 2006; Memish *et al,* 2016). Culture is still considered the "gold standard" for diagnosis of trichomoniasis. However, it has many disadvantages, as it is costly, laborious and time-consuming as up to 7 days are needed for identification of positive cultures (Nye *et al,* 2009). The wet mount preparation of vaginal discharge was the commonest method for diagnosis. However, examination of specimens must be performed within 30 min after collection to enable visualization of viable and motile protozoa; a limitation that may affect its sensitivity (Radonjic *et al,* 2006). In set up lacking immediate microscopic facilities, staining techniques like Giemsa and Gram staining are very useful where prepared and fixed smears can be transported to laboratory for staining and diagnosis. With large number

of patients attending gynecological outpatient clinics an immediate examination of vaginal swab samples is virtually impossible. Unlike wet mount examination, delay in transport has no significant impact on staining methods reliability for diagnosis.

The present study aimed at detection of *T. vaginalis* in vaginal swab specimens from clinically suspected women in Jazan, KSA, using wet mount examination, Giemsa stain, Gram stain and culture, and evaluation of the performance of staining techniques in diagnosis of *T. vaginalis* compared to culture as the reference method.

Subjects and Methods

The study subjects were women in Jazan City who had clinical manifestations suggestive of trichomoniasis. Each woman was asked to fill a questionnaire covering the personal data, clinical symptoms, marital status and obstetric and gynecological history. Vaginal swab samples were obtained from women fulfilling the following inclusion and exclusion criteria using sterile vaginal swabs provided to the patients.

Inclusion criteria: a- Married female ≥18 years old, b- Complaining of one or more trichomoniasis symptoms (yellowish or green color vaginal discharge with or without itching, foul smell vaginal discharge, lower abdominal colic, pain in urination, pain in intercourse).

Exclusion criteria: a- Unmarried female, b- Pregnant female, c- During menstrual period, and d- During puerperal period.

Vaginal secretions were collected from the available responders to participate in the study who attend the gynecology outpatient clinics at different health centers in Jazan. Patients` self-obtained vaginal swabs were collected (Crucitti *et al*, 2003; Van Der Pol *et al*, 2014). The patient was informed to insert a single swab into the vagina and to rotate the swab three times. Each swab was suspended in 2ml of 0.85% normal saline supplemented with two drops of 5% glucose. Each sample was labeled and quickly transported to the Parasitology Laboratory, Faculty of Applied Medical Sciences, Jazan University, for complete testing within 30 minutes. Before testing, the swabs were vigorously shaken to displace all the parasites into the saline solution.

Procedures: Each vaginal specimen was subjected to the following:

Wet mount Examination: A drop of saline with suspended vaginal secretions was placed on a 22×40 glass slide and covered with a cover slip. The preparation was examined microscopically for motile *T. vaginalis* under ×10 & ×40 objectives. The vaginal secretion was characterized by the presence of the squamous epithelial cells while the trichomonads were identified by their size (10-20 μm), round or oval shape, and characteristic quivering or twitching motility (Mahmoud *et al*, 2015).

Giemsa staining: A drop of saline with vaginal secretions was smeared on a 22 × 40 glass slide and allowed to air- dry then fixed by immersion in methanol for one minute. The slides were stained with stock Giemsa stained diluted 1:9 with phosphate buffer solution (pH 7.2) for 10 min. Slides were washed with running faucet water, air dried and scanned for parasites at 10 × 100 magnification. No less than 30 fields were inspected before a negative finding was recorded. Trophozoite nucleus is round or oval, the flagella and undulating membrane appeared (Mahmoud *et al*, 2015).

Gram staining: Vaginal smear was prepared and fixed as for Giemsa staining then subjected to gram staining method as follows: the slide was flooded with crystal violet for one minute, the dye was poured off and the slide was delicately washed in tap water and depleted against a paper towel. The smear was presented to Gram's iodine for one minute by washing with iodine, at that point including more iodine and leaving it on the smear until the minute is over, then, the smear was washed with faucet water and drained. The slide was washed with 95% ethanol, then, washed with tap water at the end of the 30 seconds to stop the decoloriza-

tion. The smear was stained with 0.25% safranin for 30 seconds, washed, drained, blotted, and examined by microscope. *T. vaginalis* trophozoites appeared as pear shape with a round or oval nucleus, flagella and undulating membrane & axostyle appeared, and cytoplasm might be lacked (Harrington and Williams, 1999).

Modified thioglycolate culture: The medium was used at a concentration of 29.5g in every liter of distilled water. The well mixed medium was dispensed in 10 ml amounts in glass tubes fitted with screw-caps and sterilized by autoclaving (with caps loosened) at 121°C for 15 minutes. When cool, the tubes caps were tightened and each tube top was covered with a foil cap and stored in refrigerator. Just before use, the media were taken out of refrigerator and let to take the room temperature, and then, horse serum, Amphotericin B, Penicillin G, and Gentamicin were added (Pouch *et al*, 1996). Culture tubes were inoculated with the vaginal swab samples and incubated at 37°C. The culture was microscopically examined for trophozoites after 24 hr by taking a drop of medium from the base of each tube. This was done at two days and preceded up to seventh day before negative culture was disposed of.

Statistical analysis: For evaluation of diagnostic techniques, sensitivity, specificity, PPV & NPV were ascertained for each test contrasted with culture. Chi-square test was used. A significant difference was considered when $p < 0.05$. Kappa test was used to identify agreement between diagnostic tests.

Ethical considerations: Patients were notified about the aim of study and informative written consent was obtained prior to participation. Confidentiality of the patients was maintained through the duration of the research. Each patient was informed with the test result.

Results

The present study included 40 female patients suffering from symptoms suggestive of trichomoniasis, with ages ranged from 18-52 years. Most of them were in group 18-35 years (27/40, 67.5%). The commonest clinical symptoms were vaginal discharge (17/40, 42.5%) and vaginal discharge and itching (13/40, 32.5%) while dysuria was reported in 10 out of 40 subjects (25%).

T. vaginalis was identified in 11 out of 40 samples by culture method (27.5 %), 10 samples by wet mount (25%), 8 samples by Gram stain (20%) and 7 samples by Giemsa staining (17.5%). When the results of staining methods were combined together the number of positive samples increased to 10 samples (25%) (Tab. 1; Fig. 1). Compared to culture as standard method, Giemsa stain was more sensitive and specific than both wet mount and Gram stain. Giemsa stain showed 63.6 % sensitivity compared to 54.5 % sensitivity for wet mount or Gram staining methods. Giemsa stain specificity was 100% compared to 86.2% for wet mount and 93.1% for Gram staining. Besides, a substantial agreement was found between culture and Giemsa staining (Kappa= 0.717) while moderate agreements of wet mount (Kappa = 0.419) and Gram staining (Kappa= 0.52) with culture were observed (Tabs. 2 & 3). Discrepant outcomes were seen in five samples using the different diagnostic methods in the present work. The 5 samples were negative by culture, 3 of them were positive by wet mount & 2 were positive by wet mount and Gram stain. So, only 35 samples were included in analysis of *T. vaginalis* infection among different age groups and different clinical manifestations. Trichomoniasis was more common among patients in age group 36-45 years compared to other age groups (Tab. 4) but without significant difference ($p > 0.05$). *T. vaginalis* in-fection was more commonly presented with vaginal discharge and itching, followed by vaginal discharge only and dysuria but with-out significant difference (Tab. 5).

Table 1: Comparison of different methods for detection of *T. vaginalis* (n = 40).

Diagnostic method	Positive No. (%)	Negative No. (%)
Culture	11 (27.5 %)	29 (72.5 %)
Wet mount	10 (25 %)	30 (75 %)
Gram stain	8 (20 %)	32 (80 %)
Giemsa stain	7 (17.5 %)	33 (82.5 %)
Gram stain and Giemsa stain	10 (25 %)	30 (75 %)

Table 2: Wet mount examination and staining methods for *T. vaginalis* compared to culture.

Test	Culture		Kappa value	Statistical analysis
	Positive (n= 11)	Negative (n= 29)		
Wet mount: Positive (10) Negative (30)	 6 (54.5%) 5 (45.5 %)	 4 (13.8 %) 25 (86.2 %)	0.419 (moderate agreement)	 *P* value < 0.001
Gram stain: Positive (8) Negative (32)	 6 (54.5%) 5 (45.5 %)	 2 (6.9 %) 27 (93.1 %)	0.520 (moderate agreement)	 *P* value < 0.001
Giemsa stain: Positive (7) Negative (33)	 7 (63.6 %) 4 (36.4 %)	 0 29 (100%)	0.717 (substantial agreement)	 *P* value < 0.001

Significant at $p < 0.05$

Table 3: Efficacy of different tests compared to culture.

Test	Sensitivity	Specificity	PPV	NPV
Wet mount	54.5 %	86.2 %	60 %	83.3 %
Gram stain	54.5 %	93.1 %	75 %	84.4 %
Giemsa stain	63.6 %	100 %	100 %	87.9 %

PPV: positive predictive value, NPV: negative predictive value

Table 4: Relationship of *T. vaginalis* positive cases (by culture) and age groups.

Age group (years)	Number of cases	Number of positive cases (%)
18- 25	12	4 (33.3 %)
26- 35	12	3 (25 %)
36- 45	7	3 (42.8 %)
46- 52	4	1 (25 %)
Total	35	11 (31.4%)
P–value	0.861 (not significant)	

Table 5: Relationship of *T. vaginalis* infection and t clinical manifestations.

Clinical symptom	No. of cases	No. of *T. vaginalis* positive (%)
Vaginal discharge	15	4 (36.4%)
Vaginal discharge and itching	11	6 (54.5%)
Dysuria	9	1 (9.1%)
P– value	0.099 (not significant)	

Discussion

T. vaginalis is the second most commonly sexually transmitted infection in Saudi Arabia between 2005 & 2012 (Memish *et al,* 2016). In the present study, 40 patients with clinical manifestations suggestive of *T. vaginalis* infection were examined using 4 methods. Sensitivity and specificity were calculated for wet mount, Gram stain and Giemsa stain methods using the culture as standard method for comparison. Sensitivity of wet mount examination was 54.5% & specificity was 86.2%. Sensitivity of Gram`s stain was 54.5% & specificity was 93.1% while Giemsa stain sensitivity was 63.6 % & specificity was 100%. In the present study, low sensitivity of wet mount (54.5%) agreed with others (Khatoon *et al,* 2014; Mahmoud *et al,* 2015; Menezes, *et al,* 2016). They reported sensitivity values of 33.3% to 67.6%. This might be due to deterioration of trichomonads to lose motility, retract flagella, becoming rounded and difficult to distinguish from other structures, such as leucocytes or nuclei

of vaginal epithelial cells. Khatoon *et al.* (2014) explained the low sensitivity of wet mount by presence of less number of organisms that led to negative finding.

In the present study, Gram stain results were equivalent to that of wet mount method. This finding agreed with Angelika *et al.* (2002) who reported that Gram stain was substituted for other diagnostic techniques if immediate microscopy was impracticable Detection rate of trichomonads could be increased if one was aware of recognizing them in Gram stain smears (Sobrepena, 1980). But, five positive samples by culture were not recognized by Gram stain, which could indicate that trichomonads were more like polymorph nuclear leukocytes.

In the present study, Geimsa stain gave sensitivity of 63.6%, which agreed with 41-100% sensitivity reported (Fernando *et al,* 2011; Khatoon *et al,* 2014; Paliwal *et al,* 2017)**.** The inability of Giemsa stain to detect the trophozoites in 4/11 culture positive might be due to the fact that during stain processing, trophozoites were damaged or lost (Mahmoud *et al,* 2015).

In the present study, 5 samples were negative by culture but positive by using wet mount and Gram stain. This agreed with other studies (Crucitti *et al,* 2003; Patil *et al,* 2012) which proved that the sensitivity of culture in liquid media, such as Diamonds and Trichosel was 85 to 95%. Regardless of that culture technique proved excellent for visualization of trichomonads, yet, it was not used in routine laboratories. It is time-consuming required 7 days for growth. During which patient was on spreading disease before treatment (Mahmoud *et al,* 2015).

Conclusion

Trichomoniasis is usually diagnosed clinically, staining techniques used as supportive diagnostic methods in lack of microscopic facility. *T. vaginalis* diagnosis using staining methods would not be affected by delay in transportation, which decreases morbidity and adverse outcomes of trichomoniasis.

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Explanation of figure

Fig. 1: Vaginal secretion samples. *T. vaginalis* trophozoites (Red arrow), Squamous epithelial cells (Black arrow). A & B: wet mount smears. C& D: Giemsa stained smears. E & F: Gram stained smears.