

Detection of *Trichomonas vaginalis* among women with abnormal vaginal discharge by PCR technique targeting TVK3 and TVK7 genes in Basrah province

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ABSTRACT:

A total of 552 samples of vaginal discharge were collected from women that visited the Maternity and Pediatrics hospital in Basrah Province .Microscopic examination of vaginal discharge revealed that 4.1 % of these women were infected with Trichomoniasis .

In present diagnostic study, two technique were used to diagnosed the same sample for the first time in Iraq , these include microscopic examination (wet preparation) and Polymerase chain reaction (PCR) .PCR technique was the highest sensitivity (100 %) which diagnosed three samples were considered negative in the other diagnostic technique and microscopic recorded sensitivity 88.4 % .In the same time diagnosis by vaginal discharge were found to be highly percent (100 %) than the urine (1.9%) .

Introduction

Trichomoniasis is a sexually transmitted disease (STD) with important health ramification ; it has been associated with vaginatis , Urethritis , and pelvic inflammatory disease (PID) . Trichomoniasis also impacts upon birth outcomes and is co – factor in human immunodeficiency

virus (HIV) transmission and acquisition (Swygard *etal.*, 2004) . Symptoms in women with Trichomoniasis include vaginal discharge , dysuria , and pruritus . in men symptoms include the urethral discharge , urethral pruritus , and dysuria (Schwebke and Burgess 2004)

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Approximately 180 million women worldwide may be infected with *T. vaginalis*. Prevalence estimates vary between population studies, but ranging from 5-74% in women and 5-59% in men, with the highest rate reported in either sex from sexually transmitted infection (STI) clinic and in other high risk population (Karyakarte and Damle 2003). Diagnosis of *T. vaginalis* depends on the observation of a motile organism in fresh vaginal discharge microscopically (Lawing et al., 2000). The current study used polymerase chain reaction (PCR) which has become increasingly attractive for diagnosing infection of *T. vaginalis* and compared with another diagnostic method.

Materials and methods:

Sample collection:

High vaginal swab (HVS) was collected from 552 women attending the maternity and pediatrics hospital in Basrah province with and without symptoms after the insertion of speculum (Verteramo et al., 2008). Two vaginal swabs were taken from each woman, first swab was placed in 500 µl of Tris-EDTA (pH:8) and stored at -20 for PCR, and second swab was mixed with a drop of normal saline and examined microscopically at 40X.

Diagnostic method:

Wet mount preparation was prepared through the mixing of vaginal discharge which collected above with a drop of normal saline and examined directly under 40X for observation the movement of organism. (Verteramo et al., 2008).

DNA extraction and PCR for *T. vaginalis*:

PCR was also used in this study to compare with another diagnostic method. DNA from *T. vaginalis* were extracted based on SDS-Proteinase K method (Sambrook et al., 1989). A set of primers (TVK3/TVK7) targeting a conserved region of *T. vaginalis* were used to amplify 300 bp piece of genome by PCR procedure. The sequences were as follows: for TVK3 (5'ATTGTCTCGAACATTGGTCTTACCCTC3') and for TVK7 (5'TCTGTGCCGTCTTCAAGTATGC3'). A total volume of 25 µl of PCR reaction was performed in 0.2 µl microtube which consist of: 1 µl of each primer set, 5 µl of DNA sample, 12.5 µl of Go Taq Green master mix and 5.5 µl of distilled water and mixed well, finally about 25 µl of mineral oil were added to reaction. PCR protocol was include: 5 min of denaturation at 94°C, followed by 30 cycle of 1 min of denaturation at 90°C, 30s of annealing at 60°C and extension at 72°C for 2min. final extension for 7min at 72°C were also included (Lawing et al., 2000).

Results:

Results show that the rate of infection with *T. vaginalis* is 4.1% among women in Basrah province using direct microscopic examination but 4.8% with PCR technique .

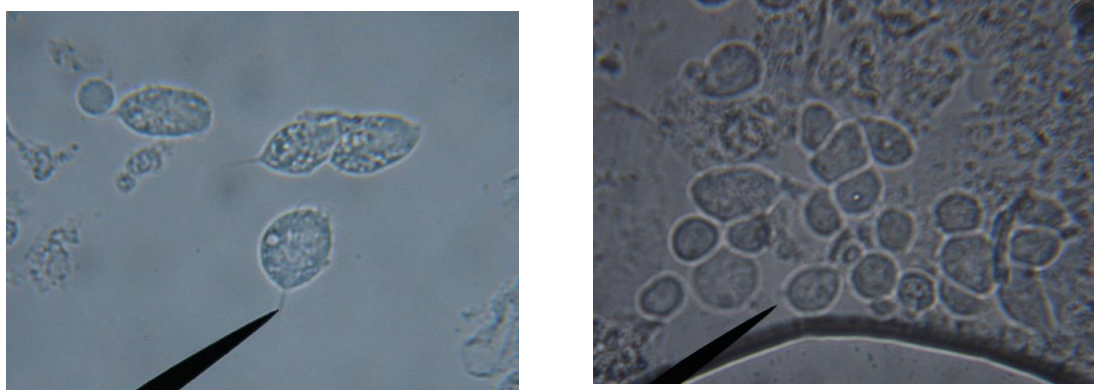


Figure (1): show high number of *T. vaginalis* from vaginal discharge in different shape (40X)

According to color of vaginal discharge , women with yellow greenish discharge show a high rate (34.7%) of infection with *T. vaginalis* .Table (1)

Table (1) the association between infection with *T. vaginalis* and color of vaginal discharge

Color of vaginal discharge	No. positive sample (%)
Yellow	6(26.0)
Greenish	4(17.3)
Creamy	3(13.0)
Yellow greenish	8(34.7)
Normal	2(8.6)

PCR show a high sensitivity and specificity in detection of *T. vaginalis* (100%) than microscopic examination since it was diagnosed three sample give negative results with microscopic examination , Table (2).

Table (2) comparison between method used in detection of *T. vaginalis*

Method	No. positive sample (%)	Sensitivity (%)	Specificity (%)
PCR	26(100)	100	100
Microscope	23(88.4)	88.4	100

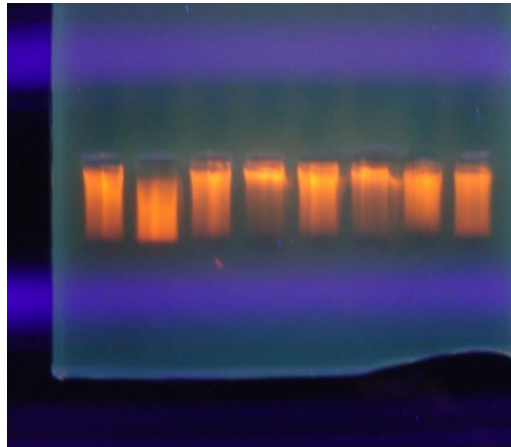


Figure (2): The amount of DNA electrophoresed gel extracted from vaginal discharge

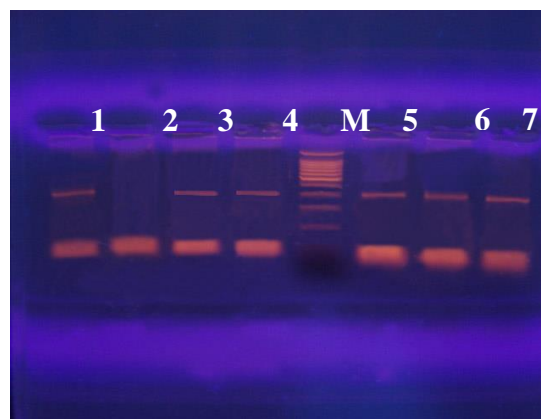


Figure (3): show 300 bp amplification of TVK3/7 gene with PCR where 1,3,4,5,6,7 are positive sample for infection with *T. vaginalis*, 2 is negative sample and M is DNA ladder to compare the results

Discussion :

T. vaginalis is a parasitic protozoan that causes Trichomoniasis, a sexually transmitted disease. It is a cosmopolitan and common in female (Graves and Gardner, 1993; Sena *et al.*, 2007). The present study shows that the rate of infection with Trichomoniasis among women who visited the Maternity and Pediatric hospital in Basrah province was 4.1% using microscopic examination of vaginal discharge. Similar prevalence of *T. vaginalis* among women in Iraq has already been established by Miteb (2000) who found that the overall incidence to be 4.9% in Najef city. Al-Saadi (2003) has shown prevalence as 22.3% in Al-Dewaniya city. However, in Kirkuk and Basrah women, Kadir and Aziz (1989) and Gani (2000) reported infection rates 8.05% respectively. Accordingly, the rate of infection among Iraqi women was low comparing with other rates of infection which reported in the world like Britain 32% (Caterall, 1970), New York 27% (Dettovitz *et al.*, 1994) and 15.3% in Turkey (Yazar *et al.*, 2002) and among Arab population the rate of infection was 18% in Syria (Yasmmench, 1998) and 15% in Saudi Arabia (Abdus and Talukder, 1986). The epidemiology of *T. vaginalis* is influenced such as personal hygiene, sanitation and good use of water (Davis and Clays, 1992). Islamic rules and habits prevent all the non-marital sexual relationship (Safe sex) which is common in non-Islamic countries and that decreasing the rates of sexually transmitted infection (Madani, 2006). Bowden and Garnett (2000) reported a high rate of infection among sexually active women,

and this accepted Verteramo *et al.*, (2008) who mentioned that multiple of sexual partners and lifestyle leads to increasing of the sexually transmitted infection. Rosenberg *et al.* (1999) stated that in disadvantage communities another factors related to the lowering of the infection that the using of condom during sexual intercourse and metronidazole in treatment of venereal disease and microorganism infections. Among the main clinical signs of infected women were abnormal vaginal discharge which contains a large number of pus cells and microorganisms such *T. vaginalis* which infect the vagina and utilized the iron and lipids during RBC lyses (Fiori *et al.*, 1993). Abnormal discharge form a problem for women which have genital tract infection (GTI) when this sign consist 95.6% of infected women with *T. vaginalis* in present study. Similar percentage was reported by Lo *et al.* (2002) in his review of infection in Auckland sexual health clinics abnormal vaginal discharges are appear with different color like yellow and green with offensive odors. Yellow greenish discharge consist a high rate of percentage in our result and this may be explained by the heavy infection and huge number and parasite T.V. in vagina which lead to increases of stains resulting from cell lyses. This result was well documented when examined sample contain small numbers of parasite. the vaginal discharge was normal and when the number of parasite increased the color changed from normal to yellow and finally greenish and this certain through examine the patients for long time, however color of vaginal discharge may be due to another

infection such as *candida albicans* , therefore the diagnosis of the infection by T.V. must be don't totally depends on the color of vaginal discharges. In the present diagnostic study , two diagnostic methods were use to detect *T. vaginalis* , PCR , wet mount preparation ,each of these methods have advantage and disadvantage . wet mount preparation is easy and possible to work quickly but it is required at least 10^3 motile parasite per ml for diagnosing and this should be work quickly in order to prevent the lyses of sample through transporting (Petrin *etal.*1998; Kengne *etal.*, 1998)

The PCR which used for the first time in Iraq is highly sensitive (100%) .All microscopic positive specimens moreover three sample diagnosed were negative in the other diagnostic methods were detect by PCR . This technique (PCR) is also highly specific (100%) , PCR able to detect *T. vaginalis* in concentration one cell in vaginal secretion , so PCR able to detect each viable and non viable organism (Reily *etal.*,1999) . This study gives a good picture about the epidemiology of urogenital Trichomoniasis in Basrah city .

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تشخيص الاصابة بطفيلي المشعرة المهبليية بين النساء اللاتي يعانين من الافراز المهبلي غير الطبيعي في محافظة البصرة باستخدام تقنية PCR مستهدفا الجين TVK3,7

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الخلاصة

جمعت 550 عينة افراز مهبلية من النساء اللواتي راجعن مستشفى النسائية والطفل في محافظة البصرة . تبين من خلال الفحص المجهرية المباشر ان 4.1% منهن مصابات بداء المشعرات المهبليية. استخدم في الدراسة الحالية طريقتين في التشخيص ولاول مرة في العراق , والتي تضمنت الفحص المجهرية وتفاعل البلمرة التسلسلي . وتبين من خلال الدراسة ان تفاعل البلمرة التسلسلي اكثر حساسية من الفحص المجهرية في الكشف عن الاصابة , اذ شخصت ثلاثة عينات اظهرت نتائج سلبية عند الفحص المجهرية .