Short Communication

Molecular Identification Based on β-Tubulin of *Trichomonas vaginalis* Infection among Women in Babylon Province

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Abstract

Background: *Trichomonas vaginalis* (*T. vaginalis*) is an anaerobic flagellated parasite that usually infects the female genitourinary system. The traditional diagnostic tools of *T. vaginalis*, like wet-mount microscopy, have several limitations regarding technical requirements and accuracy. Consequently, to control the spread of trichomoniasis, fast and accurate detection of trichomoniasis represents a crucial factor in the treatment plan. **Objectives:** This study was performed to utilize polymerase chain reaction (PCR) assays as a diagnostic tool for *T. vaginalis.* **Materials and Methods:** Vaginal swabs were obtained from 186 women who were admitted to the gynecology clinic in Al Sadiq Hospital in Babil Governorate in Iraq. Additionally, a questionnaire was gathered from the female participants regarding their age, residency, history of abortion, and symptoms. DNAs were extracted from the swabs, and conserved β-tubulin genes were amplified using specific primers. **Results:** Of the 186 participant females, 40 women showed positive PCR outcomes of *T. vaginalis* β-tubulin gene versus only seven positive cases detected by wet-mount microscopy. Residency and age-related prevalence of *T. vaginalis* infection were not statistically significant, despite the higher positive cases reported in urban areas. Additionally, higher percentages of history of abortion were seen in infected females versus noninfected females. Clinical diagnosis demonstrated that vaginal discharge and itching were the most prevalent symptoms reported by infected females. **Conclusion:** PCR assay used in the current study showed a beneficial and fast diagnostic ability of *T. vaginalis* because of its high sensitivity and specificity. Consequently, this would blunt the transmission of trichomoniasis among individuals, especially infected patients with asymptomatic properties.

Keywords: Diagnosis, PCR, *T. vaginalis*, trichomoniasis, wet-mount

Introduction

Trichomonas vaginalis is a flagellated anaerobic parasite that was initially detected in women's vaginal secretions by Alfred François Donné in 1836. The infection by this microorganism causes a mild sexually transmitted disease named trichomoniasis.[1] Recently, an elevated prevalence, as well as incidence rate, have been reported. Furthermore, an elevated resistance was seen to the treatment options. Additionally, important complications were demonstrated because of the trichomoniasis infection.[2] Complex interaction was exhibited between the host cells and *T. vaginalis* based on direct and/or indirect mechanisms.[3] Global epidemiological data revealed that *T. vaginalis* infects greater than 170 million individuals.[4] Trichomoniasis infection may be asymptomatic, representing the most common form of infection, which makes it a challenge in calculating the incidence rate as well as the early diagnosis and treatment of infection.[5] On the other hand, symptomatic *T. vaginalis* infection, although mild, has characteristic itching and swelling in the tissues of the genitourinary tract of infected patients.

The tissues of the genitourinary tract in women, as well as men, are the preferred sites of infection by this corresponding parasite.[6] *T. vaginalis* possesses characterized by a well-developed membrane with four

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anterior flagella; these flagella have important motility and diagnostic function. The frequently utilized diagnostic test for *T. vaginalis* is wet-mount microscopy.[7] The wet-mount technique is fast and economical; however, this test possesses a modest sensitivity (20%–60%).[8,9] Recently, the golden standard diagnostic test for *T. vaginalis* infection is the culture; however, it is not commonly used in laboratory examinations.[10] Culturing is expensive and slow, which takes up to 7 incubation days with daily microscopic examination. Additionally, studies showed that the sensitivity of the commercial InPouch culture test of *T. vaginalis* in Australia was no more than 63%.[11]

The clinical diagnosis of females infected with trichomoniasis relays on symptoms lacking specificity, including discharge from the genital tract, vaginitis, and irritation. Furthermore, both the wet-mount test, which requires visualization of the parasite, and the culture test, performed in reference labs, require viable parasite and fast preparations because they are not applicable in case the transportation of the specimens taken from infected females is delayed.[8] Interestingly, targeting different sites in the genome of *T. vaginalis* using polymerase chain reaction (PCR) was demonstrated by several studies to be a good option for diagnosis. Indeed, these PCR-based tests exhibited higher diagnostic sensitivity toward trichomoniasis (89%–98%) compared with culture.[6,8]

Based on that, this study was done to assess the PCR assays in the diagnosis of *T. vaginalis* by detecting specific DNA regions in the parasite, the β-tubulin (Btub) gene.

Materials and Methods

Specimens

Swabs from posterior fornix of vagina were obtained from 186 female individuals through speculum examination. These females were admitted to the gynecology clinic in Al Sadiq Hospital in Babil Governorate in Iraq from February 1, 2022 to January 1, 2023. After taking verbal consent for participation in the study, a questionnaire was used to extract data from the participants regarding their age, residency, history of abortion, and symptoms. Then the vaginal swabs were used for wet-mount test directly and then kept for PCR assay at -20°C.[12]

Regarding the wet-mount microscopic examination, swabs from the vaginal tract were taken using sterile cotton swab sticks by a gynecologist. Swabs were transferred into 1 mL of sterile normal saline. Following mixing, only one drop of the corresponding solution was placed on a grease-free microscopic slide and then covered with a coverslip and observed using a light microscope. Three slides were used for each sample. Each microscopic examination was done within a period of 30 min after collecting the sample.

DNA extraction

The solution of DNGTM-Plus (CinnaGen Inc., Tehran, Iran) was utilized to extract DNA for PCR analysis. A total of 300 µL of DNGTM-Plus solution was warmed at a temperature of 37°C for exactly 20 min and then added to a volume of 100 µL of the sample, shaked by vortex for 15 s, and then isopropanol (300 µL) was added and kept at -20°C for 30 min followed by centrifuge for 10 min at 12,000 rpm. After removing the supernatant, ethanol 75% (1 mL) was mixed with the pellet, shaked by vortex, and then centrifuged for 5 min at a speed of 12,000 rpm; these steps were repeated two times. Ethanol was poured off, and the pellet was left for drying at room temperature. Later, the pellet (DNA) was dissolved in sterile water (50 mL) using gentle shaking and kept for 5 min at 65°C. Finally, Purified DNA present in the supernatant was utilized for PCR assay.

PCR primers

The β-tubulin genes of the *T. vaginalis* were targeted on their conserved region by specific primers to amplify these 112 bp length regions. The utilized primer sequences involved the forward primer: 5′ CAT TGA TAA CGA AGC TCT TTA CGA T3′, and reverse primer: 5′ GCA TGT TGT GCC GGA CAT AAC CAT 3′.

β-Tubulin gene detection by PCR assay

The PCR procedure was done based on a previously reported protocol.[12,13] PCR mixtures used in the assay included 12.5 µL of master mix, 2.5 µL of both forward and reverse primers, 3 µL of template DNA, and 4.5 µL of nuclease-free water. PCR assay conditions involved five cycles of initialization (94°C for 5 min), denaturation (94°C for 1 min), annealing (56°C for 1 min), extension (72°C for 1 min), and final extension (72°C for 1 min).

Agarose gel electrophoresis

The PCR products were subjected to 3% agarose gel electrophoresis containing DNA stain. A commercial marker (Fermentase) of 100 bp was utilized to assess the size of the PCR product.

Statistical analysis

Descriptive statistics are presented in the form of frequencies and relative frequencies. The Chi-square test was used to study the association between the outcome and different factors. MedCalc version 20 software (MedCalc Software Ltd, Ostend, Belgium) was used for the statistical analysis. P value <0.05 is considered statistically significant.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbally, and also the analytical approval was done before samples were

taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee according to document number 1522 on February 16, 2022, to get this approval.

Results

In total, 40 women showed positive PCR outcomes of *T. vaginalis* β-tubulin gene, as demonstrated in Table 1.

The samples of vaginal swabs were identified by molecular technique (PCR) using BTUB 9/2 primer. The 40 positive samples were identified by agarose gel electrophoresis; samples of agarose gel electrophoresis examinations are shown in Figure 1. Sets of tests involved both positive as well as negative control in addition to DNA marker. The 112 bp product was amplified in all positive samples.

However, of the 40 PCR-positive samples, only seven patients demonstrated positive wet-mount microscopy. According to the geographical area, the Hilla city center had the greatest number of samples, and it showed the most positive cases; indeed, the percentage of women infected with *T. vaginalis* of the total examinations was higher in the city center (22.3%) when compared with that in the rural area (20.3%), as shown in Table 2.

Furthermore, the ages of infected women were between 16 and 50 years, and the prevalence of trichomoniasis among females based on their age was listed in Table 3. According to results, higher prevalence was seen in women aged between 21 and 40 years old, and it started o decline after the age of 40 years old.

Table 1: PCR identification of *Trichomonas vaginalis* among all studied samples using BTUB9/2 gene-specific primer

Results	No.	Infection percentage	95% Confidence interval
Positive	40	21.5%	15.85%–28.11%
Negative	146	78.5%	71.89%–84.17%
Total	186	100%	–

According to a questionnaire, out of the 146 negative samples women, 31 had a history of abortion, and 11 out of the 40 infected ones had an abortion history. As demonstrated in Table 4, infected women possessed the highest history of abortion than noninfected women.

Regarding the clinical symptoms, vaginal discharge was the most common clinical symptoms among patients, and about 70% of positive patients were complaining of clinical symptoms listed in Table 5.

Table 2: Residence-related prevalence of *Trichomonas vaginalis* infection

Residence	Examinations (%)	Infections (%)	*P* value
Rural	74 (40%)	15 (37.5%)	0.740
Urban	112 (60%)	25 (62.5%)	
Total	186 (100%)	40 (100%)	

Table 3: Age-related prevalence of *Trichomonas vaginalis* infection

Age	Examinations (%)	Infections no.	Infection (%)	*P* value
16–20	15 (8.1%)	1	2.5%	0.180
21–25	27 (14.5%)	8	20%	
26–30	49 (26.3%)	11	27.5%	
31–35	43 (23.1%)	8	20%	
36–40	24 (12.9%)	9	22.5%	
41–45	16 (8.6%)	2	5%	
46–50	12 (6.5%)	1	2.5%	
Total	186 (100%)	40	100%	

Table 4: History of abortion among noninfected and infected women with *Trichomonas vaginalis*

Results	No.	History of abortion (%)	*P* value
Positive	40	11 (27.5%)	0.40
Negative	146	31 (21.2%)	

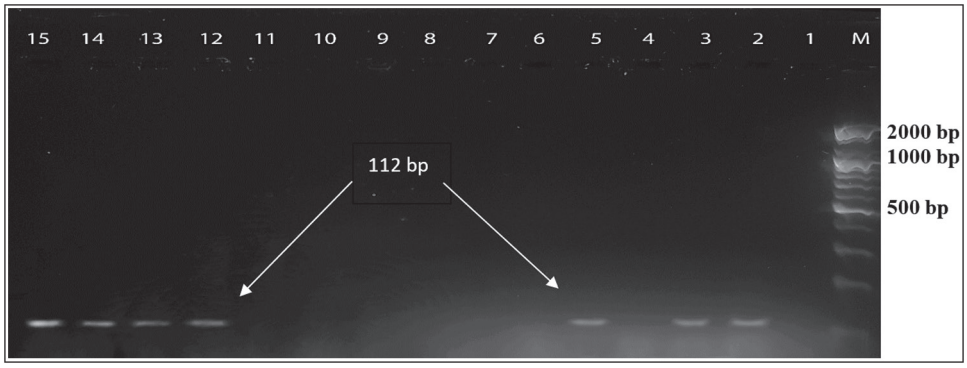

Figure 1: Agarose gel electrophoresis of Uniplex-PCR products (112 bp) using *Trichomonas vaginalis*-specific primers. Lane M represents universal DNA ladders. Lanes 2, 3, 5, 12–15 represent positive isolates

Table 5: Clinical symptoms in females infected with *Trichomonas vaginalis*

Symptoms	Negative cases	Positive case	*P* value
Discharge	112 (76.7%)	38 (95%)	0.001
Itching	65 (44.52%)	25 (62.5%)	
Dysuria	48 (23.8%)	12 (30%)	
Others	27 (18.5%)	0 (0%)	

Discussion

In this study, PCR analysis helped in offering a fast diagnostic technique to recognize *T. vaginalis*. Similarly, studies showed that PCR analysis reported a high and accurate prevalence of 48% positive cases out of 155 total examinations.[12] Inversely, only 3.2% of samples taken from women with vaginal discharge were reported as positive utilizing wet mount and culture analysis.[14]

The current study demonstrates that PCR assays represent an advanced molecular technique characterized by high sensitivity and specificity because it depends on the amplification of specific highly conserved DNA regions in the parasite genome, including the β-tubulin (Btub) gene.

Furthermore, based on the residence-related prevalence of *T. vaginalis* infection, it was found that the higher number of admitted women to the gynecology clinic was from the urban area. Moreover, the percentage of females diagnosed with trichomoniasis using PCR assay out of the total examinations was greater in the urban area, although statistically insignificant, in comparison to that in the rural area. Similarly, the difference between the urban and rural prevalence of trichomoniasis was prominent in a comparable study performed in Korea; higher prevalence was seen in urban areas, which had the greatest average population as well as the biggest number of positive cases.[15] However, no significant differences were reported between urban and rural areas in some studies.[10]

Determination of age-related prevalence of *T. vaginalis* infection exhibited that the majority of females infected with trichomoniasis were sexually active women, particularly those aged between 21 and 40 years old, as seen in previous studies.[16]

Preconception infection with trichomoniasis significantly elevated the risk of abortion, as previously reported. In this study, the history of abortion determination among noninfected and infected women with *T. vaginalis* showed that the infected females revealed a slightly higher percentage of previous abortion events in comparison with the negative cases, but these results were statistically insignificant. It was reported that the impact of trichomoniasis on the increasing risk of spontaneous abortion in pregnant women is correlated with coinfection by other reproductive tract infections.[17]

Based on clinical symptoms in females infected with *T. vaginalis*, clinical diagnosis of trichomoniasis revealed that 38 women of the 40 positive cases were complaining of vaginal discharge (*P* value = 0.001), 23 women were suffering from itching (*P* value = 0.044), and 10 women were feeling of burning in their genital tract that is statistically not significant. These clinical symptoms were reported by other studies as the main clinical symptoms seen in women diagnosed with *T. vaginalis*.[18–20] However, some females still did not complain of clinical symptoms (asymptomatic), despite the fact that these women were infected with the corresponding parasite. Consequently, these findings highlight the important role of PCR assay as an essential and accurate diagnostic tool for *T. vaginalis*.

The high prevalence of trichomoniasis infection among female individuals recently probably results as a consequence of the elevated sensitivity of detection by PCR in comparison to wet-mount tests. PCR assay was a considerably fast diagnostic technique for *T. vaginalis;* in addition to that, it was highly sensitive and specific. Consequently, this would reduce the transmission of the infection, especially from asymptomatic infected patients.

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Conflicts of interest

There are no conflicts of interest.

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