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A Comparison of Cytological and Parasitological Methods in the Diagnosis of *Trichomonas vaginalis*

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SUMMARY: *Trichomonas vaginalis* (*T. vaginalis*), which causes urogenital system infections in humans, develops symptomatically and asymptomatically. *T. vaginalis* in females is diagnosed using direct microscopy, Giemsa staining, and cultivation methods for examination of samples derived from the vaginal posterior fornix. Serologic methods can also be employed. In cytological diagnosis, the ectocervical smear is examined using the Papanicolaou (PAPS) stain. The aim of the present study was to compare the efficacy of the methods used in cytological and parasitological diagnosis. For this purpose, 506 female patients who visited the Obstetrics and Gynecology policlinic of the Academic Hospital of the Faculty of Medicine, Inönü University during a course of six years were involved in this study. The samples derived from the vaginal posterior fornix were examined in the parasitology laboratory, while the ectocervical samples were examined in the cytology laboratory. *T. vaginalis* was detected in 4.6% of the samples examined in parasitology laboratory, while parasites were found in only 0.9% of the samples taken to the cytology laboratory. The statistical analysis revealed a significant difference ($P<0.05$). It was concluded that parasitological methods are more sensitive than cytological methods in the diagnosis of *T. vaginalis*.

Key Words: *Trichomonas vaginalis*, parasitological diagnosis, cytological diagnosis

Trichomonas vaginalis'in Tanısında Sitolojik ve Parazitolojik Yöntemlerin Karşılaştırılması

ÖZET: İnsanlarda ürogenital sistem enfeksiyonlarına neden olan *Trichomonas vaginalis* (*T. vaginalis*) semptomatik ve asemptomatik seyreder. *T. vaginalis*'in tanısı, kadınlarda vagen arka forniksinden alınan örnegin; direkt mikroskobi, Giemsa boyama ve kültür yöntemleri ile incelenmesi sonucu konulur. Ayrıca serolojik yöntemlerden de yararlanılır. Sitolojik tanıda ise serviks ağzından alınan semear, Papanicolaou (PAPS) boyası ile incelenir. Sunulan çalışma sitolojik ve parazitolojik tanıda kullanılan yöntemlerin etkinliğinin karşılaştırılması amacıyla yapılmıştır. İnönü Üniversitesi Tıp Fakültesi Araştırma Hastanesi Kadın Hastalıkları ve Doğum Polikliniğine altı yıllık bir zaman sürecinde gelen 506 kadın hastanın vagen arka forniksinden alınan örnekler parazitoloji laboratuvarında, serviks ağzından alınan örnekler ise sitoloji laboratuvarında incelenmiştir. Parazitoloji laboratuvarına gelen örneklerin%4,6'sında *T. vaginalis* bulunmuş fakat sitolojiye gelen örneklerin ancak %0,9'unda bu parazite rastlanılmıştır. Yapılan istatistiki değerlendirmede anlamlı bir fark ($P<0.05$) bulunmuştur. Sonuç olarak *T. vaginalis*'in tanısında parazitolojik yöntemlerin sitolojik yöntemlere oranla daha hassas olduğu belirlenmiştir.

Anahtar Sözcükler: *Trichomonas vaginalis*, Parazitolojik tanı, Sitolojik tanı

INTRODUCTION

Trichomoniasis can be confused with all diseases concerning urinary tract and reproductive system in males and females (15, 16). Definite diagnosis can only be made after defining the agent (3, 15, 16). Trichomoniasis diagnosis can not be established based on clinical findings (15, 16).

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Although the symptoms of trichomoniasis in females include itching in vulva, yellow-green musty discharge from vagina and stomachache, not all of these symptoms are necessarily specific to the infection (3). Moreover in 90% of the trichomoniasis cases vaginal pH is over 4.5. This finding is not specific to trichomoniasis, however, since vaginal pH increases in 90% of women with bacterial vaginitis (3).

It is possible to detect *T. vaginalis* in women in urine sediment, vaginal discharge, and vaginal scrapings (2, 3). In the parasitological diagnosis of the *T. vaginalis*, the urine sample is centrifuged for urine sediment and preparation for microscopic examination is obtained from the bottom sedimentation. Diagnosis is made following the detection of trophozoites of parasite at 40-time-magnification under the light micro-

scope. For the detection of the agent in vaginal discharge or scraping, the sample derived from the vaginal posterior fornix with a sterilized swaps with the help of a speculum, is put into a tube containing 2-3 ml physiological solution or media (Csytein- Peptone- Liver- Maltose). The swaps put into the physiological solution are exposed to direct microscopic examination. In the cultivation method, however, samples are checked for trophozoites at 40-time-magnification under the light microscope 48 hours after cultivation (2, 3).

Cytological diagnosis is made by the examination of the Papanicolaou (PAP)-stained ectocervical cytology smear sample at 10-20-time-magnification under the light microscope (9).

Parasitological methods are believed to be more sensitive in the diagnosis of *T. vaginalis*, since the examination of trophozoites of *T. vaginalis* is spread at 100-times-magnification under stained microscopy (2, 3, 10). In cytological diagnosis, on the other hand, PAP-stained preparations are usually examined at 10-40-times-magnification (10, 13). Moreover the activities of the agent can be easily seen at 40-times-magnification under direct microscopy and cultivation in parasitology laboratory (2, 7).

This study intends to compare the efficacy of the methods used in cytological and parasitological diagnosis.

MATERIALS AND METHODS

Materials: The materials examined in this study include the samples derived from 506 female patients aged 20-60 living in and around Malatya province who visited the Obstetrics and Gynecology policlinic in Academic Hospital of Faculty of Medicine at Inonu University between 1999-2005. Two samples were derived from each woman for parasitological and cytological diagnoses. The female patients consulting at the policlinic with a discharge complain were informed about the *T. vaginalis* as the possible cause of their discharge and about the process of obtaining two samples. The patients brought both of the samples to the laboratory themselves. The patients were informed about the results and treated later on.

Parasitological Diagnosis: Dissections, taken from vaginal posterior fornix with eküvyon bar, were put into sterilized test tube containing serume fisiologic. Patient came to the laboratory with this tube. Dissections were analyzed by direct microscopy, giemsa stain ve culture (Csytein- Peptone- Liver- Maltose (CPLM) methods in parasitology laboratory.

Cytological Diagnosis: Dissections, taken from servical samples with smear brush. Patient came to the laboratory with these samples. They were coloured with Papanicolaou (PAPS) stain and were observed with light microscobe by enlarging 10 and 20 times.

Statistical Analyses: Values were given as numbers and percentages. The results were tested using dependent samples chi-square test. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 10.0 for Windows.

RESULTS

A significant difference was found between the rates of *T. vaginalis* infection in samples derived from 506 female patients in terms of two distinct methods of diagnosis used in Parasitological and Cytological laboratories (X^2= 18.0, $P<0.05$, Table 1).

Over 23 patients diagnosed with *T. vaginalis* infection, 23 positive cases were determined by using culture method and 22 cases were determined by using direct examination and stain method.

Table 1. The distribution of positive cases of *T. vaginalis* according to Laboratories

		Pathology Laboratory				Total	
		Positive		Negative			
		n	%	n	%	n	%
Parasitology Lab.	POS	5	0.9	18	3.5	23	4.5
	NEG	0	0.0	483	95	483	95
Total		5	0.9	501	99	506	100.0

Dependent samples X^2= 18.0, $P<0.05$

Images of *T. vaginalis* obtained in parasitology laboratory are shown in figures 1-3, while images obtained in pathology laboratory can be seen in figure 4.

DISCUSSION

Although the length of *T. vaginalis* varies depending on the pH of the habitat, it usually measures around 5-15 μm in length, with occasional cases of 30 μm (10, 14). Under favorable conditions it is small in acidic habitat and big in alkaline habitat (10, 14). Its shorter undulating membrane surrounds the axostyle and is about 1/3 or 2/3 of the body (10, 14). The axostyle, which keeps the body stretched, is a thin form starting as adjacent to nucleus and finishing with a sharp end penetrating the posterior extremity (10, 14).

The prevalence of the parasite is considerably high in women and societies with poor sexual hygienic measures (10, 14, 16). According to the relevant literature *T. vaginalis* infection cases vary between 10-90% (1, 10, 14, 16). Rassjo *et al*. (17), detected the infection at a rate of 8.0% in Kampala using polymerase chain reaction (PCR) method. Chakraborty *et. al*. (4), detected the parasite at a rate of 34% in Surat Aronud with cultivation method. And Klinger *et. al*. (12), reported the prevalence of *T. vaginalis* was 10.7% in women and 6.3% in men. Moreover Chen *et. al*. (5) reported to have found the presence of parasite at a rate of 43.2% in China using enzyme-linked immunosorbent assay (ELISA) method. No previous study was found, however, in the literature about the comparison of the cytological and parasitological methods used in the diagnosis of the parasite.

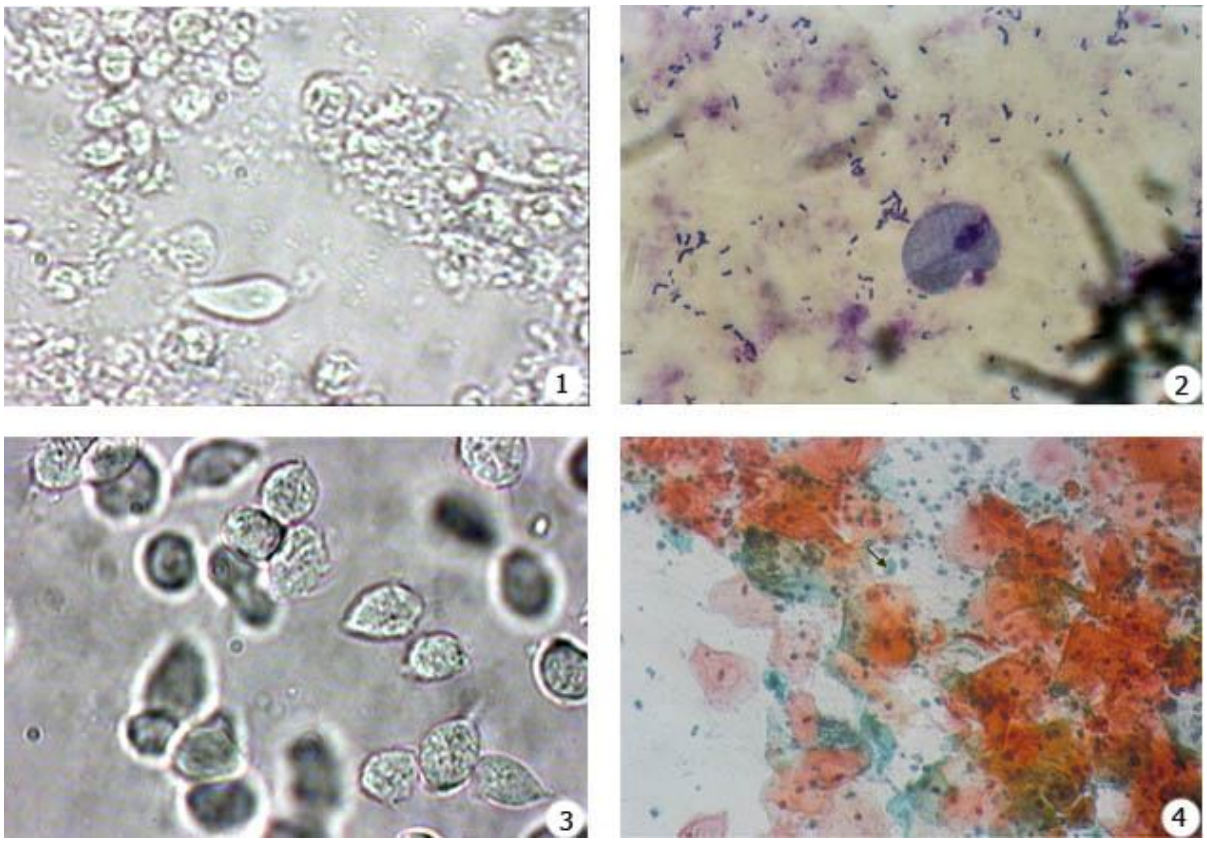

Figure 1. *T. vaginalis* in direct microscopy 400X; **2.** *T. vaginalis* in Giemsa 1000X;
3. *T. vaginalis* in cultivation (CPLM) 400X; **4.** *T. vaginalis* in PAPS stain. 100X

Using parasitological diagnosis method, Karaman *et al.* (16), found the rate of the parasite in Malatya as 8.1% in a comprehensive study to identify the epidemiology of *T. vaginalis*. Similarly Daldal *et al*. (8), detected parasite in 14 of 33 bar girls working in the same region. In the present study 4.5% of the samples brought to parasitology laboratory were found to be infected.

The methods used for the diagnosis of the parasites in the parasitology laboratory included direct examination, Giemsa staining, and cultivation methods. Cultivation methods are important in the diagnosis of *T. vaginalis*. It has been reported that cultivation method coupled with direct examination method increases the sensitivity of the test (2, 3, 7, 16, 17). Likewise Churakov *et al*. (6), reported to have obtained similar results from PCR and cultivation methods. Again Chakraborty et al. (4), reported that cultivation method is more sensitive in the diognosis of the *T. vaginalis* in and around Surat.

Demirezen (9), reported that in cytological diagnosis of the *T. vaginalis,* parasite in the smear is seen as inactive and without flagella due to the fixation procedure, and what is unique to cytological diagnosis is the ability to see the oval nucleus and basophilic cytoplasm of the parasite. Malkawi *et al*. (13), also reported to have detected parasites in cervical smears at a rate of 0.9%. In the Parasitological diagnosis, on the other hand, parasite can be observed as active in direct microscopy and cultivation (2, 3). In the Giemsa method, trichomonasis are seen in oval form, nucleus in red, and cytoplasm in purple and granular form, while the flagellas, undulating membrane, and axostyle are stained well (2, 3). This makes the diagnosis of the parasite easier. The methods used in Parasitology laboratories are both cost effective and highly sensitive.

As a result of the examinations *T. vaginalis* was found in 23 (4.5%) of 506 samples brought to the parasitology laboratory. However, parasite was detected in 5 (0.9%) of the samples brought to the cytology. And a significant difference was found in the statistical analysis ($P<0.05$).

The smears of the 23 samples found positive in parasitology laboratory were examined once again in cytology laboratory, which revealed 20 positive at 100-times-magnification. It was thought that the remaining three negative samples were either taken from the patients under unfavorable conditions, then not fixed duly causing poor parasite density or affected by intensive erythrocyte.

The possible reasons for the failure of cytological diagnosis to

detect the parasite may include its lack of a characteristic ground for *T. vaginalis*, extreme bleeding on the sample, thick nature of spread, and negligence of the parasite at older ages.

Consequently, it was concluded that parasitological methods are more sensitive than cytological methods in the diagnosis of the *T. vaginalis*.

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