

The Frequency of *Trichomonas vaginalis*, *Gardnarella vaginalis* and *Candida* ssp. Among Infertile Men and Women with Vaginitis ^[1]

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Summary

Trichomonas vaginalis, *Gardnarella vaginalis* and *Candida* spp. were known as important causes of sexually transmitted infection in developing countries. The prevalence and spectrum of trichomoniasis, *Gardnarella vaginalis* and *Candida* spp. in infertile men and women with vaginitis are not yet fully elucidated. We analyzed the presence of *T. vaginalis*, *G. vaginalis* and *Candida* spp. in 80 infertile males and 160 females with a diagnosis of vaginitis using wet mount microscopy, Giemsa staining, culture and PCR methods. The specimens were obtained from posterior vaginal fornix in woman and prostatic expression fluid or semen in men. The ages of the men were ranged from 16 to 60 (median±SD, 31±9), and that of women were ranged from 17 to 70 (median±SD; 37±11). Among female patients, *G. vaginalis* was positive in 110 (68.8%), *Candida* spp. was positive in 39 (24.4%) and *T. vaginalis* was positive in 7 (4.5%). Among male patients, *G. vaginalis* was positive in 20 (25%), *Candida* spp. was positive in 8 (10%) and *T. vaginalis* was positive in 3 (3.8%). Our findings suggest that *T. vaginalis*, *G. vaginalis* and *Candida* spp. should be considered for the etiology of the infertile male patients and female patients with complaints of discharge, pain, dysuria, itching and dry mouth.

Keywords: *Trichomonas vaginalis*, *Giardia vaginalis*, *Candida* spp., Infertile males, Females, Sexually transmitted diseases

İnfertil Erkek ve Vajinitli Kadın Hastalarda *Trichomonas vaginalis*, *Gardnarella vaginalis* ve *Candida* ssp. Sıklığı

Özet

Gelişmekte olan ülkelerde *Trichomonas vaginalis*, *Gardnarella vaginalis* ve *Candida* spp. cinsel ilişki ile bulaşan enfeksiyonların önemli etkenleri olarak bilinir. İnfertil erkeklerde ve vajinitli kadınlarda trichomoniasis, *G. vaginalis* ve *Candida* spp. yaygınlığı henüz tam olarak aydınlanmamıştır. Bu çalışmada 80 infertil erkek ve 160 vajinit tanısı almış kadın hastada *T. vaginalis*, *G. vaginalis* and *Candida* spp. varlığı direct mikroskopi, Giemsa boyama, kültür ve PCR yöntemleri kullanılarak araştırılmıştır. Örnekler kadınlarda posterior fornixten veya vajinal akıntıdan, erkeklerde ise prostate sıvısı veya semenden alınmıştır. Erkek hastaların yaşı 16-60 (31±9) kadın hastaların yaşı ise 17-70 (37±11) arasında değişmekte idi. Kadın hastaların 110'unda (%68.8) *G. vaginalis*, 39'unda (%24.4) *Candida* spp. ve 7'sinde (%4.5) *T. vaginalis* saptanırken erkek hastaların 20'sinde (%25) *G. vaginalis*, 8'inde (%10) *Candida* spp. ve 3'ünde (%3.8) *T. vaginalis* saptanmıştır. Bulgularımız erkek hastalarda infertilite etiyolojisinde ve vajinal akıntı, ağrı, disüri ve kaşıntı yakınması olan kadın hastalarda *T. vaginalis*, *G. vaginalis* ve *Candida* türlerinin dikkate alınması gerektiğini göstermektedir.

Anahtar sözcükler: *Trichomonas vaginalis*, *Giardia vaginalis*, *Candida* spp., İnfertil Erkekler, Kadınlar, Cinsel yolla bulaşan hastalıklar

INTRODUCTION

Infectious agents can impair various important human functions, including reproduction ¹. Infection with the protozoan parasite *Trichomonas vaginalis* is the most

common nonviral sexually transmitted infection (STI), with prevalence estimate frequently surpassing those for *Gardnarella vaginalis* (one of the causative agents of



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bacterial vaginosis) and *Candida* species in both sexes. Infection of the female genital tract can result in vaginitis, cervicitis and urethritis, and trichomoniasis has been associated with adverse pregnancy outcomes. Though it was once virtually ignored, *T. vaginalis* infection in men is now recognized as an important cause of nongonococcal urethritis and is associated with and male factor infertility but can be frequently asymptomatic ². In addition to this infected men are accepted as important vector for transmission of the parasite. Definition of the correct agent causing vaginal discharge is of utmost importance for successful way of treatment. We previously demonstrated that *T. vaginalis* should be kept in mind as a rare cause of male factor infertility at least in developing and underdeveloped countries ³. Several suggestions have been made to expand our study with the addition of *G. vaginalis* and *Candida* spp. both in male and female patients. In this study, we aimed to evaluate microbial causes of suspected genital infections in men and women, and to define the frequency of genital infection.

Trichomonas vaginalis, *G. vaginalis* and *Candida* spp. infections are highly prevalent worldwide ⁴. The incidence of trichomoniasis in general low risk population is less than 1%. *T. vaginalis* infection in men is suggested as an important cause of nongonococcal urethritis ⁵, though most of the trichomoniasis infected males are asymptomatic. Infected men are accepted as important vector for transmission of the parasite. A white urethral discharge and itching may develop. The infection in men can progress to prostatitis, urethritis, epididymitis, and superficial penile ulcerations ⁶. *In vitro* analysis reported that *T. vaginalis* by product rapidly killed sperm, this may contribute to infertility in infected couples ⁷. Decreased sperm motility and morphology in the trichomoniasis were reported, and that both abnormalities in sperm improved significantly after treatment ^{8,9}. Since nucleic acid amplification analysis more sensitively detects the microorganisms, we examined the presence of *G. vaginalis*, *T. vaginalis* and *Candida* spp. in infertile male and women with vaginitis applied to outpatient department of Firat University Hospital, located at conservative East Anatolian region.

MATERIAL and METHODS

From 1 June to 31 December 2009, fresh semen was collected from 80 male patients with complaints of infertility and/or prostatitis at the Outpatient Department of Firat University Hospital and fresh vaginal discharge was collected from 160 female patients with a complaint of vaginitis at the outpatients departments of Sarahatun Obstetrics and Gynecology Hospital. The ages of the male patients were ranged from 16 to 60 (mean±SD; 31±09), and that of female patients were ranged from 17 to 70 (mean±SD; 37±11). Informed consents were explained to the patients, and approvals were obtained. A brief

questionnaire was filled in, including questions about demographics, signs and symptoms, history and toilet habits. The preferred birth control method was also included for female patients. The patients taken antibiotics during the preceding 14 days were excluded. From male patients, semen samples and prostate massage fluids obtained by digital rectal massage were collected into sterile beakers. From female patients, three samples of vaginal discharge were obtained with a sterile swab from the posterior vaginal fornix during pelvic examination. One of the samples was inoculated to the freshly prepared and brought to room temperature TYM (Tripticase-Yeast extract-Maltose) culture medium without delay. The two other swabs were transported to the laboratory by putting into the tubes containing 1 ml sterile saline. The same procedures were also applied to the semen and prostatic massage fluid samples within sterile bakkers. The inoculated culture mediums were kept in oven for 7 days and reproductive controls were evaluated. The presence of *G. vaginalis*, *T. vaginalis* and *Candida* spp. were determined by using wet mount microscopy, Whiff test, Gram and Giemsa staining, culture and PCR. The diagnosis of *G. vaginalis* was established by observation of clue cells on stained and unstained preparations with direct microscopic examination, and whiff test was evaluated together with markedly diminished *Lactobacillus* and despite scarcity of leucocytes roughly presence of maximum one leukocyte per each epithelial cells. *Candida* spp. was investigated by direct microscopic examination of yeast cells and pseudohyphae on painted and unpainted preparations. *T. vaginalis* was investigated by direct microscopic examination of painted and unpainted preparations, culture and PCR. Negative wet mounts were examined for at least 10 min.

Culture

Tripticase-yeast extract-maltose (TYM) medium was used for culture of *T. vaginalis*. Cultures were incubated at 37°C and examined daily for up to 7 days for the presence of motile trichomonads.

DNA Isolation

Semen and vaginal discharge samples for DNA isolation processed by use of the blood lysate method of pure link Genomic DNA kits (Kat.No:K1820-02; Invitrogen). For PCR positive control, we used DNA isolates by use of gram-negative bacterial cell lysate method of pure link Genomic DNA kits from positive culture of the patient and frozen DNA samples were kept at -20°C.

Beta Globin Spesifik PCR

The presence of DNA in vaginal discharge and semen preparation were verified by using beta (β)-globin (oligonükleotid) primers on PCR ¹⁰. The sequences were as follows: β-globin forward: 5'-GAA GAG CCA AGG ACA GGT AC-3' and (β)-globin reverse: 5'-CAA CTT CAT CCA CGT

TCA CC-3'. The resulting PCR products were 268 bp. PCRs were performed using a gradient thermocycler (Biometra professional). The final reaction mixture (50µl) contained 10 pmol of each primer, 2.5 mM dNTP (Bio Basic Inc) 10XPCR buffer (Bio Basic Inc), 5U/µl Tsg DNA polymerase (Bio Basic Inc) and 10 µl semen and vaginal discharge DNA in a 0.5 µl microcentrifuge tube. Cycling times were 5 m at 95°C, followed by 30 cycles of denaturation temperature 95°C for 30 s, annealing temperature 60°C for 1m and ending at 72°C for 2 m and extension temperature of 72°C for 10 min. Then, the samples were cooled to +4°C. The amplified PCR products were electrophoresed in 1.5% agarose gel and detected by staining with ethidium bromide according to Standard molecular biological procedures. The sizes of the amplified PCR products were compared with a commercial 50-bp ladder (Fig. 1).

Trichomonas vaginalis Spesific PCR

Beta (β)-globin primers were used to clarify the presence of targeted lenght DNA band in all the specimens. The DNA sequences for the primer set (TV1/TV2) were designed to target region 648 bp TV-E650 of *T. vaginalis*. The sequences were as follows: TV1 forward: 5' GAG TTA GGG TAT AAT GTT TGA TGT G-3' and TV2 reverse: 5'- AGA ATG TGA TAG CGA AAT GGG-3': the resulting PCR products were 330 bp (Fig. 2).

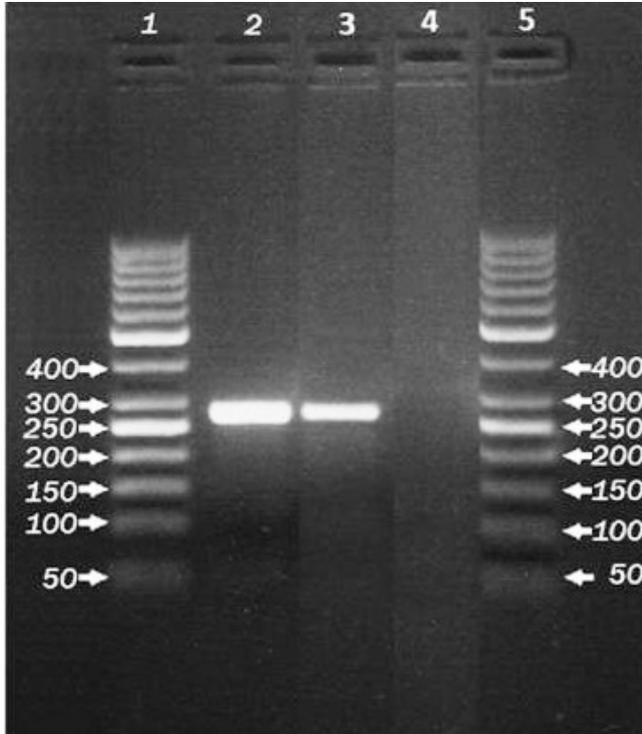


Fig 1. Agarose gel electrophoresis of Beta (β)-globin PCR products: Line1 and 5, 50 bp ladder, Line 2 and 3 amplification product of (β)-globin of patient, Line 4, negative control

Şekil 1. Beta (β)-globin PCR ürünü agaroz jel elektroforezi. 1. ve 5. sütun DNA belirteci; 2. ve 3. sütun hastanın (β)-globin amplifikasyon ürünü; 4. sütun negatif kontrol

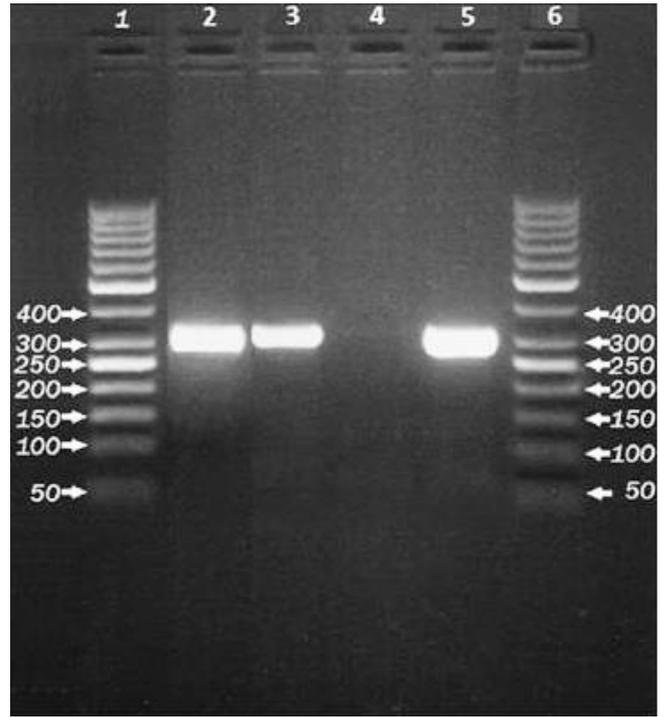


Fig 2. Agarose gel electrophoresis of *Trichomonas vaginalis* PCR products. Line 1 and 5, 50- bp ladder, Line 2, amplification product of *T. vaginalis*-positive female patient, Line 3, amplification product of *T. vaginalis*-positive male patient, Line 4 negative control, Line 5, positive control

Şekil 2. *Trichomonas vaginalis* PCR ürünü agaroz jel elektroforezi. 1. ve 6. sütun DNA belirteci; 2. sütun *T. vaginalis* pozitif kadın hasta; 3. sütun *T. vaginalis* pozitif erkek hasta; 4. sütun negatif kontrol; 5. sütun pozitif kontrol

RESULTS

The mean age of the patients was 31 years (range 16 to 60) in men, and the mean age of the patients was 37 years (range 17 to 70) in women. Patients' characteristics have been shown in Table 1. 99.4% of the female and 63.8% of the male patients were married. While none of the male patients were illiterate, 32.5% of the patients were illiterate. The overall education levels of the female patients were clearly lower than that of male patients included in our study. More than 90% of the patients were using ottoman style toilets. None of our male patients were using contraceptive methods.

The distribution of symptoms was shown in Table 2. Urethral discharge was observed in 142 (88.8%) female patients and in 7 (8.75%) male patients, disuria in 101 (63.1%) female patients and in 12 (20%) male patients, groin pain in 134 (83.3%) female patients and in 16 (20%) male patients, lumbar pain in 122 (76.3%) female patients and in 16 (20%) male patients, itching in 5 (3.8%) female patients and in 12 (15%) male patients, dry mouth in 8 (10%) male patients, and ejaculatio precox was observed in 13 (16.25%) patients.

Among female patients, *G. vaginalis* was positive in

Table 1. Patients' characteristics and habits**Tablo 1.** Hasta özellikleri ve alışkanlıkları

Patients' Characteristics and Habits	Female		Male	
	n	%	n	%
Marital Status				
Married	159	99.4	51	63.8
Unmarried	1	0.6	29	36.3
Education				
Illiterate	52	32.5	-	-
Primary education	85	53.1	10	12.8
Secondary education	10	12.5	20	33.3
High education	3	1.9	42	53.8
Toilet Habits				
Ottoman style	145	90.6	66	93.0
European style	13	8.1	2	2.8
Both	2	1.3	3	4.2
Contraceptive Method				
No	66	41.2		
Intrauterine device	30	18.8		
Condom	42	26.3		
Pills	7	4.4		
Tubal ligation	7	4.4		
Hysterectomized	5	3.1		

110 (68.8%), *Candida* spp. was positive in 39 (24.4%) and *T. vaginalis* was positive in 7 (4.5%). Among male patients, *G. vaginalis* was positive in 20 (25%), *Candida* was positive in 8 (10%) and *T. vaginalis* was positive in 3 (3.8%) (Table 3).

DISCUSSION

Vaginitis is one of the commonly observed women disease in all aged group. There is a miss believe in women with vaginal discharging regarding the improvement of disease without treatment. Thus, these patients generally neglect to attend to hospital for treatment. The co-infection, which can be resulted misdiagnosis and/or insufficient treatment in addition to this miss believe, may cause infertility¹¹. Gor et al.¹² have been reported that bacterial vaginosis (40-45%), vulvovaginal candidiasis (20-25%), and trichomoniasis (15-20%) are the main reasons in womens with symptomatic vaginitis. *G. vaginalis*, *Mycoplasma hominus* and *Ureaplasma urealyticum* are the main agents for the bacterial vaginosis. *Candidal* infections are responsible of 40% to 50% of vaginal infections and *Candida albicans* is a normal component of vaginal flora¹².

Trichomoniasis is a widely observed nonviral sexually transmitted infection. The prevalence of trichomoniasis shows variability with life style and sociocultural structure of the targeted population. Ours is the first report of the frequency of trichomoniasis in males at the Eastern part of

Table 2. Patients' complaints**Tablo 2.** Hasta yakınmaları

Complaints	Female				Male			
	Positive		Negative		Positive		Negative	
	n	%	n	%	n	%	n	%
Discharge	142	88.8	18	11.2	7	8.8	73	91.0
Dysuria	101	63.1	59	33.0	18	22.5	62	76.5
Groin pain	134	83.3	26	17.0	16	20.0	64	80.0
Lumbar pain	122	76.3	38	24.0	16	20.0	64	80.0
Itching	5	3.8	155	95.7	2	15.0	68	85.0
Dry mouth					8	10.0	72	90.0
Ejculatio precox					13	16.25	67	83.7

Table 3. Incidence of *T. vaginalis*, *G. vaginalis* and *Candida* spp. in Patients' samples**Tablo 3.** Hasta örneklerinde *T. vaginalis*, *G. vaginalis* and *Candida* spp. sıklığı

Infectious Agents	Female				Male			
	Positive		Negative		Positive		Negative	
	n	%	n	%	n	%	n	%
<i>Candida</i> spp.	39	24.4	121	76.6	8	10.0	72	90.0
<i>G. vaginalis</i>	110	68.8	50	31.2	20	25.0	60	75.0
<i>T. vaginalis</i>	7	4.4	153	95.6	3	3.8	77	96.2

Turkey. The frequency of *T. vaginalis* ranged from 2.18% to 72.3% among female patients in previous studies from our Country¹³⁻²⁶. This variability suggestedly may stem from risk characteristics of targeted study group²⁷. A previous report from the same geographical region reported 5.4%-8% *T. vaginalis* positivity among different risk groups¹⁹. We found 4.4% positivity of *T. vaginalis* among female patients, and for the first time reported 3.8% positivity of *T. vaginalis* among infertile male patients³.

In the male infertility case, male genito-urinary tract infections are responsible for about 15%. These infections could be seen different sites of the male reproductive tract. The *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the most commonly observed microorganisms involved in sexually transmitted infections of infertile male. However, non-sexually transmitted epididymo-orchitis, generally caused by *Escherichia coli* and *T. vaginalis* are less frequently results male infertility^{1,3}.

In the present study, we have found 10% *Candida* spp., 25% *G. vaginalis* and 3.8% *T. vaginalis* in infertile male. *T. vaginalis* incidence was similar with the results of previous studies performed in Turkey. However, it should be emphasise that existence of *Candida* spp. and *G. vaginalis* in infertile male was firstly described in our region. The observation of *Candida albicans* has been reported in semen flora of asymptomatic infertile male. Onemu and Ibeh have been reported that *Candida albicans* was found 7.7% in semen culture of Nigerian infertile male²⁸. The incidence of *G. vaginalis* has been varied between 9.6-38% in different studies performed in infertile male²⁸⁻³². There is no sufficient data concerning the *Candida* spp. and *G. vaginalis* in infertile male in Turkey. However, our results concerning the incidence of *G. vaginalis* were similar with the reported results from other countries.

Wet mouth microscopy examination is an easily applied practical method for the diagnosis of *T. vaginalis*. Because of low sensitivity of the wet mouth microscopy, culture test have been added. PCR was suggested as more effective for *T. vaginalis* than wet mount microscopy and culture tests⁴.

We observed no association of patients characteristics depicted in [Table 1](#) with the positivity of the *T. vaginalis*, *G. vaginalis* and *Candida* spp. Others³³ reported relationships of *T. vaginalis* positivity with marital status, education levels, occupation and toilet habits.

Our findings suggest that *T. vaginalis*, *G. vaginalis* and *Candida* spp. should be considered for the ethiology of the infertile male patients and female patients with complaints of discharge, pain, dysuria, itching and dry mouth.

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