Use TVK 3/7 gene as a target to detect Trichomonas vaginalis from urine of women in Southern Iraq

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ABSTRACT

A total of 662 samples of urine and vaginal discharge from women with and without symptoms for Trichomoniasis (STD) were examined during the period from September 2007 to February 2009 who entered the Maternity and pediatrics hospital , private clinic and laboratories in each Basrah and Thiqar Province (Southern Iraq) using TVK3/7 gene as target by PCR technique. The results shows that the urine samples has 90% of sensitivity to detect the parasite and that mean the possibility of use the urine to detect infection with trichomoniasis by PCR as use of vaginal discharge , suggesting that urinary tract get infection in rate 90% during infection of vagina .

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INTRODUCTION

Trichomonas vaginalis is a flagellate protozoan infect the urogenital tract of men and women [1], with more than 170 million cases worldwide[2]. It is transmitted mainly by sexual intercourse , rarely by non venereal means such as sharing of contaminated towels , underclothing , or using of non sterile medical examination tools [3],[4] . In women it cause vaginitis and cystitis ,whereas in men it cause urethritis and prostatities [5] ,[6]. Trichmoniasis has important medical , social , and economical implication . women who are infected during pregnancy are predisposed to premature rupture of the placental membrane , premature labor and low- birth – weight infants [6] .Complications of this disease are cervical cancer , a typical pelvic inflammatory disease and infertility [7] . Traditionally diagnosis of *T. vaginalis* has depend on the observation of motile organism in vaginal discharge or cervical secretions [8] . Most studies built them results highly based on vaginal discharge examination [9],[10] whereas current study used urine sample to detect the parasite by various methods such as microscopic examination , polymerase chain reaction (PCR) , culturing and Geimsa staining comparison with vaginal discharge examination .

MATERIAS AND METHODS :

Samples collection :

Urine samples were take from 662 women under current study who entered the Maternity and pediatrics , private clinics and laboratories in South of Iraq (Basrah and Nassiriyah provinces) during the period from September 2007 to February 2009 , informations about age , marital status , education , economic status , locality , symptoms , pregnancy , type of labor , infertility and use of contraceptive were also collected .

Diagnostic methods :

Urine sample were collected by test tube from women, centrifuged at 1000 xg and the sediment divided into four groups one examined directly by light microscope (40X) [8] , second were inoculated into Kupferberg Trichomonas broth (Ph:6) and incubated at 37C˚ [11] , third were washed twice with distilled water and stored at -20 for PCR assay and the fourth were stained with Geimsa stain .Four high vaginal swab (HVS) were obtained from the same women above , first vaginal swab were examined directly by light microscope at 40X , the second were also inoculated in the same culture media and incubated at same temperature, the third were stained with fixed Geimsa stain , Geimsa stain was used and prepared according to method used by [9] , briefly , the vaginal swab was mixed with a drop of normal saline , fixed with 70% ethanol and dried then Geimsa stain were add and left to dry for hour , finally washed with water ,dried and examined under 40X to observe the fixed organisms and the fourth were placed in 500 µl of Tris – EDTA (Ph:8) and stored in -20 C˚ for PCR assay .

DNA extracted from urine sample and vaginal discharge :

Proteinase K/ SDS method [12] were used to extract DNA of *T. vaginalis* from urine sample and vaginal discharge as following: stored Tris- EDTA containing sediment of urine or vaginal discharge were centrifuged at 12,000 rpm for 5 minutes at 4C˚ , discard the upper layer and add 500-600 µl of Tris – EDTA to the pellet in eppendorf and mixed well (by vortex for 2 min) , add 70 µl of 10% SDS (Ph:7.2) follow by 6 µl of proteinase K to eppendorf and mixed well , incubated at 65C˚ for one hour in water bath , add 10 µl of sodium chloride and mixed well, then add 750 µl of chloroform : isoamyl alcohol in the ratio 24:1 , centrifuged at 12,000 rpm for 5min and carefully transferred the upper layer to another eppendorf and add 550 µl of chilled iso- propanol follow by gentle shaking to precipitate the DNA , keep the tube in a freezer overnight and then centrifuged at 9 – 10 K rpm for 10-15 min in a cooling centrifuged , drain the supernatant and dissolve the pellet in 50 µl of Tris – EDTA , finally store at -20 C˚ after checking in 0.8% agarose gel

Primer use and PCR protocol for T. vaginalis :

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 sequence were as follow : for TVK3 (5' ATTGTCGAACATTGGTCTTACCCTC 3') and for TVK7 (5' TCTGTGCCGTCTTCAAGTATGC 3') . 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 add to reaction . PCR protocol was include : 5 min of denaturation at 94C˚ , followed by 30 cycle of 1 min of denaturation at 90C˚ , 30s of annealing at 60C˚ and extension at 72C˚ for 2min . final extension for 7min at 72C˚ were also included [13] , [10].

RESULTS

The result of examination of 662 women in present study by various methods and in different ways show 27 (4.0 %) of infection with *T. vaginalis* from urine whereas 30 (4.5 %) of infection from vaginal discharge . Table (1) PCR compared with traditional diagnostic method has revealed more sensitive technique to detect *T. vaginalis* from both urine sample and vaginal discharge which is diagnose 27 (90%) from urine and 30 (100%) from vaginal discharge , also PCR detects 14 urine sample that give negative results with another methods and four vaginal discharge sample . Table (2) , Figure (1) There are

differences in a mount of DNA extracted from both of urine and vaginal discharge samples, DNA from urine has revealed as a smear and less amount whereas DNA from vaginal discharge is high. Figure (2). Microscopic examination of urine and vaginal discharge show high differences in characters , activity and number of parasites in sample, during microscopic examination of urine the parasite appear in less activity , hidden to the most characters like undulating membrane which is recognize and move actively in vaginal discharge (about 3 waves) and flagella occasionally has been recognize for a short time , furthermore the number of parasites in urine is very small, while in vaginal discharge is different which high number of parasite can be recognize and move actively with four flagella that equal in length for a long time . Figure (3) (4) Staining method consist a low sensitivity in both urine sample (20%) and vaginal discharge (53%) compared with another diagnostic methods in spite of the possibility in recognizing most characters of *T. vaginalis* such as flagella , nucleus , undulating membrane and costa . Figure (5) Information of each women under current study especially who get infection with *T. vaginalis* are important to know the reason that related to infection . symptoms associated with infection are record 55.5% , 60% with vaginal discharge , dysuria , low abdominal pain and dyspareunia among women have infection in urinary tract and vagina whereas dysuria and urethritis show high rate among women have infection with urinary tract (29%) , vaginal discharge and pruritis consist high rate among women get infection in vagina . Table (3) Women who have age ranging from 26 – 30 years show high rate of infection with *T. vaginalis* for each urine sample (44.4%) and vaginal discharge (43.3%) followed by women in age group 31 – 35 years . Table (4) Infection with *T. vaginalis* is restrict among married women for both urine sample and vaginal discharge when study the association between infection with *T. vaginalis* and marital status . Table (5) Results are record high rate of infection with *T. vaginalis* among non pregnant women for both urine sample and vaginal discharge followed by women with infertility . Table (6) among pregnant women , women with preterm labor consist the total rate of infection for both urine sample and vaginal discharge .Table (7) Recent years the use of contraceptive is increase , among women used for contraceptive current study has been explain that the infection with *T. vaginalis* are decrease during the use of contraceptive except four of infection is record among women who use of IUCD as contraceptive for both urine sample and vaginal discharge . Table (8) Occupation , education , economic status and locality also included in this study **,** worker women show high rate of infection with *T. vaginalis* for both urine and vaginal discharge except in less differences . Table (9), whereas ignore women record high rate of infection in both urine and vaginal discharge . Table (10), and women who are high economic status explain 36.0% rate of infection in urine and 40% in vaginal discharge Table (11) ,and finally results return to locality show in Table (12) .

DISCUSSION

Data on detection of *T. vaginalis* from urine of women are very limited because of most studies are prefer to using of vaginal discharge in diagnosis of parasite depending on traditional diagnostic methods and using of urine sample only to comparison with vaginal discharge [14] , [15] . Current study is used urine samples mainly beside vaginal discharge by PCR for each and for the first time in Iraq ,the total rate of infection with *T. vaginalis* among women in south of Iraq by urine are near to that record by vaginal discharge 27 (4.0%) and 30 (4.5%) so urine sample is useful to detect

the parasite by PCR depending on TVK3/7 gene as a target .Recent years molecular techniques are provide anew method in detection the parasitic infection such as *T. vaginalis* [16] , [13] . PCR is one of these molecular method which is allow to amplify one molecules of DNA for one cell in vitro for millions times [17] ,PCR able to detect *T. vaginalis* in concentration one cell at least from urine sample and vaginal discharge so the ability of PCR to detect each viable and nonviable organism [18] . In present study PCR record sensitivity about 90% in urine and 100% in vaginal discharge this is differ from [14] and [15] because they were build them results depend on traditional methods only but the less differences in results between both sample return to three urine sample get negative results with PCR and this may related to contamination and chemical component of urine which may inhibits PCR reaction [13] . Traditional methods has low sensitive in detection the parasite from both urine sample and vaginal discharge compared with PCR , microscopic examination for both sample get rather results in vaginal discharge than urine since microscopic examination depends on observation of a motile organism in fresh sample [19] , [5] and this different in both urine sample and vaginal discharge where *T. vaginalis* appears in vaginal discharge jerky motile and actively but in urine is less activity and loss more it characters such as undulating membrane , axostyle and flagella occasionally in contrast to the presence of it in vaginal discharge , the characters of parasite is clear like four equal flagella , 3-4 waves of undulating membrane and axostyle furthermore number organism is more in vaginal discharge than urine since microscopic examination require 10^3 per ml of viable parasite to get results [5] , [20] , [21] . culturing and staining have disadvantage more than advantage like time consumption , skull of workers and lost of the most parasite characters during fixation and staining process [1]. The rate of infection if by urine sample or vaginal discharge is low compared with studies were present in the world such as 27% in New York [22], 15.3% in Turkey [9] , and 22 % in Nigeria [23]. In Iraq and other Islamic countries sexually transmitted diseases (STD) like *T . vaginalis* are rare since Islamic roles and values prevent all the illegal sex relationship through application no age limited for marriage as law [24], and this is seen during recorded most infection of Trichomoniasis among married women in current study and each of [14] , [15] , [25] since infection is getting mainly by sexual intercourse which is may return to husband responsibilities and rarely from contaminated towels [2] , therefore women who are age at 26 – 30 consist high rate of infection because all of them were married so infection with *T. vaginalis* related to lifestyle and high among disadvantage communities and among women with multiple sexual partners [26] . Some studies reported some association between infection and pregnancy [14] , [25] and some of them explain that *T. vaginalis* may causes premature rupture of the placental membrane , premature labor and low-birth – weight infants [5] , only three pregnant women are infected with and get premature labor and this may leads to release prostaglandins in vagina and then in amniotic fluid [27] but the infection in non pregnant women is high since most of them are not use of contraceptive which increase the thickness of cervix and closed it preventing *T. vaginalis* and other microorganisms from reaching and prevent the following of menstrual blood which provide iron and source of organism alive [28] , [5] . Non worker , ignore , and rural women consist high rate of infection since infection may return to ignorance , low use of contraceptive such as condoms by their ale , sanitation and personal hygiene [1] .

Figure (1): 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

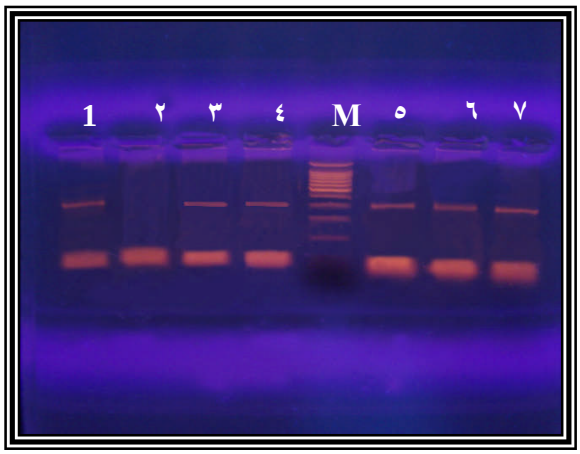


Figure (2): The amount of DNA extracted from urine sample (left) and vaginal discharge (right)

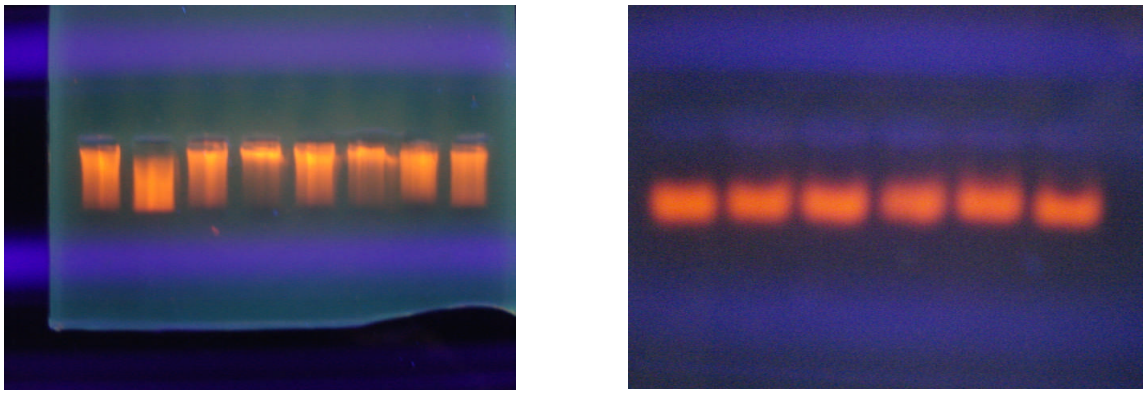


Figure (3): The shape of *T. vaginalis* in urine sample (left) and vaginal discharge (right) 40X

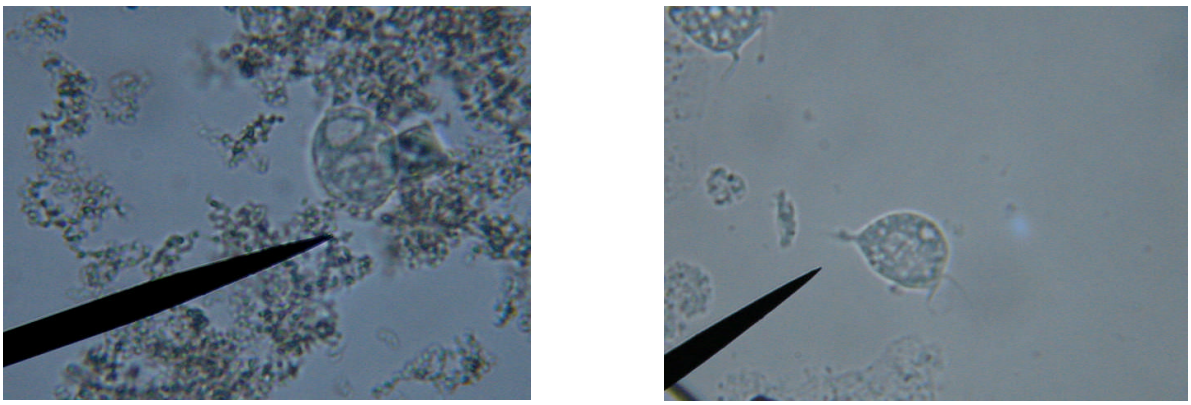
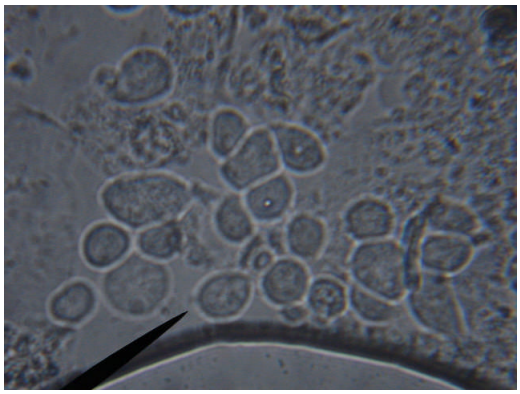


Figure (4): show high number of *T. vaginalis* from vaginal discharge in different shape (40X)



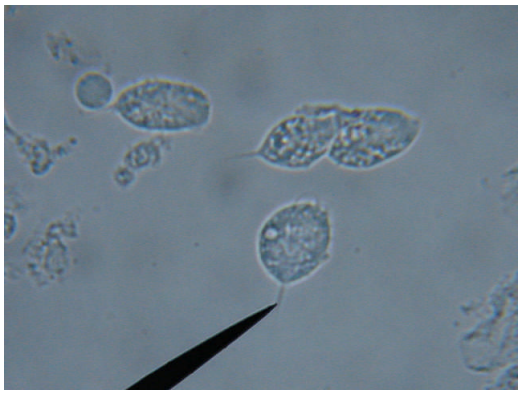


Figure (5): The shape of *T. vaginalis* after staining with Geimsa stain (40X)

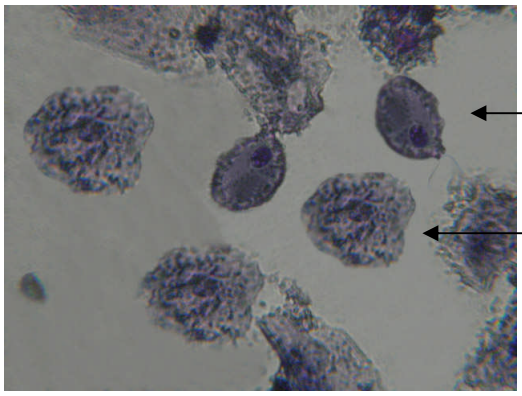


Table (1): The rate of infection from urine compared with vaginal discharge

Type of sample	No. positive sample (%)	Sensitivity (%)	Specificity (%)
Urine	27 (4.0)	90	100
Vaginal discharge	30 (4.5)	100	100

Table (2): comparison between diagnostic methods in Both of urine sample and vaginal discharge

Diagnostic methods	No. positive sample from urine	Sensitivity in urine (%)	No. positive sample from vaginal discharge	Sensitivity in vaginal discharge (%)
PCR	27	90	30	100
Microscope	13	43.3	26	86.6
Culturing	10	33.3	24	80
Staining	6	20	16	53

Table (3): The association of infection with *T. vaginalis* and symptoms for both urinary tract and vagina

Symptoms	No. positive sample in urinary tract (%)	No. positive sample in vagina (%)
Vaginal discharge , dysuria , low abdominal pain and dyspareunia	15 (55.5)	18 (60)
Vaginal discharge , dysuria and urethritis	8 (29)	3 (10)
Vaginal discharge and pruritis	4(14)	9(30)
Total	27	30

Table (4): The association between infection with *T. vaginalis* and age group for each urine sample and vaginal discharge

Age group	No. positive sample in urine sample (%)	No. positive sample in vaginal discharge (%)
15 – 20	0	0
21 – 25	3(11.1)	3(10)
26 – 30	12(44.4)	13(43.3)
31 – 35	7(25.9)	9(30)
36 – 40	5(18.5)	5(16.6)
> 40	0	0
Total	27	30

Table (5): The association between infection and marital status for each urine sample and vaginal discharge

Marital status	No. positive sample in urine (%)	No. positive sample in vaginal discharge (%)
Married	27(100)	30(100)
Single	0	0
Widow	0	0
Divorced	0	0

Table (6): The association between infection with *T. vaginalis* and pregnancy for both urine sample and vaginal discharge

State of pregnant	No. positive sample in urine (%)	No. positive sample in vaginal discharge (%)
Non pregnant	20(74)	23 (76.6)
Pregnant	3(11.1)	3(10)
Infertile	4(14.8)	4(13)
Total	27	30

Table (7): The association between infection with *T. vaginalis* and type of labor for each urine and vaginal discharge

Type of labor	No. positive sample in urine (%)	No. positive sample in vaginal discharge (%)
Nature	0	0
Preterm	3(11.1)	3(10)
Cesarean	0	0
Total	3	3

Table (8): The association between infection with *T. vaginalis* and use of ontraceptive for each urine sample and vaginal discharge

Type of contraceptive	No. positive sample in urine (%)	No. positive sample in vaginal discharge(%)
Not use	23(85.1)	26(80)
IUCD	4(14.8)	4(13.3)
Injection	0	0
Condom	0	0
Tubal ligation	0	0
Pills	0	0
Total	27	30

Table (9): The association between infection with *T. vaginalis* and occupation for each urine sample and vaginal discharge

Occupation	No. positive sample in urine (%)	No. positive sample in vaginal discharge (%)
Worker	5(18.5)	6(20)
Non worker	22(81.4)	24(80)
Total	27	30

Table (10): The association between infection with *T. vaginalis* and Education for each urine sample and vaginal discharge

Education	No. positive sample in urine (%)	No. positive sample in vaginal discharge (%)
High	3(11.1)	4(13.3)
Secondary	5 (18.5)	5(16.6)
Primary	8 (29.6)	8(26.6)
Ignore	11(40.7)	13(43.3)
Total	27	30

Table (11): The association between infection with *T. vaginalis* and Economic status for each urine sample and vaginal discharge

Economic status	No. positive sample in urine (%)	No. positive sample in vaginal discharge (%)
High	10(37.0)	12(40)
Mediate	10 (37.0)	11(36.6)
Low	7 (25.9)	7(23.3)
Total	27	30

Table (12): Distribution of infection with *T. vaginalis* among women based on their locality .

locality	No. positive sample in urine (%)	No. positive sample in vaginal discharge (%)
Rural	18(66.6)	19(63.3)
Urban	9(33.3)	11(36.6)
Total	27	30

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استخدام الجين TVK3/7 كمستهدف في الكشف عن طفيلي المشعرة المهبلية *Trichomonas vaginalis* من إدرار النساء في جنوب العراق

***امل خضير خلف و * خالد جميل كاظم**

الخلاصة :

فحصت ٦٦٢ عينة ادرار وافراز مهبلي من النساء اللواتي يظهرن اعراضا للاصابة بداء المشعرات المهبلية والاتي لايظهرن الاعراض . المراجعات لمستشفى النسائية والاطفال ، العيادات والمختبرات الاهلية خلال الفترة من شهر ايلول ٢٠٠٧ الى شهر شباط ٢٠٠٩ في محافظتي البصرة والناصرية / جنوبي العراق وباسخدام تقنية تفاعل البلمرة التسلسلي PCR مستهدفا الجين TVK3/7 . تبين من النتائج ان حساسية فحص الادرار باستخدام PCR كانت ٩٠% وهذا يعني امكانية اسخدام الادرار في الكشف عن طفيلي المشعرة المهبلية مثلما يستخدام الافراز المهبلي ومقترحا اصابة القاة البولية بنسبة ٩٠% عند اصابة المهبل .

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