

Diagnosis of Trichomoniasis by Polymerase Chain Reaction

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Abstract

The clinical usefulness of polymerase chain reaction (PCR) for the diagnosis of trichomoniasis was evaluated in comparison with other conventional tests. PCR was used for specific detection of *Trichomonas vaginalis* by primers based on the repetitive sequence cloned from *T. vaginalis* (TV-E650). Between June 1996 and August 1997, 426 patients visited the department of obstetrics and gynecology, Hanyang University Kuri Hospital and were examined for trichomoniasis using wet mount examination, Papanicolaou (Pap) smear, culture and PCR. One hundred and seventy-seven patients (group A) visited with the symptom of vaginal discharge and 249 patients (group B) visited for regular cervical Pap smear with no vaginal symptoms. From group A (n=177), 3 infections (2.0%) were detected by wet mount, 6 infections (3.3%) by Pap smear and culture, and 17 infections (10.4%) by PCR. From group B (n=249), 4 patients (1.6%) were found to have *T. vaginalis* by culture and 6 infections (2.4%) were detected by PCR. Therefore, in both groups, PCR for *T. vaginalis* showed a higher detection rate compared with conventional wet mount, Pap smear or culture. The detection by PCR was specific for *T. vaginalis* since no amplification was detected with DNAs from other protozoa and *Candida albicans*. The sensitivity and specificity of PCR were 100%. This method could detect *T. vaginalis* in vaginal discharge at a concentration as low as 1 cell per PCR mixture. These results indicate that PCR could be used as a specific and sensitive diagnostic tool for human trichomoniasis.

Key Words: *Trichomonas vaginalis*, polymerase chain reaction (PCR), diagnosis of trichomoniasis

INTRODUCTION

Trichomonas vaginalis commonly causes vaginitis and perhaps cervicitis in women, as well as urethritis in both sexes.¹ In pregnant women, trichomonads may be associated with the premature rupture of membranes, premature delivery, and delivery of low-birth weight infants.^{2,3} As well, trichomoniasis has been implicated as a risk factor for human immunodeficiency virus transmission.⁴ Worldwide, more than 180 million people get the infection with this parasite annually.⁵ The prevalence rate was recently 7.6% (478/6262) in the Kangwon area of Korea.⁶

Various methods have been used for the diagnosis of trichomoniasis such as wet mount, culture, Papanicolaou smear, and serologic test.^{7,8} Wet mount examination is an easy, simple, and rapid method but more than 10³/ml and live protozoa are required for detection.¹ Culture demands a specialized medium

and takes 2–5 days for the diagnosis. Accurate interpretation of *T. vaginalis* by Papanicolaou smear also necessitates a skilled observer. A major limitation in the serological detection of *T. vaginalis* by indirect immunofluorescence test is the lack of sensitivity and/or, specificity.⁷

In recent years, molecular biological techniques have provided a new approach to the diagnosis of parasites. Polymerase chain reaction (PCR) that permits *in vitro* amplification of DNA fragments and increases the level of detection has opened new possibilities, using it for the diagnosis of numerous infectious agents including parasites.^{9–12}

In the present study, we developed a highly-sensitive and specific PCR using primers based on the repetitive sequence cloned from *T. vaginalis* (TV-E650) by Paces et al.¹³ This technique was applied to 426 women who visited Hanyang University Kuri Hospital and its diagnostic value was compared with that of other detection methods.

MATERIALS AND METHODS

Patients and diagnostic tests

Between June 1996 and August 1997, vaginal discharges were sampled from women who visited

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Hanyang University Kuri Hospital, Korea. The women included 177 patients with the symptom of vaginal discharge (group A) and 249 asymptomatic patients who visited for Pap smear screening (group B). For group A (n=177), (1) wet mount, (2) culture, (3) polymerase chain reaction (PCR), and (4) Papanicolaou (Pap) smear were performed, while for group B (n=249), the same tests with the exception of wet mount were performed. As well, each of the 177 suspected vaginitis patients had a problem-directed history taken and a physical examination performed.

Wet mount and culture

Two sterile cotton-tipped applicators were used for the swab of vaginal discharge for each patient, and one applicator was gently agitated in one drop of normal saline on a slide for wet mount, and the other applicator was put into a tube containing 5 ml of TYM media for culture.¹⁴ Inoculum in TYM media was cultivated in an incubator at 37°C for 2–5 days and the presence of parasites was confirmed with a microscope.

Papanicolaou smears

Pap smears were done by scraping the cervix with a cytobrush and spatula.

Polymerase chain reaction (PCR)

Collection of sample: For the PCR, 3–5 ml of normal saline was injected into the posterior vaginal fornix using a pasteur pipette, and after pipetting several times with vaginal discharge, it was all recollected in a 15 ml tube and stored at –70°C after washing twice with PBS.

Pretreatment of the samples: Five μ l of vaginal discharge was mixed with 20 μ l of Gene Releaser[®] (BioVenture Inc., Kensington, TN, USA) and the mixture was boiled for 5 min using a microwave oven. Then, it was centrifuged and 3 μ l of supernatant were added to the PCR reaction mixture. *T. vaginalis* isolate KT9 cultivated in our laboratory was used for the positive control.

Production of primers: Primers were designed based on *Trichomonas vaginalis*-specific DNA repetitive sequence in clone TV-E650-1 cloned by Paces et al.¹³ The Nucleotide sequence of a pair of PCR primers was as follows:

Primer 1 : 5' gagttaggggtataatgtttgatgtg 3'

Primer 2 : 5' agaattgtatagcgaaatggg 3'

PCR conditions: Reaction mixture contained primer 1 (10 pmol/ μ l) 1.5 μ l, primer 2 (10 pmol/ μ l) 1.5 μ l, dNTPs (2.5 mM each) 2 μ l, Taq polymerase (5 U/ μ l) 0.1 μ l, pre-treated vaginal discharge 3 μ l, 10 \times PCR buffer 2 μ l, and distilled water 9.9 μ l. Before entering the PCR cycle, predenaturation was performed for 5 min at 95°C. Then thermal cycles for PCR consisted of 30 sec. denaturation at 94°C, 10 sec. annealing at 50°C, and 30 sec. extension at 72°C. A total of 35 reaction cycles were done. After amplification, 5 μ l of each sample and 1 μ l of DNA size standard marker were mixed with 1 μ l of 6 \times loading dye and the mixture was electrophoresed on a 2% agarose gel stained with 0.5 μ g/ml ethidium bromide for 15 min at 100 V. The presence of amplification products was observed under UV illumination. The 330 bp band on electrophoresis was identified as a positive result.

To avoid product carryover, PCR reactions were set up in an area physically separate from all activities involving amplified target sequences, thermocycling, and the running of gels.

Studies on the sensitivity and specificity of the PCR

To test the detection level of the PCR, the *T. vaginalis* were counted with a haemocytometer and the number of trophozoites with PBS was adjusted to 1, 3, 5, 10, 50, 100, 1000 and 36,000 cells. 17 μ l of PCR reaction mixture were added to each tube containing a different number of trophozoites.

We tested the specificity of PCR using DNAs extracted from *Trichomonas foetus* strain KV1, F2, *Candida albicans*, *Acanthamoeba culbertsoni* strain A1, and *Acanthamoeba* sp. YM-3.¹⁵

RESULTS

Comparison of the detection rate of PCR with other tests

In group A, detection rates of wet mount, Pap smear, culture and PCR were 2.0% (3/150), 4.6% (6/130), 3.3% (6/177) and 10.4% (17/163), respectively. In group B, those of Pap smear, culture and PCR were 0% (0/248), 1.6% (4/249) and 2.4% (6/249), respectively. Therefore PCR showed a higher detection rate than other tests in both groups. Group A had a higher overall positive rate of *T. vaginalis* than group B, especially by PCR ($p < 0.05$, Pearson's Chi-Square test) (Table 1).

Table 1. Comparison of Diagnostic Methods for Trichomoniasis in Patients with Suspected Vaginitis (A Group) and Women for Screening by Papanicolaou Smear (B Group)

Lab. Method	Positive rate of <i>T. vaginalis</i>	
	A group	B group
Wet mount	2.0% (3/150)	ND
Papanicolaou smear	4.6% (6/130)	0% (0/248)
Culture	3.3% (6/177)	1.6% (4/249)
PCR	10.4% (17/163)*	2.4% (6/249)

ND, not done.

*P<0.05 by Pearson's Chi-square test.

Table 2. Comparison between PCR and Papanicolaou Smear for Diagnosis of *T. vaginalis* Infection in Patients with Suspected Vaginitis

	PCR		Total
	+	-	
Papanicolaou smear	5	1	6
	12	112	124
Total	17	13	130

κ -index = 0.393, $p < 0.01$

In 150 wet-mount specimens of symptomatic patients, trophozoites were identified from 3 (2.0%) patients. All 3 patients also had positive results by Pap smear, culture and PCR.

In group A, Pap smears were interpreted as positive for trichomonads in 6 (4.6%) of 130 suspected vaginitis patients, but one of these 6 cases was not confirmed by PCR as well as culture. The overall proportion of agreement between PCR and Pap smear was 0.9 (κ -index=0.393, $p < 0.01$) (Table 2). In group B, compared with the 6 positive patients by PCR, there was no positive patient by Pap smear.

In group A, 6 patients showed positive culture and all of these positive results were consistent with that of PCR. In group B, 4 women showed positive results by culture and all of them also showed positive results by PCR. The overall proportions of agreement between PCR and culture were 0.93 (κ -index=0.429, $p < 0.01$) and 0.99 (κ -index=0.75, $p < 0.01$) in the A and B groups, respectively (Table 3).

The sensitivity and specificity of the PCR were 100%.

Table 3. Comparison between PCR and Culture for Diagnosis of *T. vaginalis* Infection in Patients with Suspected Vaginitis (A Group) and Women for Screening by Papanicolaou Smear (B Group)

	PCR		Total
	+	-	
Culture	6 (4)*	0 (0)	6 (4)
	11 (2)	146 (243)	157 (245)
Total	17 (6)	146 (243)	163 (249)

*B group.

A Group: κ -index = 0.429, $p < 0.01$

B Group: κ -index = 0.75, $p < 0.01$.

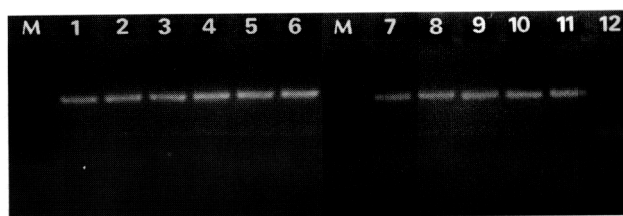


Fig. 1. Agarose gel electrophoresis of PCR products (330 bp) by increasing number of *T. vaginalis*. Lanes 1-11: 1, 1, 1, 3, 3, 5, 10, 50, 100, 1,000 and 36,000 cells, respectively. Lane 12: distilled water (negative control).

Studies on the sensitivity and specificity of PCR

To evaluate the sensitivity of the reaction, PCR was undertaken with 1, 3, 5, 10, 50, 100, 1000 and 36,000 trophozoites. The expected product of 330 bp was obtained from as few as 1 organism (Fig. 1). When DNAs from different organisms such as *Trichomonas foetus* strain KV1, F2, *Candida albicans*, *Acanthamoeba culbertsoni* and *Acanthamoeba* sp. YM-3 were tested for specificity of PCR, no specific amplification was obtained.

To confirm whether or not the 330 bp band obtained by PCR originated from *T. vaginalis*, nested-PCR was undertaken using primer 1: 5'aaattg-gaaccagtgtcaga 3' and primer 2: 5' gaagaaattta-aatcaaac 3', confirming the 200 bp band (Fig. 2).

Signs and symptoms of patients with suspected vaginitis

The mean ages of patients of group A and group B were 37.8 years and 52.7 years, respectively. Group A patients showed various symptom and/or signs of vaginitis that included vaginal discharge, pruritus,

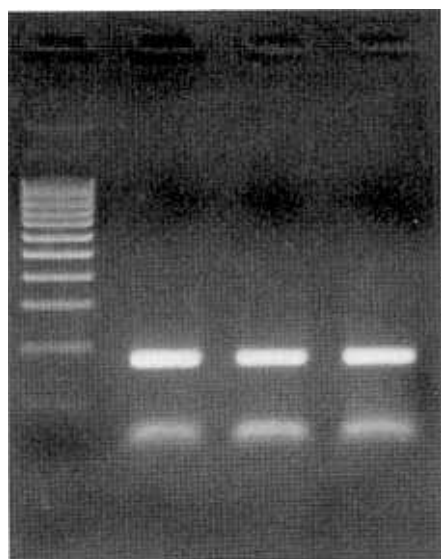


Fig. 2. Agarose gel electrophoresis of nested-PCR product (200 bp) of *T. vaginalis*. All of the lanes were about 40,000 cells.

erythema, edema on vaginal wall, dyspareunia, dysuria, postcoital bleeding, burning sensation and strawberry patch (Table 4).

There was no correlation between these variables and the results by PCR for the diagnosis of trichomoniasis (Table 5).

DISCUSSION

Several investigators have developed PCR for the diagnosis of trichomoniasis, and primers used by them were based on multiple genomic loci¹¹ or repeated, dispersed DNA of *T. vaginalis*¹⁶ or adhesion gene of *T. vaginalis*¹⁷ or Tv-E650 repetitive sequence¹⁸ or β -tubulin¹⁹ of *T. vaginalis*.

Primers used in our study and also by Shaio et al.¹⁸ were derived from Tv-E650 repetitive sequence cloned by Paces et al.¹³ They estimated that the *T. vaginalis* genome contains about 10^2 – 10^3 of the Tv-E650 repeat. Therefore, primers used in this study and by Shaio et al.¹⁸ were expected to be very sensitive for detecting *T. vaginalis*. However, the PCR product in this study showed one band (330 bp) irrespective of the number of trophozoites. In contrast, Shaio et al. reported that multiple bands of PCR product in cases of more than 100 cells applied by nested-PCR.¹⁸ Therefore, PCR using our primer set may be easier and faster for interpreting the PCR result compared with the nested-PCR of Shaio et al.¹⁸

Shaio et al. and Paterson et al. compared the detection rate of trichomoniasis by PCR with other conventional diagnostic methods and reported that

Table 4. Symptoms and Signs of Patients with Suspected Vaginitis

Characteristics	Ratio of patients with symptoms or signs
Vaginal discharge	72.4% (126/174)
Pruritus	24.6% (43/175)
Erythema, edema	4.6% (8/174)
Dyspareunia	4.0% (7/175)
Dysuria	3.4% (6/175)
Postcoital bleeding	2.3% (4/174)
Burning sensation	1.7% (3/175)
Strawberry patch	0.6% (1/174)

Table 5. Comparison of Symptoms and Signs between Women with Positive Result and Women with Negative Result by PCR for *T. vaginalis*

Symptoms and signs	PCR	
	Positive	Negative
Vaginal discharge	64.7% (11/17)	71.5% (103/144)
Pruritus	35.3% (6/17)	23.6% (34/144)
Erythema, edema	17.6% (3/17)	2.8% (4/144)
Dyspareunia	5.9% (1/17)	4.2% (6/144)
Dysuria	0% (0/17)	4.2% (6/144)
Postcoital bleeding	0% (0/17)	2.8% (4/144)

PCR was very sensitive compared with other conventional tests.^{18,20} In this study, of 177 women with suspected vaginitis (A group), positive rates by wet mount, Pap smear, culture and PCR were 2.0%, 4.6%, 3.3%, and 10.4%, respectively. Among 249 women undergoing Pap smear screening (B group), four (1.6%) and six (2.4%) trichomoniasis patients were confirmed by culture and PCR, respectively, although Papanicolaou smear couldn't detect *T. vaginalis*. Therefore, PCR was the most sensitive method for the diagnosis of trichomoniasis irrespective of symptomatic and asymptomatic group. The results of culture and Pap smear were also in agreement with those of PCR with the exception of one case.

In this study, the positive rate (10.4%) of 177 symptomatic samples was significantly higher than that (2.4%) of 249 asymptomatic samples by PCR. Therefore, trichomonas infection may be related with symptomatic women. However, clinical symptoms and signs were also analyzed in association with PCR results. Although frequent symptoms and signs were vaginal discharge (72.4%) and pruritus (24.6%), they did not correlate with trichomoniasis. This result was consistent with the report that information taken

from patient history and clinical examination were poor predictors with *T. vaginalis*.²¹

Pretreatment of vaginal samples in this PCR was a quick and easy process by heating with Gene releaser (BioVenture Inc., USA) for 5 min compared to incubation with proteinase K for 60 min as described elsewhere.^{22,23} When 1, 3, 5, 10, 50, 100, 1000 and 36,000 trophozoites were tested for evaluation of PCR sensitivity, all of the samples containing even one trophozoite showed amplified PCR product. The 330 bp bands on all of the electrophoresis lanes undertaken for analysis, PCR results had similar densities irrespective of trophozoite numbers, suggesting that the PCR is highly sensitive for detecting trichomoniasis. It may also be possible to detect less than a single trophozoite because a definite band was shown with one trophozoite.

DNAs extracted from *Tritrichomonas foetus* strain KV1, F2, *Candida albicans*, *Acanthamoeba culbertsoni*, and *Acanthamoeba* sp. YM-3 were used for template DNA in order to examine the specificity of the PCR. However, none of these protozoa showed false positive reactions, indicating excellent specificity of the PCR.

In conclusion, it is suggested that the PCR set up in our laboratory could be a sensitive and specific method for diagnosis of human trichomoniasis.

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