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## Molecular investigation of Trichomoniasis in women in Al-Muthana province /Iraq

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### Abstract

This study was conducted to determine the effect of factors as synthetic contraceptives used by women on the incidence of trichomoniasis, (150) vaginal swap samples were collected from women who complained of vaginal infections only (no miscarriage or pregnancy) who visit : the Children's and Women's Hospital in Al-Muthana governorate and private clinics during the period from January 2018 to June 2018, the parasite was diagnosed by using a wet smear and recorded 39/150 (26%) positive infection, after that the samples conducted to molecular method PCR on the basis of 18SrRNA gene to reach more accurate results and showed to rate infection (32%) 48/150, then collect information from each patient about the use of contraceptives:(did not use any method, contraceptives pills, condom, IUCD) and has been recorded infection rates (34.80%), (30.10%), (21.11%), and (6.40%) respectively, these results indicate the importance of the use of contraceptives for the incidence of a parasite as risk factors, the statically analysis for this results showed there were significant differences between rate of infection and type of contraceptives under probability level ( $p \leq 0.05$ ).

Keywords: Trichomoniasis, women, PCR.

### 1. Introduction

It is one of the most sexually transmitted diseases (STDs) caused by parasitic infection *Trichomonas vaginalis* infection, the sexual pathogenesis is the most common method of transmission [1]. The female vagina is characterized by being an environment suitable for many forms of microbial and parasitic infections. The symptoms of the disease in females are yellow, green, The smell also contains epithelial cells, white and red blood cells. The patient also feels itching in the vaginal area, burning, dysmenorrhea, pain when urinating urine and abdominal pain [2] Showing small hemorrhagic spots in the membrane [3], vaginal infections are common in all ages, resulting in a natural imbalance in the bacterial vagina and a dysfunction of beneficial bacteria known as lactobacillus [4], Trichomoniasis begins with simple symptoms and continues to become acute, causing some other health problems as a result of poor comprehension and awareness of the disease. Therefore, the risk is exacerbated and the treatment becomes difficult. It may cause cervical cancer, vaginal wall erosion, and most important infertility [5; 6]. Therefore, it is important to study the effect of some factors on the spread of parasite intensive study to determine its role in the spread of this society scourge [7], especially that Trichomoniasis is a disease of men and women as well as children, and transmitted in many ways (direct intercourse), or may move illness through the use of unhealthy public toilets [5], Therefore, awareness and factors are important to prevent the occurrence and spread of the disease, the aim of this study is to investigate the effect of contraceptives on the trichomonas as an important and specific risk factor for this serious parasitic infection.

### 2. Material and methods

(150) vaginal specimens were collected from January 2018 to June 2018 from women referred to : the Children's and Women's Hospital in Al-Muthana governorate, who suffer from infections in the genital area, aged between (20-45) years, vaginal fluid samples were collected using vaginal fold swabs by a specialist doctor



[8], the information was collected for each review in a questionnaire containing information about the age and the contraceptive they used.

-Preparation of the moist swab: A drop of saline solution was placed on a clean and sterile slide, the cotton piece containing the sample was then wrapped on the glass slide to mix well with the salt solution and examined at 400X magnification force [9].

Polymerase chain reaction: DNA was extracted from the vaginal smear according to the DNA genome extraction kit [10] Trich Find™ kit, the parasite was diagnosed by using 18SrRNA (312) bp, in database Gen Bank the with a registration in Accession Number (U17510), and the primers according to [5], as shown in a table (1), were produced by Bioneer and the method of operation was done according to the manufacturer's equipment.

Table (1): the primers used in this study with their sequence examination of nucleotide and PCR:

The gene	Sequence	
18SrRNA	F	5'ATTGTTCAACATTGGTCTACCCTC3'
	R	5'TCTGTGCCGTCTTCAAGTATGC 3'

-statically analysis: the results were statistically analyzed according to(5.04) Graph pad software Inc. (USA) for (2010) by using a significant level (P. Value <0.05).

**3. Results**

Trichomoniasis is a high rate of infection among humans, WHO statistics have indicated that millions of infections worldwide each year [11], the results by the wet swab showed to 39/150 (26%) positive samples, as in a figure (1).

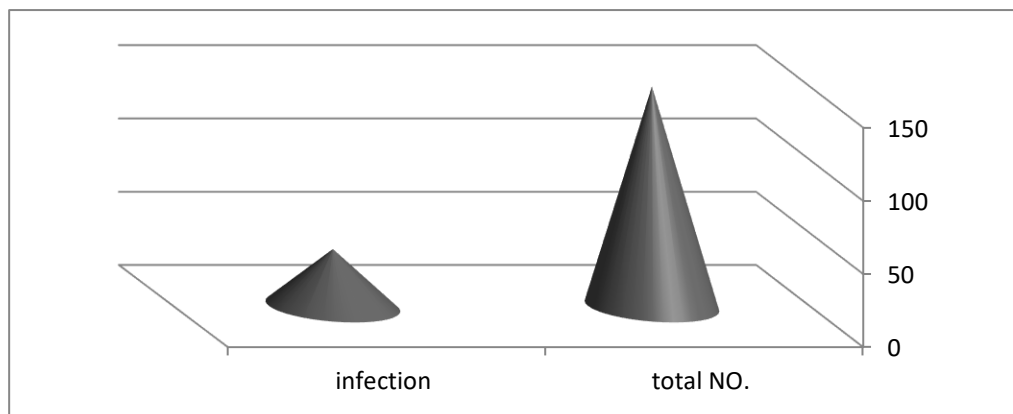


Figure (1): rate of positive and negative infection with trichomoniasis

Polymerase Chain Reaction (PCR) as all molecular methods of diagnosis, they are highly sensitive in diagnosis because they can diagnose one parasite in the sample, so they are hyper sensitive and hyper-accurate [12 ; 13], the current study shows that the rate of infection (32%) 48/150 depending on [18SrRNA gene] (312

pb), as shown a figure (2):

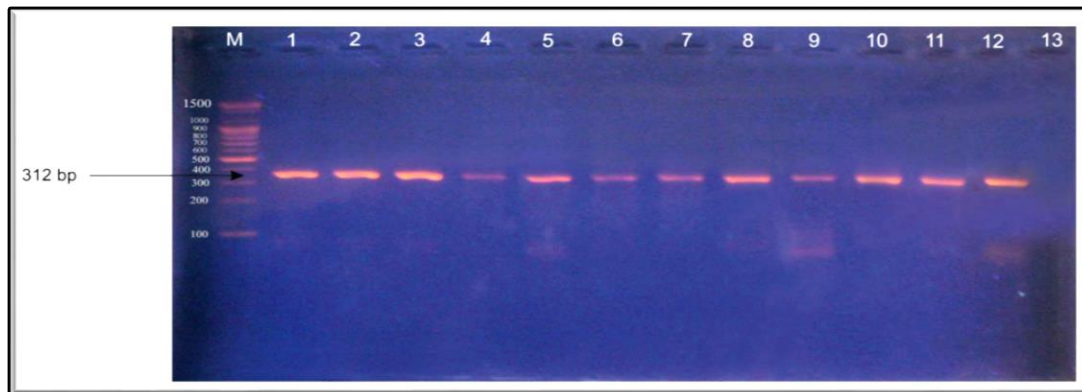


Figure (2): Interaction product PCR by using the 18SrRNA gene for *T. vaginalis* parasites

The current study investigated the relationship between contraceptives methods used by women suffering from inflammation, with the possibility of *T. vaginalis* infection and indicated to highest rate of infection among women who did not use artificial contraception (34.80%), while the lowest rate in women who used the IUCD (6.20%), a figure (3) shows rates of infection with trichomoniasis based on the contraceptive.

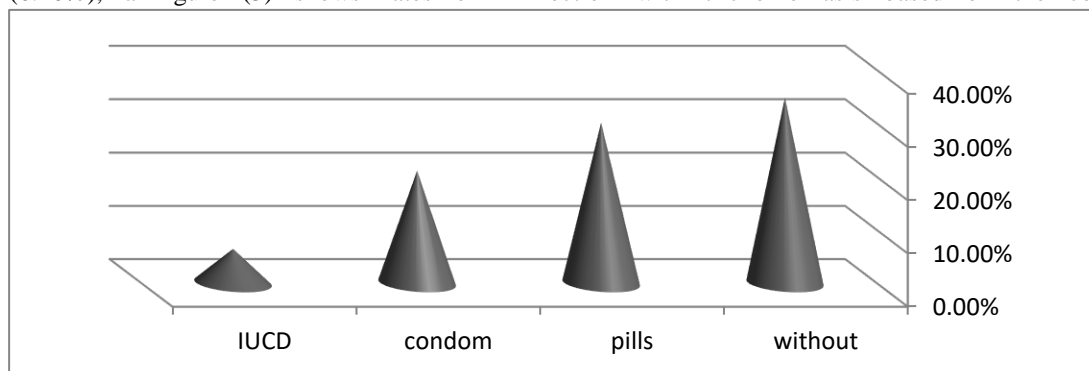


Figure (3) shows rates of infection with trichomoniasis based on the contraceptive methods used by women.

#### 4. Discussion

This study interested on the prevalence of the disease in women in Al-Muthanna governorate, has been tested the virginals' samples by using a wet swab and recorded 39/150 (26%) positive infection with Trichomoniasis, but this method the lack of accuracy and low sensitivity when we compared to other methods as a result the parasite quickly loses movement if exposure for any delay before this examination [14] so, molecular methods used in the diagnosis of the parasite in study samples.

Molecular diagnostic are new methods in detection any infection such as trichomoniasis [12], PCR is one of these molecular techniques which are allowed to amplify molecule of nucleic acid DNA for the one target cell for a million duplicates [13], this study showed to (32%) 48/150, and more than study of [15] when recorded (2.8%) by using PCR targeting 18S rRNA with  $\beta$ -tubulin genes, and study of [16] 20/1478 of childbearing women by using real-time PCR technique recorded a rate infection (1.3%) in Italy, and [7] when they recorded (11.1%) by used PCR technique depending on a pair of oligonucleotide PCR primers based conserved region of the parasite *T. vaginalis* GenBank (No. TVU86613), the studies indicated that vaginal swabs were suitable for the parasite screening as in [4], the differences of ratios recorded in this study and with other studies due to difference number of samples, uncontrolled laboratory conditions [7].

The high incidence of parasitic infection in the current study among women who do not use the contraceptive, which amounted to (34.80%) may be attributed to the fact that they are less reviewed to clinics for the purpose of periodic examination and thus increase the proportion of infection while the use of contraceptives more review of women's clinics, continuous treatment status before the development of the infection, this is consistent with the results of some studies that indicated that the highest incidence of Trichomoniasis was in women who did not use any contraceptive method as in a study [5] in Baghdad (30%), a study of [17] in Thi-Qar governorate (25.2%), as well as [18] in Baquba city (24.1%), and [19] in Al-Nasiriyah governorate (3.1%), however, its disagree with [20] in Egypt that the highest percentage of registered infection was among women who used contraceptive pills, this difference is due to the fact that the study in Egypt did not target the women who did not use contraception during their study.

The infection rate that is almost high in this study is also recorded among women who use oral contraceptives (30.10%), and may be due to the effect of contraceptive contents of hormones (progesterone and estrogen) that affect the natural flora in the vagina, causing inflammation and the hormone effect of the pills leads to an increase in the collagen stored in the membrane of the vagina, which ferments to the lactic acid, which in turn changes the pH of the vagina to become an appropriate medium for the growth of different types of microorganisms, including *T. vaginalis*, this is fully consistent with [5] which recorded (11.67%), [17] showed to (4.34%), as well as [18] that recorded (10.8%), and study of [Mahmoud *et al.*, 2015] with (14.3%) infection rate, but the current results differ with the study of [19] which indicated that there was no parasitic infection among women who took oral contraceptives and expressed in value (0%), it was not possible to know the cause of the lack of infection in the study, despite the presence of infection in women who did not use contraceptive methods and who used the condom, because the number of samples infected in the search, which prevented the possibility of knowing the cause.

The results indicate that the incidence of trichomoniasis among women who use condoms during sexual intercourse as a means of contraception was (21.11%), but this percentage differs in principle with differ from what was cited in a study in Ecuador on the incidence of parasitic infection among women who have sex with partners who use condoms to avoid pregnancy, being less than (20%) of women Others who did not adopt On the condom to the partner [21], In order to determine the cause of the difference in the rate of infection between the current study and other studies, patients were asked women who uses their husbands condoms during sexual intercourse as a means of contraception., in most cases it has been found that the husband has not used a condom since sexual intercourse began, but they used it only in the stage of ejaculation of sperm within the vagina as a means to prevent the entry of sperm into the female reproductive system thus contraception, as for start sexual intercourse the condom is not used, that is allowing direct contact between the genitals and thus allowing transmission of the pathogenic infection if it is present in one of the partners.

The lowest rate of infection was recorded in women who used IUCD (6.40%), this is different from studies what has been reported by several different studies that the IUCD is an important cause of the various infections of the female genital area the thread of the IUCD, the medical devices used for the IUCD may be sterile and thus cause inflammation [17], this is consistent with the study [5] when recorded (6.67%), [17] at (22.3%), as well as [19] with the rate of infection (0.67%), and [20] which indicated to (4.3%), the cause of the lowest incidence may be due to a review of women who use IUCD to private medical clinics for the purpose of periodic examination.

## 5. Conclusion

Trichomoniasis begins with simple symptoms and continues to become acute, causing some other health problems as a result of poor comprehension and awareness of the disease. Therefore, the risk is exacerbated and the treatment becomes difficult. It may cause cervical cancer, vaginal wall erosion, and most important infertility, the parasite was diagnosed by using a wet smear and recorded 39/150 (26%) positive infection, after

that the samples conducted to molecular method PCR on the basis of 18SrRNA gene to reach more accurate results and showed to rate infection (32%) 48/150, then collect information from each patient about the use of contraceptives:(did not use any method, contraceptives pills, condom, IUCD) and has been recorded infection rates (34.80%), (30.10%), (21.11%), and (6.40%) respectively, these results indicate the importance of the use of contraceptives for the incidence of a parasite as risk factors, the statically analysis for this results showed there were significant differences between rate of infection and type of contraceptives under probability level ( $p \leq 0.05$ ).

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