Use PCR technique to detect the infection with *Trichomonas vaginalis* among women with preterm labor

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Abstract:

The aim of the following study was to explain the association between the infection with *Trichomonas vaginalis* and preterm labor between women with preterm labor in Nassiriyah city \ south of Iraq by PCR technique. The results revealed that *T. vaginalis* has a role or co – factor in causing of this action among pregnant women through the reporting about 4.8% of infection with *T. vaginalis* among this female.

Introduction:

Trichomonas vaginalis is a flagellate protozoan infects the urogenital tract of men & 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, underclothing, towels or usingmof non sterile medical examination tools [3, 4]. In women it causes cystitis and vaginalis, whereas in men it causes prostatitis and urethritis [5, 6].

Trichmoniasis has important medical, social, and economical implication. Women who are infected during pregnancy are predisposed to premature rupture of the fetal membrane, premature labor and low- birth – weight infants [6]. Complications of this disease are cervical cancer, a typical pelvic inflammatory disease & infertility [7]. A wide spectrum of causes and demographic factors have been implicated in the preterm labor, one of this result from amniotic fluid infection caused by a variety of micro-organisms located in the genital tract: approximately one-third of preterm births are associated with chorioamniotic infection.

Traditionally diagnosis of T. vaginalis has depended on the observation of motile organism in vaginal discharge or cervical secretions [8, 9, 10]. The current study used vaginal discharge to detect the parasite by polymerase chain reaction (PCR).

Materials and Methods:

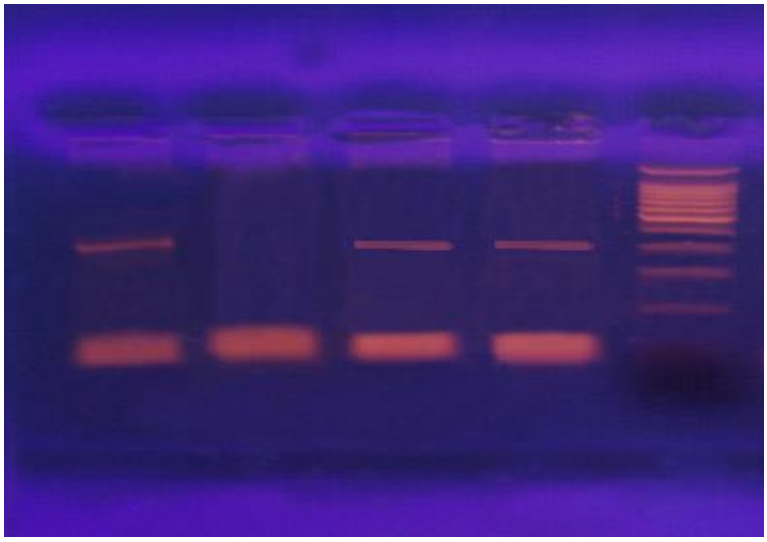
Samples collection: Highly vaginal swab (HVS) were obtained from 103 of women with preterm labor whom attending the Maternity and pediatrics hospital and private clinics in Nassirriyah city, from 1st of January 2013 to 1st of December, vaginal swab were placed in 500 µl of Tris – EDTA (Ph:8) and stored in -20 C° for PCR assay.

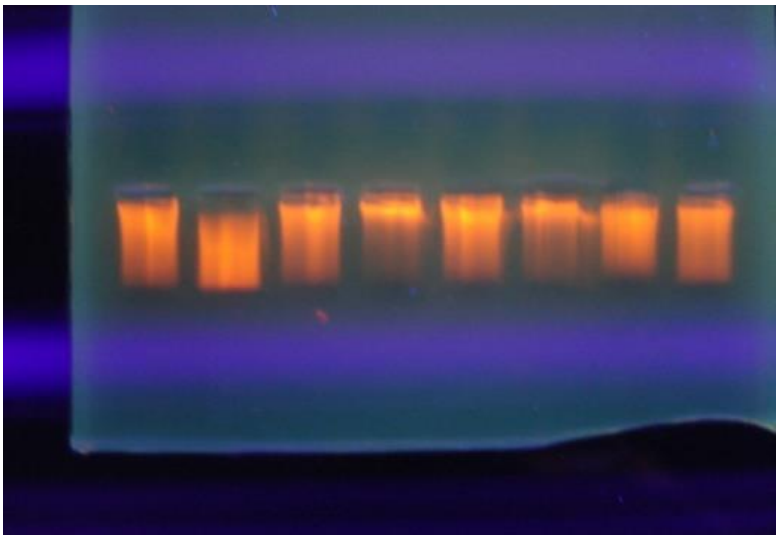
DNA extraction and PCR program for T. vaginalis:

DNA from T. vaginalis was 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 sequences were as follow: for TVK3 (5' ATTGTCGAACATTGGTCTTACCCTC 3') and or.TVK75'TCTGTGCCGTCTTCAAGTATG C 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).

Results:

The current study has been explained that T. vaginalis has responsible for preterm labor in about 5 (4.8%) of women with preterm labor in Nassiriyah city by PCR technique, further condom is not associate with T. vaginalis infection. Figures (1, 2)





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 results, M is DNA Ladder to compare results

Discussion:

Data on detection of T. vaginalis from women with preterm labor 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 preterm labor is 5 (4.8%) 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]. 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, 27, 26, 5].

Recently, molecular techniques are providing a new 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]. These results are different from [14, 15] because they were building them results depend on traditional methods only.

Traditional methods have 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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استخدام تقنية تفاعل البلمرة التسلسلي في الكشف عن طفيلي المشهرة المهبلية Trichomonas vaginalis بين النساء ذوات الولادة المبكرة

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المستخلص:

استهدفت الدراسة الحالية العلاقة بين الاصابة بطفيلي المشعرة المهبلية والولادة المبكرة بين النساء والمراجعات لمستشفى بنت الهدى للنسائية والطفل والعيادات الخاصة في مدينة الناصرية لمحافظة ذي قار باستخدام تقنية تفاعل البلمرة التسلسلي PCR. بينت النتائج بان الاصابة بالطفيلي ايضا من العوامل المؤدية للولادة المبكرة اذ سجلت حوالي ٥(٤,٨%) من النساء كانت مصابه بالطفيلي من خلال فحص الافرازات المهبلية لهن.