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Determination of *Trichomonas vaginalis* Frequency Among Symptomatic Cases in Muğla Sıtkı Koçman Hospital Using Different Methods (Direct Microscopy, Culture, PCR and Immunochromatographic Method)

Muğla Sıtkı Koçman Üniversitesi Hastanesi'ndeki Semptomatik Olgularda *Trichomonas vaginalis* Görülme Sıklığının Farklı Yöntemler (Direkt Mikroskobi, Kültür, PCR ve İmmünokromatografik Yöntem) ile Araştırılması

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Abstract

Objective: *Trichomonas vaginalis* (*T. vaginalis*) is a sexually transmitted protozoan parasite that colonizes the urogenital system. Because of the various health risks it poses, the diagnosis of the parasite is crucial. Therefore, the current study investigated the incidence of *T. vaginalis* in cases with vaginal discharge complaints.

Materials and Methods: A total of 150 cases presenting with vaginal discharge complaints were included in the study conducted at Muğla Sıtkı Koçman Hospital's Gynaecology and Obstetrics Clinics. The demographic characteristics and clinical findings of the cases were recorded. Swab samples obtained from the cases were evaluated for the detection of *T. vaginalis* using direct microscopy (DM), culture, polymerase chain reaction (PCR), and immunochromatographic rapid diagnostic test (RDT).

Results: Among the 150 women studied, two cases (1.3%) were positive for *T. vaginalis* with DM, whereas three cases (2%) were positive using other methods, including culture, PCR, and RDT. There was no statistically significant difference between the methods ($p>0.05$). In addition to vaginal discharge, the most common symptoms observed were itching (45%) and abdominal pain (41%).

Conclusion: We reported the frequency of *T. vaginalis* in Muğla province. In addition, RDT was preferable for routine *T. vaginalis* diagnosis because of its ease of use, lack of equipment requirements, and rapid results.

Keywords: *Trichomonas vaginalis*, direct microscopy, culture, PCR, immunochromatographic technique

Öz

Amaç: *Trichomonas vaginalis* (*T. vaginalis*), cinsel yolla bulaşan bir protozoon olup ürogenital sisteme yerleşmektedir. Neden olduğu çeşitli sağlık risklerinden dolayı parazitin tanısı çok önemlidir. Bu nedenle mevcut çalışmada vajinal akıntı şikayeti olan hastalarda *T. vaginalis* varlığının araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya Muğla Sıtkı Koçman Üniversitesi Hastanesi Kadın Hastalıkları ve Doğum Poliklinikleri'ne vajinal akıntı şikayeti ile başvuran 150 olgu dahil edilmiştir. Olguların demografik özellikleri ve klinik bulguları kayıt altına alınmıştır. *T. vaginalis* saptanması için hastalardan alınan sürüntü örnekleri direkt mikroskopi (DM), kültür, polimeraz zincir reaksiyonu (PZR) ve immünokromotografik hızlı tanı testi (RDT) ile değerlendirilmiştir.

Bulgular: Olgularda saptanan akıntı semptomunun yanında en sık kaşıntı (%45) ve batın ağrısı (%41) olduğu belirlenmiştir. İki (%1,3) olgu DM yöntemi ile, üç (%2) olguda ise kültür, PZR ve RDT ile *T. vaginalis* pozitifliği saptanmıştır. Kullanılan tanı yöntemleri arasında istatistiksel olarak anlamlı fark saptanmadı ($p>0,05$).

Sonuç: Bu çalışmada Muğla ilinde *T. vaginalis* görülme sıklığı araştırılmıştır. Ayrıca, RDT yöntemi uygulamasının kolay olması, ekipman gerektirmemesi ve hızlı sonuç vermesi nedenleriyle *T. vaginalis*'in rutin tanısında kullanılabilceği düşünülmüştür.

Anahtar Kelimeler: *Trichomonas vaginalis*, direkt mikroskobi, kültür, PZR, immünokromatografik yöntem

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Introduction

Trichomoniasis, caused by *Trichomonas vaginalis* (*T. vaginalis*), is a prevalent sexually transmitted infection worldwide and infects only humans (1). It is an anaerobic protozoan, pear-shaped, measuring 10-20 µm in size, and possesses flagella (2). According to the World Health Organization, 156 million new cases of trichomoniasis were reported worldwide in 2016 (3).

Trichomonas vaginalis infection can manifest as asymptomatic or symptomatic, with a range of symptoms observed. In women, these symptoms may include vaginitis, urethritis, cervicitis, and endometritis, while in men, prostatitis and epididymitis can occur. Furthermore, trichomoniasis in women is characterized by yellow-green vaginal discharge, dysuria, genital itching, and burning sensations (4). Apart from sexual transmission, it has been reported that transmission of *T. vaginalis* can also occur through the use of shared bathwater and equipment, as well as during passage through the birth canal from an infected mother (5).

The prevalence of *T. vaginalis* varies depending on features such as sexual activity, age, presence of other sexually transmitted infections, socioeconomic factors, and diagnostic methods used (6). The prevalence of *T. vaginalis* infection has been reported as 5.3% in women and 0.6% in men (3). Studies conducted in Turkey have reported *T. vaginalis* prevalence rates ranging from 1.9% to 72.3% (7,8).

Direct microscopy (DM), culture methods, staining techniques, serology-based methods, molecular methods, such as polymerase chain reaction (PCR), immunochromatographic rapid diagnostic tests (RDT), and immunocytochemical methods are used in the diagnosis of *T. vaginalis*. Each of these methods has been reported to have unique advantages and disadvantages (9,10).

Our study aimed to investigate *T. vaginalis* using DM, culture, PCR, and immunochromatographic RDT in cases presenting with vaginal discharge complaints at the gynaecology and obstetrics outpatient clinic.

Materials and Methods

This study has been approved by the Muğla Sıtkı Koçman Universtiy Scientific Research Ethics Committee (decision no: 125, date: 21.08.2015). After obtaining ethics committee permission, cases who accepted the informed consent form were included in the study. A total of 150 cases between the ages of 18 and 45, presenting with vaginal discharge complaints, were included in the study at Muğla Sıtkı Koçman Universtiy Hospital Gynaecology and Obstetrics Clinics. For each included case, a patient information form was completed, which included personal information, complaints, and findings. Three vaginal swab samples were collected from the posterior fornix by a gynaecologist. The presence of *T. vaginalis* was investigated in the swab samples using DM, culture, PCR, and RDT.

An examination for *T. vaginalis* was performed at the patient's bedside using the RDT method from the swab sample obtained with a Dacron swab. The other swab samples obtained with a cotton swab were processed as follows: one sample was placed in tubes containing 8 mL of trypticase-yeast extract-maltose (TYM) with 10 µg/mL gentamycin, 100 U/mL penicillin-streptomycin, and another sample was placed in tubes containing 1 mL of physiological saline for DM and PCR. All samples were rapidly transported to the microbiology laboratory.

A drop of the vaginal swab sample, obtained in a tube containing 1 mL of physiological saline, was examined under a microscope using 20x and 40x objectives for DM examination. Samples in which *T. vaginalis* trophozoites were detected were considered positive. After the swab sample was inoculated onto TYM culture medium, it was incubated at 37 °C, and the presence of growth was microscopically examined daily for a week. The swab samples were collected in tubes containing 1 mL of physiological saline, centrifuged at 2,000 g, and stored at -20 °C until DNA isolation. DNA isolation was performed using the Nucleospin Tissue DNA Isolation Kit® according to the manufacturer's recommendations.

Trichomonas vaginalis specific primers TV3 (5' TTG TCG AAC ATT GGT CTTA CCC TC 3') and TV7 (5' TCT GTG CCG TCT TCA AGT ATG C 3') were used for PCR amplification. The reaction was set in a 30-µL volume containing 0.4 pmol of each of the primers, 0.3U of Taq DNA polymerase, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 2 mM magnesium chloride (MgCl2), and 1× Taq buffer with ammonium sulfate $(NH_4)_2SO_4$. The amplification was performed using the following protocol: initial denaturation step at 96 °C for 2 min and 30 cycles (1 min at 90 °C, 30 s at 60 °C, and 2 min at 72 °C) with a final extension step at 72 °C for 7 min. The PCR product was run on a 1.5% agarose gel and the gels were visualized using a gel documentation system (Vilber Lourmat, Basel, Switzerland).

The commercial RDT (OSOM) method (Sekisui Diagnostics, LLC, Lexington, PMA) was performed according to the manufacturer's recommendations. The swab obtained from the posterior fornix using a Dacron swab was placed in plastic tubes provided in the kit containing 0.5 mL of sample buffer. The swab was rapidly mixed in the sample buffer and left to stand for approximately one minute. Then, using the plastic tube, the swab was squeezed thoroughly and discarded. After, test strips were inserted into the plastic tubes and left for 10 minutes. At the end of this time, strips viewing only a red control line were measured negative, strips showing both a red control line and a blue test line were considered positive, and strips with no visible lines for both were considered invalid.

Statistical Analysis

The results of the methods were compared with the non-parametric McNemar test in SPSS statistics version 22.

Results

Vaginal swab samples from 150 different cases presenting with vaginal discharge at the Muğla Sıtkı Koçman Universtiy Hospital Obstetrics and Gynaecology Clinics were evaluated. Patient information forms containing personal details, complaints, and examination findings of the cases were reviewed. The age range of the cases was 18-45 years, with a mean age of 34.8±6.7. Of the cases, 91.4% were married, and 49% were working. The measurement data of the education level of the cases, the history of sexually transmitted disease in themselves or their partners, and the level of knowledge about *T. vaginalis* are given in Table 1. Evaluating the cases in terms of symptoms, the most common complaint following vaginal discharge was itching (45%). The characteristics of the discharge were determined as follows: yellow-green discharge (37%), curd-like discharge (33%), grey discharge (21%), and foul-smelling discharge (9%). The overall clinical findings of 150 women are given in Table 2.

In the current study, out of a total of 150 vaginal swab samples, two (1.3%) samples were positive for *T. vaginalis* in DM, while three (2%) samples were positive in culture, PCR, and RDT methods. The results of DM, culture, PCR and RDT diagnostic methods used in the detection of *T. vaginalis* are presented in Table 3. There was no statistically significant

Table 1. The analysis of demographic characteristics, clinical features and knowledge about trichomoniasis

		n	%
Job status	Working	73	49
	Housewife	77	51
Educational status	Primary school	44	29.4
	Middle school	20	13.3
	High school	50	33.3
	University	36	24
History of sexually transmitted disease	Exist	1	0.6
	None	149	99.4
History of sexually transmitted disease in partner	Exist	1	0.6
	None	149	99.4
T. vaginalis knowledge level	Exist	6	4
	None	144	96
Preterm labor	Exist	1	0.6
	None	149	99.4
Total		150	100
	Minimum-maximum	Mean ± SD	
Age (years)	18-45	34.8±6.7	

SD: Standard deviation

Table 2. The clinical findings of study population (n=150)

Symptoms	Number	%
Vaginal discharge	150	100
Vaginal itching	67	44.6
Lower abdomen pain	61	40.6
Genital pain	48	32
Vulval erythema	41	27.3
Dysuria	35	23.3
Punctate hemorrhagic spots (strawberry cervix)	29	19.3

Table 3. Evaluation of diagnostic methods in the detection of *T. vaginalis

	DM	OSOM	Culture	PCR
Positive	2 (1.3%)	3 (2%)	3 (2%)	3 (2%)
Negative	148 (98.7%)	147 (98%)	147 (98%)	147 (98%)
Total	150 (100%)	150 (100%)	150 (100%)	150 (100%)

p>0.05. DM: Direct microscopy, PCR: Polymerase chain reaction

difference between the methods (Mcnemar test, p>0.05). The ages of these cases were 35, 45, and 45, respectively. All positive cases presented with yellow-green discharge, along with symptoms and findings such as itching, lower abdominal pain, and vulvar erythema. The presentation of clinical findings of *T. vaginalis*-positive three cases were given in Table 4.

Discussion

Trichomoniasis represents a considerable global public health concern due to its associated health risks. Studies have indicated that the presence of the parasite is linked to infertility, cervical neoplasia, premature membrane rupture, and preterm labour (11). Also, trichomoniasis infection increases the risk of HIV-1 transmission by approximately 1.5-3 times, highlighting the importance of the infection (12). The use of more advanced diagnostic techniques, early diagnosis and appropriate behavioural approaches in developed countries has led to a decrease in the prevalence of *T. vaginalis* in the general population. However, in developing countries, it is still detected at high rates (13). Due to the asymptomatic nature and challenging detection of *T. vaginalis* infection, many diagnostic methods such as microscopy, molecular techniques, culture, and RDT are employed for the detection of the parasite in today's medical practice. However, these methods have disadvantages. Particularly, although the culture method is considered the gold standard for diagnosing the parasite, it has drawbacks such as susceptibility to contamination and a turnaround time of 2-7 days (14,15). Therefore, in recent years, the use of rapid point-of-care diagnostic tests has been increased because of easy handling and implementation for *T. vaginalis* diagnosis (16). One of these commercial RDT methods is OSOM that is FDA-approved and can be used at the point of care.

Multiple methods have been utilized in global studies for the diagnosis of *T. vaginalis*. However, there are only a limited number of studies that employ immunochromatographic-based rapid tests. In India, *T. vaginalis* investigation was conducted on 418 cases using DM, the rapid test, and culture. While the culture method detected 68 (16.3%) positive samples, wet mount microscopy identified 56 of the culture-positive samples along with four false positive samples. The OSOM test identified 60 of the culture-positive samples and two false-positive cases. This straightforward test has the potential to enhance the screening and diagnosis of *T. vaginalis* infection in settings where microscopy and culture are not available and resources are limited (17). In our study, one DM negative sample was positive with the OSOM test. In another study, vaginal swabs were collected from 835 female patients. The rates of positivity were determined as follows: 5.4% with DM, 8.1% with culture, 7.5% with the same rapid test, and 6.3% with acridine orange staining. The study concluded that due to its superior performance compared to other methods, the *Trichomonas* Rapid Test could be favoured in laboratory settings (18). In the United States, the prevalence of *T. vaginalis* among 449 cases with vaginal symptoms was determined to be 23.4%. The study highlighted the potential use of the RDT, especially in situations where microscopy and culture are not available (19). There are few studies in Turkey regarding the methods used for the detection of *T. vaginalis*, and no study comparing RDT has been identified. In the gynaecology clinic of Hatay province, among 104 symptomatic patients, 12 (11.53%) tested positive through DM, 14 (13.4%) through the culture method, 12 (11.53%) through Giemsa staining, and 5 (4.8%) through Papanicolaou staining. The sensitivities of direct microscopic examination, Giemsa staining, and cytological diagnosis were reported as 85.7%, 85.7%, and 35.7 % respectively, with specificities of 100% for all three methods (20). In a study conducted in Sivas, positivity was found in 258 cases with a pre-diagnosis of vaginitis at a rate of 1,9% with DM and 1,5% with culture method (7). Vaginal discharge samples were collected from 233 women with symptoms of vaginal discharge and vulvar itching in Manisa, as well as from 100 women in a control group who sought routine gynaecological examination without presenting vaginal discharge or vulvar itching. The samples were examined using the TYM medium, DM, and culture methods. Through both direct examination and culture, *T. vaginalis* was detected in 11 out of 233 (4.7%) patients with vaginitis. None of the 100 women in the control group showed evidence of the parasites in either method (21).

In the present study, vaginal swab samples from 150 cases presenting with vaginal discharge at Muğla Sıtkı Koçman Universtiy Hospital Gynaecology and Obstetrics Clinics were investigated for *T. vaginalis* using DM, TYM, PCR, and RDT methods. *T. vaginalis* was detected in two (1.3%) samples by DM and three (2%) samples by the other three methods. Limited studies are comparing multiple diagnostic methods for *T. vaginalis* in our country. Ertabaklar et al. (22) reported in their study in 2011 that PCR was used for the first time in our country for the diagnosis of *T. vaginalis*. In the current study, *T. vaginalis* positivity rates were found 2.9% by DM, and 4.9% by culture and PCR. Moreover, our

Table 4. Clinical presentation of *T. vaginalis* positive cases (n=3)

	Case 1	Case 2	Case 3
Vaginal discharge	+	+	+
Vaginal itching	+	+	-
Lower abdomen pain	-	+	-
Genital pain	+	+	-
Vulval erythema	-	+	+
Dysuria	-	+	+
Punctate hemorrhagic spots (strawberry cervix)	+	+	-

+: Present, -: Absent

study is the first study in Turkey to use the commercial RDT (OSOM) method for the diagnosis of *T. vaginalis.*

Many studies in the literature use more than one method for the diagnosis of *T. vaginalis*. However, limited studies are using immunochromatographic tests. The sensitivity of the rapid test has been reported to range from 83.3% to 100% (23,24), while the specificity ranges from 96% to 99.6% (18,24). In our study, although we did not find statistical differences between the methods. DM caused misleading of a *T. vaginalis* case, giving false negative results.

Conclusion

In conclusion, OSOM, which was used for RDT in this study, offers several advantages in antigen detection. These include being a point-of-care test, which means it is not affected by delays during transportation, and not requiring experienced personnel or specialized equipment. Additionally, it provides rapid results, and its high sensitivity, as reported in other studies, was also confirmed in our study. It can be concluded that the RDT test may a better option than DM for the diagnosis of *T. vaginalis*. However, further studies are needed to compare it with the reference methods, culture, and molecular methods.

Ethics

Ethics Committee Approval: This study has been approved by the Muğla Sıtkı Koçman Universtiy Scientific Research Ethics Committee (decision no: 125, date: 21.08.2015).

Informed Consent: After obtaining ethics committee permission, cases who accepted the informed consent form were included in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.S., E.T., Concept: F.S., S.E., H.E., Design: S.E., E.K., H.E., Data Collection or Processing: E.T., İ.Y., Analysis or Interpretation: S.E., E.M., İ.Y., H.E., Literature Search: F.S., S.E., E.M., Writing: F.S., E.K., H.E.

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