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Study the association between the infection with *Trichomonas vaginalis* and use of contraceptive among women with abnormal vaginal discharge by PCR technique in Nassiriyah city

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ABSTRACT

The aim of the present study was designed to determine the relationship between the infection with *T. vaginalis* and use of contraceptive among women whom suffering from abnormal vaginal discharge in Nassirriyah city \ Thiqar Province using TVK3/7 gene as target by PCR technique. all women entered the Maternity and pediatrics hospital , private clinics and laboratories.

The results are explain that the infection with *T. vaginalis* are decrease during the use of contraceptive since there are no case of infection with *T. vaginalis* among women whom used injection , pills and tubal ligation except there is three or 3(0.67) cases of infection is recorded among women who use of IUCD compared with 11 (3.1) total infection among women whom are not use the contraceptive.

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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 the current study used vaginal discharge to detect the parasite by polymerase chain reaction (PCR).

Materials and Methods :

Samples collection :

Highly vaginal swab (HVS) were obtained from about 447 of women with abnormal vaginal discharge whom attending the Maternity and pediatrics hospital , private clinics and laboratories in Nassirriyah city, vaginal swab were placed in 500 µl of Tris – EDTA (Ph:8) and stored in -20 C˚ for PCR assay .

Figure (1): microscopic examination for vaginal discharge show

T. vaginalis in different shape (40X)

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).

Results :

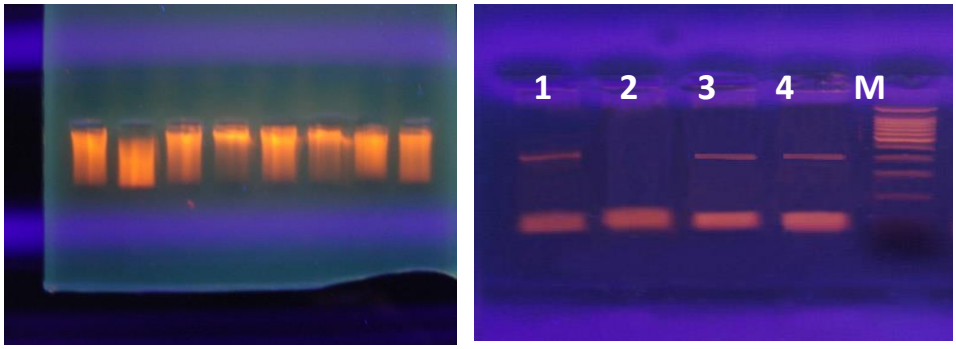
The current study has been explain that the infection with *T. vaginalis* are decrease during the use of contraceptive since there are no case of infection with *T. vaginalis* among women whom used injection , pills and tubal ligation while there is three or 3(0.67) cases of infection is recorded among women who use of IUCD as contraceptive by PCR technique , further condom are not associate with *T. vaginalis* infection . Table (1) , Figures (1,2)

DNA extraction and PCR program for *T. vaginalis* :

DNA from *T. vaginalis* were extracted based on SDS \ Proteinase K method (12) . 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

Table (1): The association between infection with *T. vaginalis* and use of contraceptive for vaginal discharge

Type of contraceptive	No. positive sample (%)
Not use	11 (3.1)
IUCD	3(0.67)
Injection	0
Condom	0
Tubal ligation	0
Pills	0
Total	14(3.1)



Figures (1,2) show DNA extraction and amplification of *T. vaginalis* after Electrophoresis in 0.2 % of agarose gel , the sample 1,3,4 show positive results , 2 show negative result , M is DNA Ladder to compare results

Discussion :

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], since infection is getting mainly by sexual intercourse which is may return to husband responsibilities and rarely from contaminated towels [2] , [25].The use of contraceptive is widely among women in Nassiriyah city and the rate of infection were increase among women whom are not use of contraceptive since it is 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

Data on detection of *T. vaginalis* from 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 vaginal discharge to diagnose *T. vaginalis* by PCR for the first time in Iraq ,the total rate of infection with *T. vaginalis* among women with and without contraceptive in south of Iraq by vaginal discharge 14 (3.1%) by PCR depending on TVK3/7 gene as a target . The rate of infection 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].

of organism alive [27] , [26] , [5] . Recently, molecular techniques are provide anew method in detection the parasitic infection such as *T. vaginalis* [16] , [13] . PCR is one of these molecular methods 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 sample so the ability of PCR to detect each viable and nonviable organism [18] . this results are different from [14] and [15] because they were build them results depend on traditional methods only .

Traditional methods has low sensitive in detection the parasite compared with PCR , since microscopic examination of vaginal discharge depends on observation of a motile organism in fresh sample [19] , [5] where *T. vaginalis* appears jerky motile in vaginal discharge the characters of parasite is clear like four equal flagella , 3-4 waves of undulating membrane and axostyle [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].

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الخلاصة

استهدفت الدراسة الحالية العلاقة بين الاصابة بطفيلي المشعرة المهبلية واستخدام موانع الحمل بين النساء اللواتي يعانين من الافرازات المهبلية والمراجعات لمستشفى بنت الهدى للنسائية والطفل للعيدات والمختبرات الاهلية في مدينة الناصرية لمحافظة ذي قار بأستخدام تقنية تفاعل البلمرة التسلسلي PCR .

بينت النتائج بان الاصابة بالطفيلي تقل عند استخدام الحقن والحبوب وعقد الانابيب كموانع للحمل ماعدا ثلاث حالات للاصابة بالطفيلي سجلت بين النساء المستخدمات للولب IUCD مقارنة بنسبة اصابة (3.1) 11 كانت بين النساء الغير مستخدمات لموانع الحمل .