

Brief communication (Original)

Investigation of the presence of *Trichomonas vaginalis* in infertile Turkish women

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Background: Trichomoniasis is a sexually transmitted vaginal infection caused by a protozoan called *Trichomonas vaginalis*. Its prevalence ranges between 3–40% among randomly tested women.

Objectives: To investigate the presence of *T. vaginalis* in infertile women presenting to a Turkish gynecology outpatient clinic using parasitological methods.

Methods: The study population comprised 51 patient participants who attended the gynecology clinic of Mustafa Kemal University Hospital between March and August 2013. The women were divided into those presenting with infertility (n = 22) and a patient control group (n = 29). Women in the control group had complaints other than infertility. Microscopic examination, Giemsa staining, and cysteine–peptone–liver–maltose culture were performed on samples taken from posterior fornix tested for *T. vaginalis*.

Results: *T. vaginalis* was observed in 18% (n = 4) of infertile patients and in none of the control group (P = 0.03).

Conclusions: Asymptomatic infertile women should be examined for *T. vaginalis* infection, which may play a role as a cause or contributing factor in infertility.

Keywords: Infertility, parasitological diagnosis, *Trichomonas vaginalis*

Trichomoniasis is a common sexually transmitted parasitic disease [1]. Its prevalence is usually higher than for other sexually transmitted diseases (STDs). Diagnosis of *Trichomonas vaginalis* infection is cumbersome, and the infection is often asymptomatic and underdiagnosed. Trichomoniasis is an STD that increases the risk of human immunodeficiency virus (HIV) infection [2]. Asymptomatic trichomoniasis is associated with pregnancy complications such as premature rupture of membranes or amniorrhexis, preterm labor, low birth weight, endometritis after cesarean section, and abortus. Furthermore, newborn girls may be infected during birth and their infection may remain silent [3]. Trichomoniasis was found to be related to infertility and cervical dysplasia [4]. *T. vaginalis* infection, though often asymptomatic, may also result in a broad spectrum of signs and

symptoms, such as foul smelling vaginal discharge, pelvic pain, dyspareunia, and dysuria. Furthermore, *T. vaginalis* infection may cause infertility because of endometritis, salpingitis, and atypical pelvic inflammatory symptoms [5, 6].

There is no simple diagnostic method with adequate sensitivity and specificity for *T. vaginalis* infection [1]. Therefore, special diagnostic tests are needed to confirm the presence of *T. vaginalis*. Diagnosis is usually made via observing parasites in a wet mount preparation. Although this method is inexpensive and fast, sensitivity is not high and the parasite is detected in only 60% of culture positive samples. Novel diagnostic methods such as nucleic acid amplification are more sensitive than routine diagnostic methods, but more expensive and not generally available [7, 8].

The aim of this study was to compare the presence of *T. vaginalis* in infertile and fertile Turkish women and to investigate the association of *T. vaginalis* with infertility.

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Materials and methods

The ethics committee of Zeynep Kamil Women's and Children's Hospital, approved the study (protocol number 30.09.2013/93) and written informed consent was obtained from all patient participants.

The study population comprised 51 participants applying to the gynecology outpatient clinic between March and August 2013. The participants were divided into 2 groups: an infertility group ($n = 22$) and control group ($n = 29$). Women in the control group had complaints other than infertility. Women having systemic disease, pregnancy, foul smelling vaginal discharge, and taking antibiotics were excluded from study. Routine gynecological examinations were performed after women were informed about the objectives of the examination and a signed consent form was obtained. Three samples were taken from the posterior fornix via sterile cotton swabs during a speculum examination. The first sample was mixed with 1 mL sterile saline and direct microscopy was performed. One drop from the mixture was put on a slide and examined using light microscopy at $\times 400$ magnification. The second sample was examined using light microscopy after Giemsa staining. The third sample was cultured in tubes containing cysteine–peptone–liver–maltose medium (CPLM). The tubes were incubated at 37°C for 48 hours and examined.

In both groups, after 12 h fasting on the third day of the menstrual cycle, the hormone profile or the patient and complete blood counts were measured.

Hormone profile included follicle stimulating hormone (FSH), luteinizing hormone (LH), and 17β -estradiol (E2). These hormones were measured using standard enzymatic methods with a fully automated random access chemiluminescence-enhanced enzyme immunoassay system (Roche Laboratory Systems, Mannheim, Germany).

The data obtained were analyzed using PASW Statistics for Windows, version 18.0 (SPSS Inc, Chicago, IL, USA). The relationship between categorical variables in groups was evaluated using a chi-square or Fisher's exact test. The relationship between continuous variables in groups was evaluated using a Student t test. $P < 0.05$ was considered significant.

Results

T. vaginalis testing was positive in 18% ($n = 4$) of women in the infertile group and *T. vaginalis* was not observed in the control group (**Figure 1**).

There was a significant difference in prevalence of *T. vaginalis* between the groups ($P = 0.03$ (odds ratio: 2.6 (95% confidence interval 1.8 to 3.7))).

The mean age, hemoglobin, hematocrit, platelet counts, FSH, LH, and E2 levels of all participants are presented in **Table 1**. Reasons of infertility are shown in **Table 2**.

While three patients with *T vaginalis* had unexplained infertility, one patient infected with *T vaginalis* also had male factor infertility.

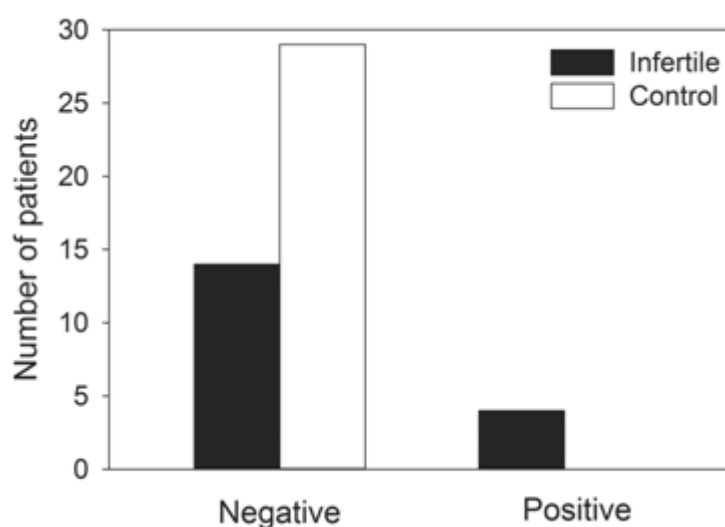


Figure 1. Incidence of *Trichomonas vaginalis* in infertile and control patient groups

Table 1. Relationship of continuous variables in between groups

	Infertility	Control	P ^a
Age (years)	29.5 ± 4.63	32.7 ± 6.45	0.06
Hemoglobin (g/dL)	12.09 ± 1.50	12.45 ± 1.48	0.40
Hematocrit (%)	35.87 ± 4.67	37.20 ± 4.63	0.32
Platelets (×1000/mm ³)	301.59 ± 136.34	242.51 ± 65.63	0.046
FSH (IU/L)	14.52 ± 17.41	10.18 ± 8.75	0.25
LH (IU/L)	7.64 ± 5.97	8.88 ± 7.88	0.54
Estradiol (pmol/L)	105.92 ± 175.92	89.35 ± 87.44	0.66

^aStudent *t* test, FSH = follicle-stimulating hormone, LH = luteinizing hormone

Table 2. Causes of infertility in infertile group

	Frequency	Percent
Unexplained	7	31
Female factor (tubal factor and low ovarian reserve)	8	36
Male factor	7	31

Discussion

Trichomonas vaginalis infection results from an anaerobic flagellated protozoan and may result in serious health consequences, but is readily treatable [9]. This infection has been related to vaginitis, endometritis, salpingitis, infertility, preterm labor, low birth weight, increased incidence of human papilloma and HIV infections, and cervical cancer [2, 10]. The incidence of infection is high in adolescent girls [5, 6, 11].

Diagnosis of trichomoniasis is made by observing parasite trophozoites using light microscopy, direct florescent antibody testing, latex agglutination, enzyme-linked immunosorbent assays, and molecular polymerase chain reaction techniques. Culture and staining techniques may be used in diagnosis of disease in samples taken from vaginal, urethral, prostatic secretions, and urine [12, 13]. Culture is considered to be the criterion standard for diagnosis of trichomoniasis with a sensitivity of 95% [12]. We performed microscopic examination, Giemsa staining, and CPLM culture on samples taken from the posterior fornix.

Prevalence of *T. vaginalis* was found to be 3.1%–8.7% in reproductive aged women in USA by nucleic acid amplification test [14, 15]. The prevalence may increase to 38% in drug using African-American women and 9%–30% in HIV positive women [16, 17]. The prevalence of *T. vaginalis* in Turkish women depends on the study population and diagnostic

methods used. Akarsu et al. reported the prevalence of *T. vaginalis* infection among women working in brothels in Ankara at 4.9% (n = 246) where the diagnosis was by direct microscopy and culture [18]. Sonmez-Tamer et al. detected *T. vaginalis* in 5.4% of women with vaginal discharge by direct microscopy and 9.3% of these women by culture (n = 128) [19]. In a retrospective study, the presence of *T. vaginalis* was investigated in random infertile women with vaginal discharge and in asymptomatic infertile women 2 weeks before in vitro fertilization. *Trichomonas vaginalis* was detected in only 3% of women with vaginal discharge using a FDA-cleared nucleic acid probe test [10]. In the present study, we found the prevalence of *T. vaginalis* was 18% in infertile women by culture in those with vaginal discharge. Consistent with our study, El-Shazly et al. [20] showed significantly higher *T. vaginalis* in a group of infertile women compared with a control group (14.58% vs 2.5% respectively).

Increased levels of IgA, IgG, Th1 cytokines and reactive nitrogen intermediates were observed in rats having experimentally induced *T. vaginalis* [8, 21]. Antibodies against *T. vaginalis* were detected in circulation and vaginal mucosa of infected women [8, 22, 23]. *T. vaginalis* cysteine proteases including CP30 may induce apoptosis of vaginal epithelial cells and multiple mucosal immune cell types [8, 24]. These immunological changes may be a cause of infertility.

The prevalence of *T. vaginalis* infection increased in infertile women having tubal factor infertility and pelvic inflammatory disease [20, 25]. Interestingly, the prevalence of *T. vaginalis* infection was 25% in women with male factor infertility and 75% in women with unexplained infertility. *T. vaginalis* was not detected in women with tubal factor infertility in our study. These findings may be related to immunological changes because of infection or independently. Our study is limited because of its small sample size.

Conclusion

Asymptomatic infertile women should be examined for *T. vaginalis* infection. Further studies are needed to elucidate the pathophysiology of infertility in relation to *T. vaginalis* infection.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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