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Diagnosis of *Trichomonas vaginalis* Infection by Urine PCR Analysis Compared to Wet Mount Microscopic Screening

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This study was conducted for parasite detection in urine samples using Polymerase Chain Reaction (PCR) and comparing the results to wet mount microscopic screening. For this, 155 urine samples were collected from symptomatic women suspected to trichomoniasis (mean age = 20±5 year) introduced to laboratory by physician. Urine samples were subjected to polymerase chain reaction to detect the parasite's genome. As a result 25 out of 155 samples (16%) were detected positive by direct microscopic observation, but 75 out of 155 (48%) by PCR. Eighty out of 155 samples (52%) were detected negative by PCR. This study confirmed that the microscopic screening with a low sensitivity must be substituted by highly sensitive screening methods such as PCR.

Key words: STD, *Trichomonas vaginalis*, PCR, β tubulin gene

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INTRODUCTION

Trichomonas vaginalis is a flagellated parasitic protozoan typically pyriform in culture and amoeboid form adhering to mammalian cells. *T. vaginalis* is site-specific, usually surviving only in human urogenital tract. *Trichomonas vaginalis* is the causative agent of pelvic inflammatory disease in HIV infected women[1,2]. Men usually display no symptoms or disease[3]. In recent years, *T. vaginalis* has been focused on, partly due to the increased general knowledge about sexually transmitted diseases and partly due to the higher risk of HIV infection in patients infected with *T. vaginalis* than none infected ones[4]. *T. vaginalis* is more prevalent in women using intrauterine contraceptive devices than others. Asymptomatic patients can also remain infected and at risk of developing symptomatic disease later on[5].

As a sexually transmitted disease, the clinical manifestations of the disease, ranges from a mild-unapparent infection to a chronic and substantial level of inflammation in the reproductive tract of women and urethritis in men[4]. Clinical features that may develop in 50-90% of infected women include purulent vaginal discharge, pruritus and dyspareunia, leucorrhoea and dysuria. Vaginal discharge is a clearer symptom observed in 50-70% of diagnosed women. Notably children born to women with trichomoniasis may suffer from low birth weight[6].

Current detection methods rely on microscopic examination of vaginal specimens and culture, which are actually insensitive and time-consuming[7]. Ryu *et al.*[8] compared sensitivity of PCR with wet mount and culture for trichomonas vaginalis screening, concluding that PCR could be used as a specific and sensitive diagnostic tool for human trichomoniasis.

Guillermo *et al.*[9] developed a PCR method using primers that produced a segment of β-tubulin sequence with 97% sensitivity and 98% specificity. Their primers did not amplify genes of other species of trichomonas, intestinal flagellates and bacterial agents of vaginitis. Patel found that the sensitivity of the polymerase chain reaction technique was 95% and the specificity was 98% compared to other diagnostic tests[10].

Based on different studies, PCR is very sensitive and specific for detection of *Trichomonas vaginalis* suggesting this method as the gold standard instead of other screening methods[6,9,11-16].

MATERIALS AND METHODS

Samples: Urine specimens (155) were collected from symptomatic women (mean age = 20±5 years.) suspected to trichomoniasis and referred to gynecology department of Mahdieh Hospital in Tehran. Clinical manifestations of infected women included purulent vaginal discharge, purities, dyspareunia, leucorrhoea and dysuria. Physician referred the infected women to laboratory and vaginal discharge was tested by direct microscopic method for detection of the Trichomonas vaginalis trophozoites. Urine samples were submitted to molecular laboratory for PCR testing.

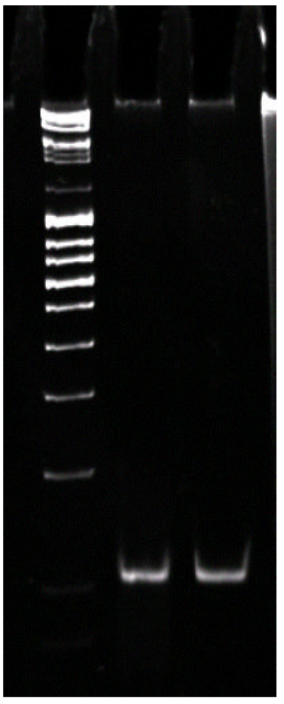

Fig. 1: DNA bands at 312 nm by UV-Tran illuminator

DNA extraction: The pellet of centrifuged urine was boiled with 100 µl of lysis buffer (150 mM sucrose, 10 mM Tris, 5 mM $MgCl_2$, 1% Triton X-100) and DNA was then recovered by ethanol precipitation.

PCR primers: The Primers based on conserved region of beta-tubulin gene of the *Trichomonas vaginalis* (gene bank accession number L05470) were used to amplify a 112 bp piece of the gene[2]. The sequence of the primers were as follows:

Forward: 5` CAT TGA TAA CGA AGC TCT TTA CGA T-3` and Reverse: 5` GCA TGT TGT GCC GGA CAT AAC CAT 3`

PCR protocol: The total volume of PCR reaction was 30 µl. The PCR mixture contained: 0.1 mM dNTP, 1U Taq DNA polymerase, 20 picomole each of the forward and

reverse primers, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.1 μg template DNA and distilled water up to 30 μl. PCR cycling parameters included initial preincubation at 94°C for 5 min, the incubation cycles consisting of 94°C for 30 sec (denaturation), 52°C for 30 sec (annealing) and 72°C for 30 sec (extension) repeated for 30 cycles and then the final incubation at 72°C for 5 min[17].

Agarose gel electrophoresis: A 15 μl volume of each reaction was subjected to electrophoresis on 3% agarose gel containing ethidium bromide[18]. The DNA bands were visualized at 312 nm by UV-Tran illuminator (Fig. 1).

RESULTS AND DISCUSSION

As shown the sensitivity and specificity of microscopic screening is 33.3% compared to PCR (Table 1). Trichomoniasis is one of the sexually transmitted diseases (STD), so sensitive and specific detection methods are needed in both sexes specially men, since they may display no symptoms or disease, causing trouble in parasite detection. Direct observation of the trophozoite in vaginal secretions has 60% identities[19]. It is known that asymptomatic patients can also carry infection and remain at risk of developing symptomatic disease later on. Shaio *et al.*[16] and Lin *et al.*[12] compared, in two separate studies, three different detection methods including PCR, culture and direct observation methods. Both of the groups reported a higher sensitivity and specificity for PCR method than the other two. Krieger *et al.*[11] detected *T. vaginalis*, *Chlamydia trachomatis* and *Mycoplasma genitalium* in prostate secretions of men with chronic prostatitis by PCR procedure. Kaydos *et al.*[7] used PCR, ELISA and PCR-ELISA methods for detection of *T. vaginalis* in urine samples and they reported a sensitivity of 66.9, 86.4 and 90.8%, respectively. Schee *et al.*[19], Lawing *et al.*[20] and Holger *et al.*[21] confirmed PCR's efficiency in detecting the parasite in the urine of both sexes. Schwebke and Lawing[22] suggested that the use of PCR techniques in urine specimen-based detection of *T. vaginalis* was highly sensitive and revealed a prevalence of infection more than three times that revealed by culture for men at high risk for STDs.

Based on these studies present research was designed on detection of *T. vaginalis* in urine samples of 155 women suffering from vaginitis by PCR. We detected the parasite in the specimens by amplifying a 112 bp segment of β tubulin gene sequence of *Trichomonas vaginalis*. Present results confirmed the high sensitivity of PCR designed protocol for parasite detection compared to routine screening tests.

Table 1: Comparison of the results of microscopic observations and PCR on 155 urine samples

PCR\WM*	Positive	Negative	Sum
Positive	25 (16%)	0 (0%)	25
Negative	50 (32%)	80 (52%)	130
Sum	75	80	155

• Wet mount

In this study a highly sensitive and specific PCR method was used as a detection method of *T. vaginalis* parasite in urine samples. Present results proposed that parasite detection in urine is a good substitute for other clinical specimens, easily provided from patients without pain and is especially preferable in men. So taking advantage of urine PCR analysis, the problem of parasite detection in carriers would be efficiently overwhelmed.

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