Detection of Trichomonas Vaginalis in Vaginal Speciemens from Women by Wet Mount, Culture and PCR

Trichomonas vaginalis Tanısında Mikroskopi, Kültür ve PCR Yöntemlerinin Karşılaştırılması

Trichomonas Vaginalis Tanısında Mikroskopi, Kültür ve PCR / Diagnosis of Trichomonas Vaginalis Microscopy, Culture and PCR

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Özet

Amaç: Trikomonosiosis, seksüel geçişli enfeksiyona sebep olan Trichomonas vaginalis nedenlidir. T.vaginalis, dünyada 180 milyon insanı etkilemektedir ve anlamlı morbidite sebebidir. T.vajinalis enfeksiyonu kadınlarda vajinit, ekzoservisit ve üretrit nedenidir. Bu çalışmanın amacı, üç değişik yöntem sonuçlarını karşılaştırarak T. vajinalis varlığını tespit etmektir. Gereç ve Yöntem: Mustafa Kemal Üniversitesi Tıp Fakültesi Jinekoloji polikliniğine ve Doğumevi hastanesine başvuran toplam 200 hastadan alınan swap örnekleri toplandı. : Mustafa Kemal Üniversitesi Tıp Fakültesi Parazitoloji bölümünde T. vajinalis genotip ve fenotipileri değerlendirildi. T. vaginalis tespiti açısından ve hasta tedavisine etkisi açısından bu çalışma bölgemizde yapılmış ilk moleküler çalışmadır. Bulgular: 200 hastanın 56 sı pozitif olarak bulundu ve bunların 24'ü (%42.8) mikroskopik olarak, 18'i (%32,1) kültür ile, 24'ü (%42,8) PCR ile tespit edildi. Tüm metodlarla tespit edilen hasta sayısı 14 idi. Bu çalışmada, Cochran's Q testi ile elde edilen üç metod karşılaştırılması anlamlı idi. (P = .022). McNemar yöntemi ile tüm yöntemler ikili olarak karşılaştırıldığında mikroskopi ile kültür arasında (P = .5),mikroskopi ile PCR arasında (P = .063), kültür ve PCR arsında (P = .25) fark bulunamadı. Tartışma: Kültür metodu rutinde kulanılan bir metod değildir ve kontaminasyon riski vardır. PCR metodu direkt olarak parazit DNA'sını göstermektedir ve diğer yöntemlere göre daha güvenilir bir yöntemdir.

Anahtar Kelimeler

Trichomonas Vaginalis; Mikroskopi; Kültür; PCR

Abstract

Aim: Trichomoniasis, a sexually transmitted infection (STI) caused by Trichomonas vaginalis, affects 180 million people worldwide and causes significant morbidity. Infection with T. vaginalis has been associated with vaginitis, exocervicitis, and urethritis in women. Material and Method: In this study, we aim to investigate the presence of T. vaginalis by using three different methods for comparing the results. Two hundred T. vaginalis isolates taken from swap samples were collected in Medical Faculty, Department of Gynecology, Mustafa Kemal University Polyclinic, and examined genotypically and phenotypically to identify T. vaginalis in Parasitology Department. This research is unique in terms of its contribution to patient treatment, being the first molecular study in Turkey/Hatay to determine Trichomonas (TV) genes stemming from Trichomonas vaginalis strains. Result: 56 out of 200 patients examined were identified as positive and 24 (42.8%) of these were identified through microscopy, 18 (32.1%) with culture and 24 (42.8%) with PCR. The number of those identified through all these methods is 14 (25%). In this study, difference was calculated using three methods (P = .022) with Cochran's Q test. When compared with McNemar two by two, no superiority in T. vaginalis diagnosis was found between microscopy and culture (P = .5), microscopy and PCR (P = .063), or culture and PCR (P = .25) methods. Discussion: Culture method is not used in routine laboratory procedures and has contamination risk. PCR method shows directly the parasite of DNAs, and so it is thought to be more reliable compared to the other two methods.

Keywords

Trichomonas Vaginalis; Microscopy; Culture; PCR

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Indroduction

The female genital system has a strange microflora with different species alive. Among them is pruning of T. vaginalis infection,[1] an important factor. It has been suggested that T. vaginalis infection plays a role in the pathogenesis of preterm birth, preterm rupture of membranes, and posthysterectomy cuff infections.[2,5] Trichomoniasis, a sexually transmitted infection (STI) caused by T. vaginalis, affects 180 million people worldwide and causes significant morbidity. Infection with T. vaginalis has been associated with vaginitis, exocervicitis, and urethritis in women.[6] Symptoms of trichomoniasis are vaginal discharge, vulvovaginal soreness, and/or irritation. Dysuria and dyspareunia are also other common symptoms. Recent studies have suggested that T. vaginalis increased the transmission of especially Human Immunodeficiency Virus (HIV) Type I. Furthermore, predisposing effect of the development of cervical cancer has been reported.

T. vaginalis infection can be asymptomatic in 10 to 50 % of women[7] and the characteristics of the vaginal discharge including color and odor are poor predictors.[8,9] Since no symptom alone or in combination is sufficient to diagnose T. vaginalis infection reliably, laboratory diagnosis is a necessary tool. Wet-mount microscopy is the most commonly employed laboratory method for diagnosing trichomoniasis.[4] Although this test is rapid and inexpensive, it has a limited sensitivity of 20–60%.[10]

Culture is the current reference standard in the diagnosis of trichomoniasis, but is rarely used in routine laboratory tests.[11] This technique is slow, taking up to seven days of incubation, requires daily microscopy and is relatively expensive. In remote areas of central and northern Australia, the commercially available InPouch culture showed a sensitivity of 63%.[8] Several Polymerase chain reaction (PCR) assays targeting various regions of the T. vaginalis genome have been described for diagnosis of this infection.[10] PCR improves detection of T. vaginalis infection compared with culture using self-collected vaginal swabs.[12]

T. vaginalis's repeated DNA is a target for highly sensitive and specific for polymerase chain reaction diagnosis. Detection of trichomonosis in vaginal and urine specimens from women is achieved by culture and PCR. 18S ribosomal DNA are the basis os PCR for diagnosis of T. vaginalis.[9] These studies showed a detection sensitivity by PCR in the range of 89-98%. A recent study showed that T. vaginalis DNA is undetectable after two weeks of treatment with metronidazole in 85% of individuals . Prevalence, incidence, natural history, and response to treatment of T. vaginalis infection among adolescent women[13] are impostant factors in its diagnosis.

Molecular methods have been shown to be the most sensitive and specific for the diagnosis of infectious agents. Recently, polymerase chain reaction (PCR) has been used for the diagnosis of parasitic infections.[14]

In this study, we aim to investigate the presence of T. vaginalis in vaginal secretion samples by wet mount microscopy, culture and PCR and compare the results of these methods.

Material and Method

Clinical samples, staining and culture procedures: The study population included 200 symptomatic women (between 20-50 years) attending out patients clinic of Mustafa Kemal University, Medical Faculty, Department of Gynaecology and Hatay Maternity Hospital between 2009-2011. Vaginal swabs taken from vaginal discharge of woman were examined by wet mount microscopy, culture and polymerase chain reactin (PCR) in Mustafa Kemal University Department of Parasitology. Ethical approval for our study was obtained from Mustafa Kemal University.

Wet mount microscopy: For each patient, vaginal discharge was carefully collected from the posterior vaginal fornix with a sterile cotton swab and polyethylene transfer pipette. A portion of each vaginal discharge specimen (100 ml) was combined with an equal volume of normal saline (0.9% NaCl). Immediately, one drop of this mixture was applied to a glass slide, covered with a coverslip, and examined at x100 and x400 magnifications with a light microscope for the presence of T. vaginalis.

Culture; Trypticase-yeast extract-maltose (TYM) medium without agar (pH:6.0) supplemented with 10% heat inactivated bovine serum, penicillin (1000 IU/ml) and streptomycin sulphate (1 mg/ml) was used for the cultivation of the organism. Before vaginal swabs were placed into the medium, culture tubes were warmed to 37 oC or 15 min. Inoculated tubes were incubated for seven days at 37 oC, and examined daily under microscope. Presence of motile organisms was accepted as positive.

DNA Isolation was performed as previously reported by Sharma et all.[17] Briefly, specimens were centrifuged at 2,000 g for 10 min. The supernatant was discarded and the pellet was resuspended with 600 ml of lysis buffer (1 M Tris, 0.5 M EDTA, 10% glucose, and 2 mg of lysozyme per ml), heated at 80°C for 5 min, and then cooled to room temperature.

The classic phenol/chloroform extraction method was used for nucleic acid extraction from the T. vaginalis samples, and DNA was precipitated in 1 ml 70 % ethanol and 90 % ethanol. The DNA precipitate was dissolved in 50 µl of TE buffer (10 mM Tris chloride-1 mM EDTA [pH 8.0], and stored at -20 °C until processing.

PCR Method of Tv3 and Tv7 Amplification were performed using primers amplifying 300 bp of T. vaginalis. The sequences of primers were as follows: Tv3 forward, 5'-ATTGTCGAACATTGGTCTTACCTC-3' and Tv7 reverse, 5'- TCTGTGCCGTCTTCAAGTATGC-3'(Table 1). The PCR mixture consisted of 5 µl 10x PCR buffer, 4 µl of dNTP (2.5 mM), 50 mol each primer, 0.5 µl Taq DNA polymerase (5 U/ml), 10 µl template DNA, Total volume completed 50 µl with steril distilled water. Positive and negative controls were included in all PCR runs. Cycling conditions were 5 min at 95 °C initial denaturation, followed 30 cycles of 1 min at 90 °C, 30 s at 60 °C, and 2 min at 72 °C and an additional extension step at 72°C for 7 min. DNA markers with defined molec¬ular weights in the range of 100 to 2000 and a reference strain were used. The PCR products were analyzed in a 2% (wt/vol) agarose gel in 1× TAE buffer (40 mM, Tris-acetate, 1 mM EDTA). Ethidium bromide (0.5 g/ml TAE) -stained DNA amplicons were visualized using a gel-imaging system (Thermo,

Table 1. The primer sequences and predicted size used in the PCR Tv

Primer	Oligonucleotide sequence	Size of amplifield product(bp)
Tv3	5'-TCTGTGCCGTCTTCAAGTATGC-3'	
Tv7	5'-ATTGTCGAACATTGGTCTTACCTC-3'	300

Sparks, NV). A size of 300 bp product was considered as positive for T. vaginalis. (Figure.1)

Statistical analyses; were assessed using SPSS (Statistical Package for the Social Sciences) statistical 19 software packaging. Mc Nemar and Cochran's Q tests were used.

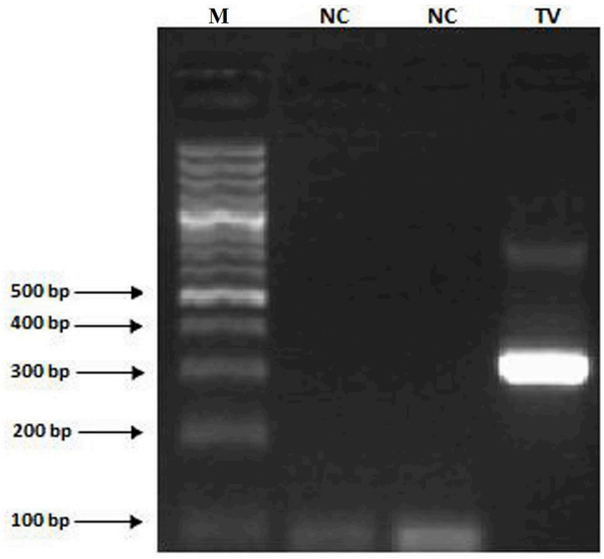


Figure 1. T. vaginalis isolates the primary TV (3) and TV (7), the electrophoresis of the PCR amplification products. NC: Negative control, M: Marker (100 bp)

Results

56 out of 200 patients examined were detected as positive, and the detection of 14 (25%) patients was with wet mount microscopy, 18 (32.1%) with culture and 24 (42.8%) with PCR (Table 2). The number of patients detected to be positive through all three methods was 14 (25%). 14 others were found both by culture and PCR methods, whereas 6 patients diagnosed negative through microscopy proved to be positive by PCR. 8 patients were diagnosed positive by both microscopy and culture.

Difference was found between three methods (P = .022) when Cochran's Q test was used. Compared with McNemar test two by two, no superiority in T. vaginalis diagnosis was found between microscopy and culture (P = .5), microscopy and PCR (P = .063), or culture and PCR (P = .25) methods.

Discussion

Trichomoniasis is the most prevalent nonviral STD in the world.

Table 2. Comparison of PCR, wet preparation and culture results for vaginal samples tested for T. vaginalis

	Microscopy (DM) n (%)	Culture (TYM) n (%)	PCR n (%)	DM-YT-PCR n (%)	p value
Positive	18 (32.1)	14(25)	24 (42.8)	14(25)	0.022*
Negative	182 (67.2)	186 (75)	176 (57.2)	193 (75)	
Total	200 (100)	200 (100)	200 (100)	200 (100)	

Direct microscopy, culture and staining methods are frequently used methods in the diagnosis of trichomoniasis. Since T. vaginalis strains show high phenotypic variation due to expression level and/or differences in genomic sequences, development of PCR based diagnostic methods were difficult. Recently, a PCR test using vaginal swab samples for the detection of T. vaginalis has been developed to add T. vaginalis infection to the growing list of STDs that could be detected by DNA amplification techniques..

Every day, gene targeted PCR primers and methods used in the new techniques (Conventional PCR, nested PCR, Real-time PCR used TaqMan probes, FRET probes used in the Real-Time PCR, PCR-ELISA) for diagnosing of T.vaginalis is reported by researchers in different regions of the world.

The first study from Turkey on T.vaginalis and PCR method is reported by Etabaklar at all[15] and T. vaginalis was found as positive in 2.94, 4.90 and 4.90% with wet mount, TYM medium and PCR respectively from 102 samples. The positivity rate reached 5.88% using the 3 methods together. The wet mount had 60% sensitivity and 100% specificity, while PCR showed 80% sensitivity and 97.95% specificity when compared with the culture method.

PCR method, the sensitivity of 34.9% compared with studies with culture varies between 78% - 92% and specificity of 100% is also reported. Similarly, the direct microscopic examination is usually high, whereas specificity is weak compared with the sensitivity of PCR and is reported to vary between 58.5% and 34.2%.

Studies for diagnosing of T. vaginalis using different samples and different primers for PCR method, the sensitivity changes between 84-100% and specificity 82-100% .

Traditionally, physicians make the diagnosis based on clinical grounds, but in women, the characteristics of the vaginal discharge, including color and odor, are poor predictors of T. vaginalis. This may be identified in vaginal secretions by using a wet preparation, but this method is only 35 to 80% sensitive compared with culture. Although culture is considered the most reliable diagnostic method, its sensitivity is 90% for detecting T. vaginalis.[6] The incidence of a disease on the prevalence of sexually transmitted trichomoniasis work done at regular intervals, as well as people who are asymptomatic carriers of the determination of this factor should be scanned and cultural methods of diagnosis in addition to staining with direct implementation of the report would be useful.[17]

As a result; no statistical superiority was found between direct microscopic examination, culture and PCR methods. However, for direct microscopic examination, experience is needed because T. vaginalis trofozoites sample should be examined as soon as ensampled. Otherwise, trofozoites loose movements and diagnosis could be difficult. Culture method was not used in routine laboratory and had contamination risk. PCR method shows directly the parasites in the DNA, and so PCR method is thought to be more reliable and practical compared to other two methods.

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Competing interests

The authors declare that they have no competing interests.

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