Diagnosis of Trichomoniasis in Male Patients on Performing Nested Polymerase Chain Reaction

Erkek Hastalarda Trichomoniasis Tanısında Polimeraz Zincir Reaksiyonu (Pzr) Kullanımının Etkinliğinin Araştırılması

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ABSTRACT

Objective: Trichomoniasis is a parasitic infection that occurs with the settlement of *Trichomonas vaginalis* in female and male urinary and reproductive tracts. This infection is generally asymptomatic in males, and males are thought to be a carrier for the transmission of infection. In this study, our aim was to detect trichomoniasis using nested polymerase chain reaction among males who were referred to a hospital with suspected urinary tract infection.
Methods: Urine samples were collected from 138 male patients between 18 and 50 years of age who were referred with suspected urinary system infection to the Urology Outpatient Clinic at Malatya University Medical Center Malatya between December 2013 and May 2014. Direct microscopy, two different culture methods, and nested Polymerase chain reaction (PCR) were performed for the investigation of *T. vaginalis* in urine samples.
Results: Urinary tract infection was diagnosed in 47 of the 138 patients according to white and red blood cell counts in the urine samples. *T. vaginalis* infection was detected in 6.5% (9/138) of the suspected patients by nested PCR, while none of the samples tested positive by direct microscopy and culture examinations. Statistical significance was found between infection of the urinary tract and nested PCR positivity for *T. vaginalis*.
Conclusions: According to our results, nested PCR is the most sensitive method for the detection of trichomoniasis in male patients. We strongly recommend using nested PCR for the differential diagnosis of urinary infections in males.
Keywords: *Trichomonas vaginalis*, direct microscopy, culture, nested PCR
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ÖZ

Amaç: Kadın ve erkekte idrar ve üreme yollarında *Trichomonas vaginalis*'in yerleşmesi ile oluşan parasite infeksiyon Trichomoniosis olarak adlandırılır. Erkeklerde bu infeksiyon büyük çoğunlukla asemptomatik seyretmekte ve parazitin bulaşmasında taşıyıcı rolü üstlendikleri düşünülmektedir. Bu çalışmada trichomoniosis tanısı koyulmayan erkeklerdeki gizli taşıyıcılığı Nested Polimeraz Zincir Reaksiyonu (PZR) yöntemi ile açığa çıkarmak amaçlanmıştır.
Yöntemler: Çalışmada Malatya İnönü Üniversitesi Tıp Fakültesi Turgut Özal Tıp Merkezi'ne Aralık 2013-Mayıs 2014 tarihleri arasında idrar yolu infeksiyonu ön tanısı ile Üroloji Polikliniğine başvuran 18-50 yaş arası 138 erkek hastadan idrar örnekleri; direct mikroskobik bakı, kültür ve Nested PZR yöntemleri ile incelenmiştir.
Bulgular: Direkt mikroskobi ve kültür yönteminde *T. vaginalis*'e rastlanılmazken, Nested PZR yöntemi ile %6,5 (9/138) hastada *T. vaginalis* DNAsı pozitif bulunmuş ayrıca pozitif bulunan hastalar ile idrar yolu infeksiyonu arasındaki ilişki de istatiksel açıdan anlamlı bir farklılık olduğu tespi tedilmiştir.
Sonuç: *T. vaginalis* ile enfekte erkeklerin çoğunda semptom bulunmadığı için *T. vaginalis* araştırılması açısından gözardı edildiği, gizli taşıyıcı olarak hastalığı yaymaya devam ettikleri, *T. vaginalis*'in saptanmasında Nested PZR yönteminin çok hassas ve gizli taşıyıcıları açığa çıkarmak amacıyla tanıda mutlaka kullanılması gereken bir yöntem olduğu kanısına varılmıştır.
Anahtar Kelimeler: *T. vaginalis*, infeksiyon, direktmikroskobi, kültür, Nested PZR
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INTRODUCTION

Trichomonas vaginalis is an important and curable sexually transmitted infection (STI), and worldwide, the number of people estimated to have this infectionis187 million adults (15 to 49 years of age) (1). *T. vaginalis* infection causes vaginitis and cervicitis with profuse, frothy vaginal discharge and dysuria; pelvic inflammatory disease; preterm birth; and low-birth weight babies in women and urethral discharge (non-gonococcal urethritis), which is often asymptomatic and can be associated with prostatitis, epididymitis, and male factor infertility, in men. In addition, *T. vaginalis* infection has been implicated as a significant risk factor in the sexual transmission of human immunodeficiency virus (HIV) and other possible bacterial and viral STIs as well as of cervical cancer (2-5).

There are limited studies on trichomoniasis among female and male patients in Turkey. *T. vaginalis* was found in 8 (7%) of 116 women (114 and 2 were in the reproductive and postmenopausal periods, respectively) showing nonspecific vaginal discharge during their gynecological examination by microscopy and the cysteine-peptone-liver-maltose (CPLM) culture method (6). A total of 253 women (aged from 20 to 48 years) with abnormal vaginal discharge who applied to the Obstetrics and Gynecology outpatient clinic were enrolled, and 22 (8.69%) trichomoniasis cases were detected by performing a direct native examination and Giemsa staining (7).

T. *vaginalis* infection in male urine samples was diagnosed in 3 (2.7%) and 1 (0.9%) out of 110 patients with urethritis and control group without any symptoms admitted to the urology clinic by direct microscopical examination of the centrifuged urine samples, respectively (8).

Urine samples of 768 male patients were examined for *T. Vaginalis* infection by microscopy, and 3 patients (0.2%) were found to be positive (9). Urethral discharge in 85 male patients with non-gonococcal urethritis was investigated for *T. vaginalis* infection, and 5 (5.8%) and 2(1.4%) positive results were found on performing microscopy and trypticase-yeast extract-maltose (TYM) culture, respectively (10). Urethral discharge and urine sediments of 100 male patients with non-gonococcal urethritis were examined for *T. vaginalis* infection, and 12 (12%) and 4 (4%) positive results were found, respectively. None of the samples showed positivity on performing CPLM culture (11).

Trichomoniasis is usually asymptomatic or shows nonspecific clinical symptoms; worldwide, diagnosis based on laboratory test results is crucial for the treatment and control of the disease (2). Conventional diagnostic techniques involved direct examination of the parasite and CPLM and TYM culture methods. Culture methods are time consuming; they have lower sensitivity than other diagnostic methods and are not standard suitable tests in routine diagnostic laboratories. Direct microscopy of the parasite is a cheap, fast, and sensitive method but requires vaginal or urethral discharge samples and an examination to be performed immediately. Because of all these reasons, there is a requirement for sensitive, time-independent, standard tests using noninvasive sampling for diagnosing trichomoniasis. In recent years, techniques using Polymerase chain reaction (PCR) have provided new approaches to increase the sensitivity as well as to use noninvasive body fluids such as urine (2, 3, 12-14). In the present study, we aimed to detect *T. vaginalis* infection in urine samples obtained from males by performing nested PCR and to compare the results with those obtained using conventional methods.

METHODS

Sampling

Samples were collected from 138 males who were admitted to the Urology Outpatient Clinic in Medical Faculty Turgut Özal Center of Malatya İnönü University located in Malatya between December 2013 and May 2014. The inclusion criteria were (**i**) males between 18 and 50 years of age, (**ii**) complaints related to the urinary tract, and (**iii**) biochemical analyses of urine (counts of white and red blood cells). Two tubes of urine samples were collected from the patients: one for direct examination and the other for culture inoculation.

Urinary tract infection criteria: The presence of more than five leucocytes with more than three erythrocytes in urine sediments of the patients in a high-power (400×) microscopic field was accepted as urinary tract infection (15-16).

Ethical permission was provided from Malatya University Medical Faculty Ethical Committee (protocol no: 2013/149), and urine biochemical analysis data of the patients were obtained from hospital records.

Direct microscopy and culture

Wet mount preparations of urine samples of the patients were prepared immediately from first-void urine samples after centrifuging at 400 rpm for 5 min, and sediments were examined under a 40× objective for the detection of *T. vaginalis* trophozoites.

Cysteine-peptone-liver-maltose and TYM cultures were prepared according to the previous literature (17). At least 1 ml of the urine sediment was inoculated into both culture tubes and was incubated at 37°C for a week. Then, tubes were examined every 2 days for the presence of *T. vaginalis* infection.

DNA isolation

First-void urine samples were centrifuged at 8000 rpm for 10 min, and the pellet was stored at −20°C until DNA was isolated. After dissolving the pellet under room temperature, DNA was isolated using a Qiagen DNA Easy Blood & Tissue kit (Hilden, Germany) according to the manufacturers protocol and was stored at −20°C until Nested PCR was performed.

Nested PCR

Before the experiments, the PCR protocol was optimized using DNA obtained from a local *T. vaginalis* isolate. The first (TVC3F/ TVC4R) and second PCR (TVC11F/ TVC12R) primer sets were used as previously reported (2). PCR was performed using ready-to-use master mix (Nano Helix Co., Ltd. Helix Amp™) with 20 ng of DNA and 10 pmol of each primer. PCR conditions were as follows: 95°Cfor 2 min, followed by 35 cycles of 20 s at 95°C, 40 s at 50°C, 40 s at 72°C, and 5 min at 72°C. PCR products were visualized after performing gel electrophoresis on GelRed™-stained 1.2% agarose gel. The detection of band in 237 bp was accepted to be positive. To eliminate DNA contamination, PCR, DNA isolation, and gel electrophoresis were performed in separate rooms.

Statistical analysis

Statistical associations between positivity and urinary tract infection were determined by applying Fisher's exact test.

RESULTS

Totally, 138 males were included; their mean age was average 36 years. According to the biochemical analyses of the urine sediment, 47 of the 138 patients tested positive for urinary tract infection.

We could not detect any *T. vaginalis* trophozoites by direct microscopy and culture methods, whereas 237 bp bands were observed in gel electrophoresis by nested PCR in 9 of the 138 patients (6.5%) (Figure1). Fisher's exact Test results revealed a significant relationship between urinary tract infection and nested PCR positivity (p=0.003) (Table 1 and 2).

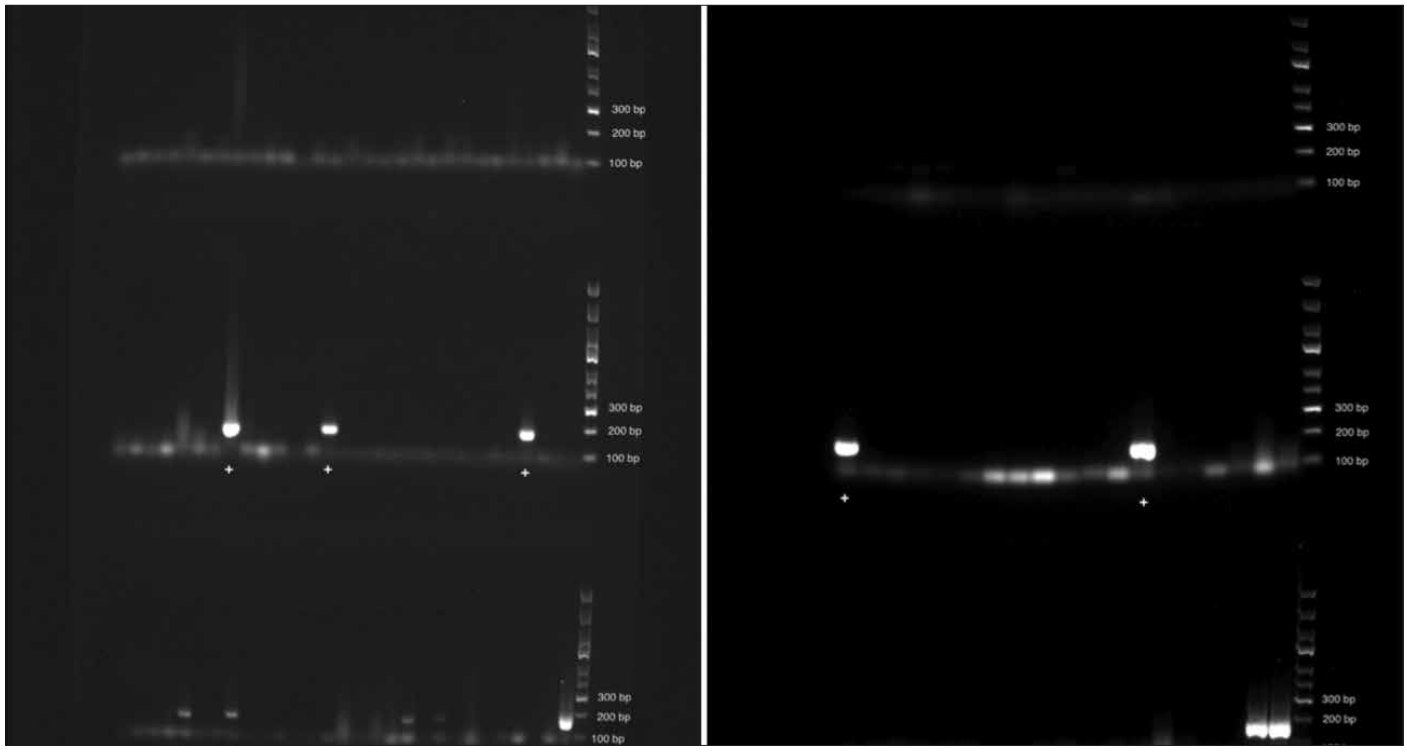

Figure 1. Nested Polymerase chain reaction gel electrophoresis showing in trichomoniasis-positive urine samples

Table 1. Results of patients positive for *Trichomonas vaginalis* infection

Patient No	Age	Direct Microscopy	Culture	PCR	Leucocyte/HPF	Erythrocyte/HPF
37	49	NEG	NEG	POS	271	12
43	34	NEG	NEG	POS	35	43
55	25	NEG	NEG	POS	965	28
63	32	NEG	NEG	POS	49	33
66	18	NEG	NEG	POS	NEG	NEG
77	48	NEG	NEG	POS	306	14
79	41	NEG	NEG	POS	25	5
105	46	NEG	NEG	POS	125	38
117	28	NEG	NEG	POS	3	7
PCR: polymerase chain reaction; HPF: highest possible frequency; NEG: negative; POS: pozitive						

Table 2. Relationship between nested PCR and urinary tract infection

		Infection		
		Negative (%)	Positive (%)	Total (%)
Nested PCR	Negative (%)	89 (69.5)	40 (30.5)	129 (100)
	Positive (%)	2 (20.0)	7 (80.0)	9 (100)
Total		91 (65.9)	47 (34.1)	138 (100)
PCR: Polymerase chain reaction				

DISCUSSION

The estimation of the total number of new patients with important STIs such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* infections and syphilis between the ages of 15 and 49 years showed that trichomoniasis is the most abundant infection globally (1).

The infection is reported to be asymptomatic in 70 to 80% of infected men; in those with symptoms, urethral discharge and dysuria are the main symptoms (4-5). Techniques for PCR have been developed to detect *T. vaginalis* infection in women; however, the detection of *T. vaginalis* infection in men by PCR has received less attention. Urine-based PCR assays for men have been described and PCR of urine samples from men was found to be much more sensitive than culture methods for detecting *T. vaginalis* infection. PCR screening of *T. vaginalis* infection among men with recurrent lower urinary tract symptoms was strongly recommended as a part of public health initiatives to control trichomoniasis (3).

There are limited studies on trichomoniasis among female and male patients in Turkey. *T. vaginalis* infection was found 4.5%, 7%, and 8.69% among women with vaginal discharge by conventional methods such as microscopy and culture in Turkey (3-6-7). *T. vaginalis* infection was diagnosed in 2.7% and 0.9% male patients with urethritis and in an asymptomatic control group by microscopy of centrifuged urine samples, respectively (8). Similarly, 0.2% of urine samples of male patients were found to be positive by microscopy (9). However, positivity rates of 5.8% and 1.4% were revealed by microscopy and culture methods using urethral discharge of male patients with non-gonococcal urethritis, respectively (10). Similarly, urethral discharge and urine sediments of male patients with non-gonococcal urethritis showed positivity rates of 12% and 4%, while none of the samples tested positivity by the culture method (11). In the present study, urine samples were chosen as their collection is a non-invasive sampling method and nested PCR was performed as a sensitive and specific method; our results were compared with those of conventional methods such as microscopy and culture method. We found no positivity using microscopy and culture methods, but a positivity rate of 6.5% was detected by performing nested PCR, which is comparable with other results obtained in previous studies that used urethral discharge and/or urine samples. To our knowledge, our study is the first on trichomoniasis among male patients in Malatya, Turkey. In a previous study in 2008 that was conducted in female patients in Malatya, *T. vaginalis* infection was detected at 4.6% from vaginal discharge samples on performing microscopy and culture methods in 2008. These results show that there is a need for standard routine diagnostic assays such as nested PCR for the correct and timely treatment as well as for the control of trichomoniasis.

The presence of more than five leucocytes and more than three erythrocytes in urine sediments of patients under a high-power (400×) microscopic field is accepted as urinary tract infection respectively. One of the reasons for microscopic hematuria is urinary tract infection (15-16). In the present study, according to white and red blood cell counts in urine, 47 out of the 138 patients tested positive for urinary tract infection. Fisher's exact Test results revealed a statistically significant relationship between urinary tract infection and nested PCR positivity (p=0.003) (Table 1, 2). There were two *T. vaginalis*-positive patients without urinary tract infection. Urine analysis of one of them was negative for leucocytes and erythrocytes and accepted as asymptomatic infection. The other patient showed three leucocytes and seven erythrocytes, which can be a sign of suspected urinary tract infection. There is a requirement for conduction further studies with trichomoniasis patients to understand the relationship between laboratory analyses and trichomoniasis.

Male trichomoniasis can be a source of infection for the partner, and trichomoniasis has been implicated as a significant risk factor for the sexual transmission of HIV and other possible STIs as well as of cervical cancer (2-4).

CONCLUSION

Direct microscopic, culture and Nested PZR methods were applied to investigate the presence of T. *vaginalis* in urine specimens of working male patients and it was concluded that Nested PZR method is more sensitive than the other methods. Although the PZR method is an expensive method, it improves the diagnostic sensitivity and makes the hidden carriers in the community more open to treatment of more cases. In addition, asymptomatic persons should also be screened to remove those affected, to determine the actual prevalence of the disease in the population, it is recommended for taking precautions.

Ethics Committee Approval: Ethics committee approval was received from Malatya Universitesi, Medical Faculty of the Ethics Committee (Decision Date: 2013 Decision No: 149).

Informed Consent: Written informed consent was obtained patient who participated in this study.

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