Detection of *Trichomonas vaginalis* by different methods in women from Dohok province, Iraq

W.M. Al-Saeed[1]

الطرق المختلفة لاكتشاف المُشَعّرة المهبلية لدى النساء في دهوك في العراق

وسام مهدي عباس السعيد

الخلاصة: قارنت هذه الدراسة بين أربعة طرق تشخيصية مختلفة لاكتشاف المُشَعّرة المهبلية في عينات المسحات المهبلية المأخوذة من النساء الـمُراجعات لمستشفى دهوك في العراق. وقد جُمِعَ ما إجماليُّه أربع مئة وخمس وعشرون مسحة مهبلية من نساء اشتكين من نَجيج مهبلي مصاحب لالتهاب المهبل، أو التهاب عنق الرحم، أو المرض الالتهابي الحوضي. وأظهرت النتائج وجود عَشْر مسحات إيجابية (2.4٪) للمشعرة المهبلية باستعمال طريقة المسحات الرطبة، وخمس عشرة مسحة إيجابية (3.5٪) تلوين اللطخة بهيماتوكسيلين-إيوزين، وسبع عشرة مسحة إيجابية (4.0٪) باستعمال طريقة لطاخة بابانيكولا، وثلاث وعشرين مسحة إيجابية (5.4٪) باستعمال طريقة مزرعة دياموند المُعدَّلة. وقد تباين المعدل تبايُناً يُعْتَدُّ به إحصائياً حسب العمر، وكان أعلى مستوى له في الشابات في عمر 20-25 سنة (7.6٪) وأقل مستوى له في النساء من الفئة العمرية 36-40 سنة (2.2٪). وكانت مزرعة دياموند المُعَدَّلة هي الطريقة التي اكتشفت أعلى معدل للعدوى بالمشعّرة المهبلية.

ABSTRACT This study compared 4 different diagnostic methods for the detection of *Trichomonas vaginalis* in vaginal swab specimens from women attending a hospital in Dohuk in Iraq. A total of 425 vaginal swabs were obtained from women complaining of vaginal discharge associated with vaginitis, cervicitis and pelvic inflammatory disease. The results showed that 10 (2.4%) swabs were positive for *T. vaginalis* by wet smear preparation, 15 (3.5%) by haematoxylin–eosin stained smear, 17 (4.0%) by Papanicolaou stain and 23 (5.4%) using Diamond modified culture. The rate varied significantly by age and was highest in young women aged 20–25 years (7.6%) and lowest in the age group 36–40 years (2.2%). The highest rate of infection with *T. vaginalis* was detected by Diamond modified culture.

Dépistage de *Trichomonas vaginalis* par différentes méthodes chez des femmes de la province de Dahouk (Iraq)

RÉSUMÉ La présente étude a comparé quatre différentes méthodes diagnostiques pour le dépistage de *Trichomonas vaginalis* dans les prélèvements vaginaux de femmes consultant dans un hôpital de Dahouk (Iraq). Au total, 425 prélèvements ont été réalisés chez des femmes se plaignant de pertes vaginales associées à une vaginite, une cervicite ou une infection génitale haute. D'après les résultats, dix prélèvements (soit 2,4 % d'entre eux) se sont révélés positifs pour *T. vaginalis* par la méthode des préparations humides, 15 (3,5 %) par la méthode de coloration à l'hématoxyline et l'éosine, 17 (4,0 %) par la méthode de coloration de Papanicolaou et 23 (5,4 %) par la méthode de culture sur milieu de Diamond modifié. Le taux variait de manière significative en fonction de l'âge. Il était plus élevé chez les jeunes femmes âgées de 20 à 25 ans (7,6 %) et plus faible dans le groupe d'âge 36–40 ans (2,2 %). Le taux d'infection le plus élevé par *T. vaginalis* a été établi par la méthode de culture sur milieu de Diamond modifié.

[1]*Department of Medical Parasitology, College of Dentistry, University of Mosul, Mosul, Iraq (Correspondence to W.M. Al-Saeed: wisamalsaeed58@yahoo.com).*

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Introduction

Trichomonas vaginalis is a sexually transmitted, flagellated protozoan that causes vaginal infections in women, including vaginitis, urethritis and cervicitis [1]. *T. vaginalis* infections are not self-limiting and produce non-ulcerative inflammation of the genital epithelium that can progress to necrosis and haemorrhage [2,3]. Pregnant women infected with *T. vaginalis* may be at increased risk of premature labour, low-birth-weight offspring and postabortion or post-hysterectomy infection [4–6].

It has been estimated that 10% to 50% of *T. vaginalis* infections in women are asymptomatic [7], and in men the proportion may even be higher. The most common tool for diagnosis of *T. vaginalis* infection is still microscopic examination of wet mount preparations, which has a sensitivity of approximately 60% [8]. Microscopic examination of cultures of the parasite in specialized media improves the sensitivity to 85% to 95% [9–11], The most sensitive of these media is thought to be modified Diamond medium [9,10]. Direct microscopic examination of vaginal secretions is the most common and rapid method used to diagnose trichomoniasis. Culture of vaginal and urethral specimens is the most sensitive, although slower, diagnostic technique [11,12].

This study compared 4 different diagnostic methods for the detection of *T. vaginalis* in vaginal swab specimens obtained from women attending a hospital in Dohuk in Iraq.

Methods

Sample and data collection

The study sample was 425 female patients attending the department of gynaecology of Azadi hospital in Dohok province between October 2006 and June 2007 with complaints of vaginal and cervical infection. Two cotton swab specimens were obtained from the posterior vaginal fornix of all patients. The swabs were inserted into the pooled vaginal secretions touching both fornices and the middle third of the vaginal wall.

Laboratory methods

All swabs from the women were examined using 4 different laboratory methods. The first swab was used to produce a wet mount after mixing with normal saline for direct microscopic examination. Another 2 smears done from the first swab were fixed with 70% ethanol for further staining with Papanicolaou and haematoxylin–eosin stains [13,14]. A second swab specimen was immediately placed in 10 mL of Diamond modified medium (Becton Dickinson Microbiology Systems). This was prepared by dissolving 35 g of the powder media in 1 L distilled water, then bringing it to boiling point in order to dissolve the powder completely, then sterilization by autoclave at 121 °C for 15 min. The medium was left to cool. The modification involved adding 50 mL of rice starch water and 50 mL of inactivated horse serum, then 1000 units of penicillin and 500 μg of streptomycin and vancomycin were added to each mL of medium to inhibit the bacterial and fungal growth. Diamond modified medium was stored at 4 °C and allowed to reach room temperature prior to use. The vaginal swab was immersed in the newly prepared culture medium in bijou bottles under aseptic conditions (10–15 mL in each bottle), then incubated at 37 °C for 72 h.

Swabs were examined by wet mount microscopy. Microscopy was performed at a magnification of 400×, and 20 fields were examined. Other unused media were stored at 4 °C until needed. Daily examination of culture for 3–5 days by taking samples from the sediment or bottom of the vial culture were examined microscopically by wet mount for detection the *Trichomonas* spp. motility and activity. Smears stained with haematoxylin–eosin and Papanicolaou stains were examined under oil immersion (1000 ×).

Analysis

The data were analysed using the chi-squared and t-test to show the significance of any differences between those 4 diagnostic methods.

Results

Of 425 women complaining of vaginal secretions, vaginitis, cervicitis and pelvic inflammatory disease, 23 (5.4%) showed the presence of *T. vaginalis* in the specimens examined by different diagnostic methods. *T. vaginalis* were detected in 10 (2.4%) of the total by wet smear preparation, 15 (3.5%) by haematoxylin–eosin stained smear, 17 (4%) by Papanicolaou stain and 23 (5.4%) in specimens cultured using modified Diamond culture method (Table 1).

Table 2 shows the frequencies of infection with *T. vaginalis* in different age groups and using different diagnostic methods. The rate varied significantly

Table 1 Frequency of detection of *Trichomonas vaginalis* infection in symptomatic women (*n* = 425) by 4 laboratory methods

Test	No. of infected women	%
Wet smear	10	2.4
H&E stain	15	3.5
Pap. stain	17	4.0
Modified Diamond culture	23	5.4

H&E = haematoxylin–eosin; Pap = Papanicolaou.

Table 2 Frequency of detection of *Trichomonas vaginalis* infection by 4 laboratory methods in symptomatic women (*n* = 425) by age group

Age (years)	No. examined	No. infected	%	Test			
				Wet smear	H&E stain	Pap. stain	Modified Diamond culture
20–25	92	7	7.6	4	5	4	7
26–30	88	6	6.8	3	4	5	6
31–35	78	5	6.4	5	4	5	5
36–40	93	2	2.2	2	1	0	2
41–45	74	3	4.1	2	2	2	3
Total	425	23	5.4	16	16	16	23
		$t = 20.45$, $P < 0.001$			$\chi^2 = 3.097$, $P = 0.995$		

H&E = haematoxylin–eosin; Pap. = Papanicolaou.

by age and was highest in young women aged between 20–25 years (7.6%) and lowest in the age group 36–40 years (2.2%).

Discussion

T. vaginalis infections are regarded as the most prevalent non-viral sexually-transmitted disease (STD) and are similar to other STDs in that the prevalence increases with increased numbers of sexual partners, presence of other STDs (especially gonorrhoea) and failure to use barrier contraceptives [12].

In the current study wet smear preparation detected the fewest number of infections with *T. vaginalis* (2.4%), followed by haematoxylin–eosin stain (3.5%) and Papanicolaou stain (4.0%). The modified Diamond culture detected the highest rate of infection (5.4% of specimens). In most settings the microscopic evaluation of vaginal discharge (wet preparation) has been the standard method used to diagnose *T. vaginalis* infections. The wet preparation is fast and convenient for clinicians and is inexpensive, but in asymptomatic women the sensitivity of the wet preparation in demonstrating motile trichomonads (definitive diagnosis) is only 60% to 80% [12]. Other stains, such as haematoxylin–eosin and Papanicolaou have better sensitivities but are more labour-intensive and take time during processing of the stain and are moderately expensive [11]. Diamond's modified medium has been shown to be the most sensitive medium for the culture of *T. vaginalis* [9–11], possibly due to the starch content of the medium which gives similar environmental conditions as the vaginal epithelium [13]. However, the method needs as long as 3–7 days for confirmation of the diagnosis and is expensive when compared with previous methods.

Overall 5.4% of women complaining of vaginal and cervical infection were found to be infected with *T. vaginalis.* This rate is consistent with another study in Iraq, in Kirkuk (7.5%) [14], but disagrees with similar studies done in Erbil (10%) and in Mosul (14%) [15,16]. The lower rate found in our study might be due to the women using vaginal washing and antiseptics after coitus with their partners or to the existence of health education programmes about STDs arranged by the maternal care office. The rates in this study and others in the Iraq, however, are lower than that reported in some other counties. The rate of infection in a hospital study New York city was reported as 41.3%, and in a community study in South Africa as 41% [17].

The highest incidence of *T. vaginalis* was found in the age group 20–30 years, which is in agreement with Al-Samarrae's study in Iraq [18], and may be related to the greater sexual activity of this age group

In conclusion, the highest rate of infection with *T. vaginalis* was detected by Diamond modified culture.

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Sexually Transmitted Diseases Diagnostics Initiative

The Sexually Transmitted Diseases Diagnostics Initiative (SDI) was founded in 1990 in response to a widely-perceived need to improve care for patients with sexually transmitted infections (STIs) in resource-limited settings through improved diagnostics. It is estimated that 80%–90% of the global burden of STIs occurs in the developing world where there is limited or no access to diagnostics. SDI aims to promote the development, evaluation and application of diagnostic tests for STIs appropriate for use in primary health care settings in developing countries. Furth information about SDI can be found at: http://www.who.int/std_diagnostics/index.htm