International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.3, pp 240-244, 2016

Polymerase chain reaction compared with wet mount for detection of *Trichomonas vaginalis* in women

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Abstract: background: Trichomonasis is a common cause of vaginitis. It is a sexually transmitted infection, and is caused by the single-celled protozoan parasite *Trichomonas vaginalis* producing mechanical stress on host cells and then ingesting cell fragments after cell death. **Objective**: The present study aimed to compared between wet mount and polymerase chain reaction for detection of *Trichomonas vaginalis* in symptomatic women.
Methods: A total of (94) Vaginal swabs were collected from symptomatic women suspected to trichomonaisis and attending to gynecology of Al-eskandaria hospital in Babylon province. All samples were examined by wet mount and PCR technique for detection of *T. vaginalis*. Specific primers used to amplify a 112bp piece of the β-tubulin gene of *T. vaginalis.*
Results: In this study 94 vaginal swaps were collected (19.1%) was positive for wet mount and (27.6%) was positive for PCR technique while (12.7%) was positive for both wet mount and PCR. the high prevalence of trichomonasis observed among the age group (30-40) followed by (20-29) then the older age group(>40). Vaginal discharge is most common symptoms among patients(13.8%) when used PCR technique while (8.5%) in wet mount. In addition of these women with contraceptive device appear more susceptible to infection with *T. vaginalis* in compare with non users contraceptive device.
Conclusion: From the results of this study we conclude that PCR technique is a best method for diagnosis of *T. vaginalis* infection in compare with wet mount.

Introduction:

Trichomonas vaginalis is a protozoa parasite that infects the urogenital tract of human, and consider the one of most common causes of sexually transmitted infection in the world[1]. Trichomonasis has important medical, social and economical implication, with an estimated 180 million infection acquired annually worldwide[2]. The clinical disease in women ranges from asymptomatic to symptomatic such as sever urethritis, vulvovaginitis, cervicitis, malodorous vaginal discharge with associated pruritus, dysuria and dyspareunia, also birth of preterm or low-birth weight infant and possibility of development of cervical cancer are associated complication in affected women[3].

Human infected with trichomonasis become more susceptible to other disease such as immunodeficiency virus, cervical cancer and aggressive prostate cancer[4]. Human are the only natural host for *T. vaginalis*. we know that trichomonasis is a sexually transmitted disease, also *T. vaginalis* transmitted without sexual contact because this parasite can survive for longtime outside the body in moisture condition, the possibility of transmission via toilet seat, shared sponges or towels, communal bathing or living under poor and overcrowded conditions had been raised[5].

Detection of *T. vaginalis* made by microscopical examination of urine and vaginal discharge and identification of the motile parasite in wet mount preparation, this method was rapid and inexpensive but fails to detect all culture positive cases in women. Culture of parasite is a more reliable diagnosis method, but requires specific and complex culture media in addition to incubation period of up to 7 days as well as a daily examination[6]. 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 diagnosis of trichomonasis. this method had sensitivity and specificity of 100% and was able to detect parasite in specimen at a concentration as well as 1 cell per PCR mixture[7]. PCR technique using specific primer sets for detection of parasite, in trichomonasis, PCR technique targeting the β- tubulin gene of *T. vaginalis* was used for detection of microorganism in vaginal swab and urine sample. The targeted gene encode the amino acid sequences of beta-tubulin protein, a major component of *T. vaginalis* cytoskeleton[8].

The aim of this study to investigate *Trichomonas vaginalis* in symptomatic women by wet mount preparation and polymerase chain reaction and compare between these methods.

Materials and methods:

Samples collection:

Vaginal swabs (94) were collected from symptomatic women suspected to trichomonaisis and attending to gynecology of Al-eskandaria hospital in Babylon province. All patients complete the questionnaire form (age, maternal state, clinical manifestations and using contraceptive device). Vaginal swabs put in sterile tube containing 1 ml normal saline and immediately examined by wet mount microscopy, other vaginal swabs were placed in 500µl of 0.01M tris buffer (pH 8) and used for the PCR assay.

DNA Extraction

PCR primer

Specific primers used to amplify a 112bp piece of the β-tubulin gene of *T. vaginalis*, the sequences of primers were as follows: Forward 5' CAT TGA TAA CGA AGC TCT TTA CGA T3'; and Reverse: 5' GCA TGT TGT GCC GGACAT AAC CAT 3'.

PCR protocol

PCR reactions were performed in 25µl volumes in PCR tubes under aseptic conditions; all tubes contained the extracted DNA, primers, DDH2O and PCR premix table (1).The temperature profile consisted of initial pre incubation at 94˚C for 5 min, and then incubated for 30 sec at 94˚C (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.

Table (1): PCR mixture

PCR reaction components	Volume (µl)
PCR premix	12.5 µl
Primer F (10 picomols/µl)	1 µl
Primer R (10 picomols/µl)	1 µl
DNA template	1 µl
DDH_2O	9.5 µl
Total volume	**25 µl**

Agarose gel electrophoresis

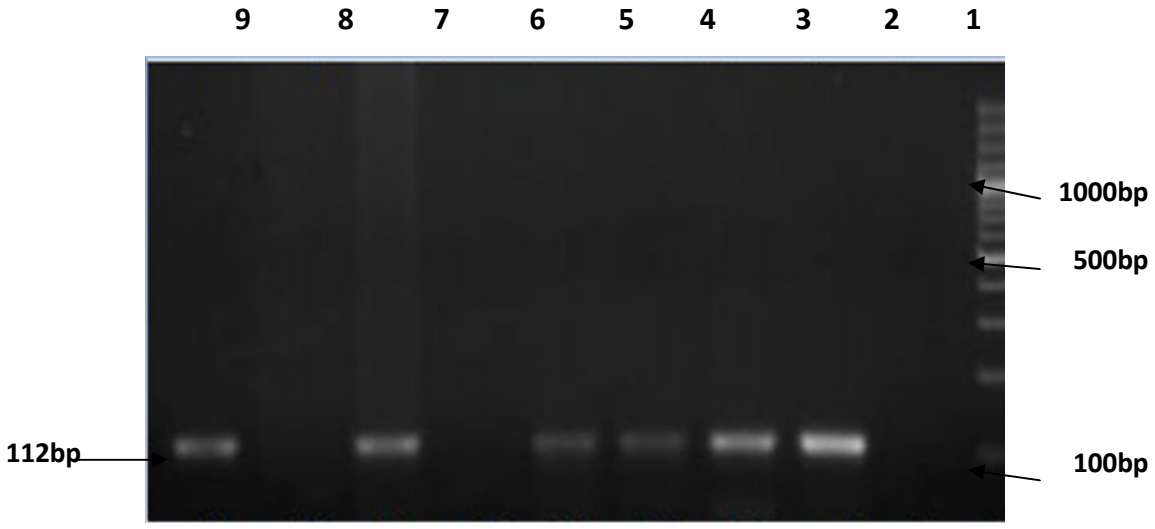
Agarose gel of 2% concentration was utilized for target gene detection. The agarose gel with 2% concentration provided by dissolve 2g in 100 ml of 1X TBE buffer using a microwave, then ten microliter of each PCR product were loaded into the wells on the gel, electrophoresis was carried out for about 1.5 hours at 5 volt/cm of the gel.

Results and discussion:

Trichomonasis is the most prevalent sexually transmitted disease (STD) in the word. Wet mount microscopical screening, culture and staining methods are frequently used in the diagnosis of T. vaginalis. Since *T. vaginalis* strains show high phenotypic variation due to expression level and differences in genomic sequencers, PCR technique was developed for diagnosis the parasite. Newly, a PCR technique using vaginal swap and urine samples for 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 technique.

In this study 94 vaginal swaps were collected from symptomatic women suffering from vaginal discharge, itching and dysuria. after investigating *T.vaginalis* by wet mount and PCR methods, 12 out of 94 sample were positive in both wet mount and PCR with percentage of infection (12.7%) where as 18 sample was positive (19.1%) to wet mount but when used PCR assay, a 26 sample (27.6%) were positive to *T. vaginalis* (table 2). Many studies showed that PCR technique is sensitive method can be used in diagnosis of *T. vaginalis* [7,8].

The results of this study agree with[9] who confirm that PCR was a good and sensitively method for diagnosis of *T. vaginalis* in compare with culture and wet mount microscopy. Twenty six sample were positive to PCR assay when amplified beta-tubulin gene(112bp) as show in figure (1).



Figure(1): Agarose gel electrophoresis for PCR product for detected β-tubulin gene. Electrophoresis was performed on 2 % agarose gel and run with a 5volt/cm for 1.30 hr. M 100bp ladder. Line (1) control negative. Line (2, 3, 4, 5,7 and 9) positive results Line(6 and 8) negative results

Table 2: distribution of *T. vaginalis* infection according to diagnostic test.

Test	Positive (%)	Negative (%)	Total
Wet mount	18 (19.1)	76 (80.8)	94
PCR	26 (27.6)	68 (72.3)	94
Both	12 (12.7)	82 (87.2)	94

Table (3) show the high prevalence of trichomonasis observed among the age group (30-40) followed by (20-29) then the older age group(>40) this results agree with the finding of previous study by[10] ,who confirm that trichomonasis is more prevalent among sexually active young people. also sexually transmitted disease awareness programs that mass media targeted at the younger generation might have contributed to the lower prevalence observed in less than 20year old group. Overall, half of all the women affected were in the 30-40-year-old age group. These older women might have lacked knowledge about health issues, might have lacked the confidence to correctly identify problems, and might have used traditional medicine rather than modern treatment[11].

Vaginal discharge is most common symptoms among patients(13.8%) when used PCR technique while (8.5%) in wet mount. 21 out of 94 suffered from vaginal discharge and itching (9.5%) in PCR and (6.3%) in wet mount, whereas 11 out of 94 with vaginal discharge and dysuria (table 4). This results were in agree with results of [8] ,whose reported that vaginal discharge was the most common symptoms in women with trichomonasis.

Table 3: distribution of *T. vaginalis* infection according to women age.

Age	Wet mount	PCR	Both
<20	1	2	2
20-29	5	8	3
30-40	9	12	6
>40	3	4	1
Total	18	26	12

Table 4: distribution of *T. vaginalis* infection according to symptoms.

Symptoms	No. patients	No. of positive		
		Wet mount(%)	PCR(%)	Both(%)
Vaginal discharge	62	8(8.5)	13(13.8)	6(6.3)
Vaginal discharge+ itching	21	6(6.3)	9(9.5)	2(2.1)
Vaginal discharge+ dysuria	11	4(4.2)	4(4.2)	4(4.2)
Total	94	18(19.1)	26(27.6)	12(12.7)

Results show the association between the Trichomonasis and marital status of women(table 5) and the reason responsible of that is the infection happen directly by sexual contact with infected men or by use of the contaminated towels . however, single women lowest prevalence(4.2%) when compared to married women (8.5%) ($p < 0.05$). other study show the sexually transmitted infection was higher prevalent in marred women than in single women[12]. In addition of these women with contraceptive device appear more susceptible to infection with *T. vaginalis* in compare with non users contraceptive device (table 6), the prevalence rate of contraceptive device user was significantly higher($p < 0.05$) (9.5%) than non user (3.1%). In study of 13 shows that the use of contraceptive device for a long period causes growth of *T. vaginalis* on the genital mucosa therefore special attention must be given to women who have prolong contraceptive device for the possible presence of *Trichomonas vaginalis*.

Table 5: distribution of *T. vaginalis* infection according to marital status.

Marital Status	No. of patients	No. of positive		
		Wet mount(%)	PCR(%)	Both(%)
Married	72	13(13.8)	19(20.2)	8(8.5)
Single	22	5(5.3)	7(7.4)	4(4.2)
Total	94	18(19.1)	26(27.6)	12(12.7)

Table 6: distribution of *T. vaginalis* infection according to using contraceptive device.

Contraceptive device	No. of patients	No. of positive		
		Wet mount(%)	PCR(%)	Both(%)
Yes	59	12(12.7)	22(23.4)	9(9.5)
No	35	6(6.3)	4(4.2)	3(3.1)
Total	94	18(19.1)	26(27.6)	12(12.7)

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