

Effect of Vaginal Trichomoniasis on Women who Suffer from Delayed Pregnancy

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History:

- Received: May 12, 2020
- Accepted: May 29, 2020
- Published: June 20, 2020

DOI: <http://doi.org/10.5334/ejmcm.278>

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ABSTRACT

This study aimed determine the effect of Trichomoniasis on women who suffering from a delay in pregnancy despite previous normal pregnancy with vaginal infection, in order to prove the relationship of *Trichomonas vaginalis* to the obstruction of pregnancy in women, also detect of effect Trichomoniasis to a level of Gonadotropic Hormone:(Luteinizing Hormone LH and Follicle Stimulating Hormone FSH) Which are the most necessary hormones responsible for the composition and maturity of sexual reproduction cells, (200)sample was taken from infected women during the period (September 2017 to October 2018), these samples examined by wet smear method for diagnostic *Trichomonis vaginalis* and recorded 51/200(25.5%) positive samples, also these samples examined using Real-time PCR on the basis of 18S rRNA gene and recorded (29.5%)59/200, this study also interested to detect of effect Trichomoniasis to a level of Gonadotropic Hormone:(Luteinizing Hormone LH and Follicle Stimulating Hormone FSH) by serological test, a results showed to a significant decrease in the level of hormones when recorded (17.313± 11.221) MUI / ml and (8.011 ± 7.001) MUI / ml respectively in patients, while their concentration was (21.623 ± 17.156) MUI / ml and (10.388 ± 9.835) in the control group.

Keywords: *Trichomonas vaginalis*, delay of pregnancy, Gonadotropic Hormone.

INTRODUCTION

Trichomonas vaginalis is a protozoan, has a simple life cycle, characterized by the presence of one phase only is trophozoite, which infected the genital path of the human male and female without need an intermediate host, Sexual contact is the common method of transmission between people (Yazar et al.2002), vaginal genitalia are characterized by a series of gaps and folds, it is an environment suitable for many pathogens, *T. vaginitis* is the leading cause of Trichomoniasis, Symptoms of the disease in females were yellowish, whitish or greenish secretions and sometimes smelly, containing on trichomonas, bacteria, cells epithelial, as well as itching, burning, dyspareunia, dysuria and ach in abdomen (Secor et al.,2014; Mairiga et al.,2011), vulva also there redness, the walls of the vagina may be congested and hemorrhagic appear in the vaginal and cervical mucosa of the uterus (Hyun et al.,2010).

Gonadotropin Hormone, which contains two hormones (Luteinizing Hormone LH and Follicle Stimulating Hormone FSH), secretion of these hormones is under the control of hormones under the hypothalamus and by mechanization of reverse feeding, which include the hormones of the basic gonads, LH is responsible for the production of corpus Latium and it controls the reproductive system of both men and women, its plays a large role in women in the process of menstruation and ovulation, and stimulates the ovaries to produce estradiol's responsible, LH is responsible of increasing blood flow in the ovaries, leading to ovulation,

sometimes called the ovulation hormone (Thackeray et al.,2010), Follicle stimulating hormone (FSH) is very necessary in the initiation of the stage of ovulation and stimulates the ovaries to produce ova and reach the appropriate size in the female, it is a hormone is responsible for the increase proteins activity of granular cells represented by progesterone and estrogen secretion which contribute to the growth and secretion of the endometrial membrane of the uterus endometrial and also it is necessary for the contractions that move sperm to the fallopian tube (Schubert et al.,2003).

this study is aimed to investigate the incidence of Trichomoniasis in women with delayed pregnancy (despite a previous pregnancy) whom a reviews to the women and children hospital in AL-Diwaniyah city/Al-Qadisiyah governorate/ middle of Iraq, also determination the level of the sex hormones associated with pregnancy.

MATERIALS AND METHODS

Sample collection

The current study included the following methods:

1- Vaginal fluid 200 sample were collected using vaginal fold swabs by a specialist doctor at period (September2017to October 2018) from the infected women and they suffer from vaginal secretions, itching in the vaginal area, redness and delayed pregnancy despite previous pregnancy, these women were aged between15 to 40 years, to investigate *Trichomonas*

vaginalis, one a drop of normal saline solution was put on a dry and sterile slide, the cotton piece containing the sample was put on slide and mix well together with the normal salt solution, after that its examined microscopically under a 400X force to ensure and detect of *T. vaginalis* (Sowmya and Mohan,2007).

2- Determination the level of the sex hormones associated with pregnancy:

(5) ml of venous blood was collected from each women, the sample was placed in a sterile dry tube, It was kept for 15 min for clotting, the serum was then separated by using a centrifuge and immediately tested for some important female sex hormones that control pregnancy In women, LH and follicle stimulating hormone (FSH) by using serological method ELISA Assay.

A- Determination the level of Luteinizing Hormone (LH) in a blood serum: (LH) hormone level concentration was estimated using the special solutions kit of Calibrators LHLH, (LH) enzyme reagent and (LH) Streptavidin coated, Center Wash Solution, Substrate-A (tetra methyl pentazin), substrate –B (hydrogen Peroxide (H₂O₂), and Stop solution ((hydrochloric acid HC11), according to manufacturer's instructions and according ELISA method in study of (Al-Samarrai,2013).

B- Determination the level of follicle stimulating hormone (FSH) in a blood serum: FSH was estimated by using the special solutions kit: FSHFSH solutions, Substrate-A(tetra methyl pentazin),Calibrators, Streptavidin Coated, H₂O₂ (substrate B), Stop solution(hydrochloric acid HC11) according to ELISA method in the study (Al- Samarrai,2013).

3- R-time PCR method was based from described protocol in study of (Caliendo et al.,2005) by PCR primersF-5'-CATTGACCACACGGACAAAAAG-3'withR-:5'-CGAAGTGCTCGA ATGCGA-3'.(200) samples were amplified in this molecular method with 2 negative and a positive control, the master mix of R-time PCR was prepared as following reagents: (10µl) of the SYBR green mix, (0.6µl) of forward , reverse primer of (10µl), (3.8µl) of deionized water in addition (5µl) of DNA template (Nangammbi et al.,2013)

4- Statistically: The results were statistically tested according to(5.04) software (2010) Inc. (USA) at probability level (P. ≤0.05).

RESULTS AND DISCUSSION

Trichomoniasis is disease spread in worldwide, transmitted by sexual, infection with *T.vaginalis* includes a range of symptoms that may not be clearly visible to the affected person, including premature birth, low birth weight in newborns, cancer of cervical, infertility, and increase the risk of other serious pathogens such as (HIV) human immunodeficiency virus (Wiwanitkit,2008;Hillier,2013), the

current study on the spread of the Trichomoniasis in women who suffer from delayed pregnancy, despite the presence of a previous pregnancy in Al-Qadisiyah province, recorded infection rate of *T. vaginalis* was(25.5%) 51/200 samples using wet swap method as in a figure (1), this results is more than the reported prevalence of infection among women in (Al-Obaidi,2010). When recorded (13.3%) positive infection in Tikrit, also (Khalil et al.,2012). Trichomoniasis was detected in 18/250 (7.2%) in women who revisions the tack care of family unit in AL-Liqaa hospital in Baghdad, and study of (Al-Tikrity and Al-Badry,2014), about Trichomoniasis distributed among a women who go to the health centers in Samarra governorate and recorded positive infection rate (14.76%), and the current incidence rate in this study is higher than that recorded in a study (Al-Kazragee,2013) in Thi-Qar governorate in Iraq, which recorded a ratio of parasitic infection amounted to 5.23% While studying epidemiology of *T. vaginalis* in the governorate, as well as study of (Oyeyemi et al.,2015), when recorded rate of *T. vaginalis* in Nigerian pregnant women (18.7%), also a study (Nangammbi et alk.,2013) which recorded (16.891%) in her study in Baghdad governorate, but the current results was lower than infection rate recorded by study of (Jarallah,2013) for women who live in marsh villages of Al-Basrah governorate south of Iraq as researcher noticed that the total prevalence of Trichomoniasis infection was (57.85%) and (53%) for Al-Mashab and Al-Nashwa villages, and study of (Al-Hussuny,2015). through examination 120 vaginal samples for Al-batool educational hospital in Baaquba city/Diyala governorate and showed to (41.6%) positive rate infection with Trichomoniasis, as for infection with *T. vaginalis* in some Arab countries such as (Mahmoud et al.,2015) when studies rate of *T. vaginalis* parasite infection between Egyptian married women by two methods and recorded infection rate (33.3%), the differences in the current results compared with other studies may be due to variation in steps diagnosis methods or delay diagnosis in some cases and environmental condition in laboratory, and may be some patients use Flagyl treatment before reviewing a doctor and causes an error in diagnosis (Al-Kazragee, 2013; Al-Tikrity and Al-Badry,2014). This diagnostic method is a quick test and is used in many of medical clinics despite the less of accuracy and sensitivity when compared with the other methods because a parasite fast loses its characteristic locomotion if exposure for any delay before examination lead to change the environment and differences in temperature and humidity leads to the loss of the parasite movement and leading to diagnostic error because its take form closest to the pus cells (Caliendo et al.,2005), so that samples should be tested within (1 hour) of collection and mention that parasite locomotion was 100% at 30 minutes of taking the sample and 99% after 60m of collected the samples and decreased by (3-15%) every subsequent hour (Al-Kazragee, 2013; Oyeyemi et al.,2015), Therefore, the current study is based on the use of molecular test in the identified of the parasite under study to reach more accurate.

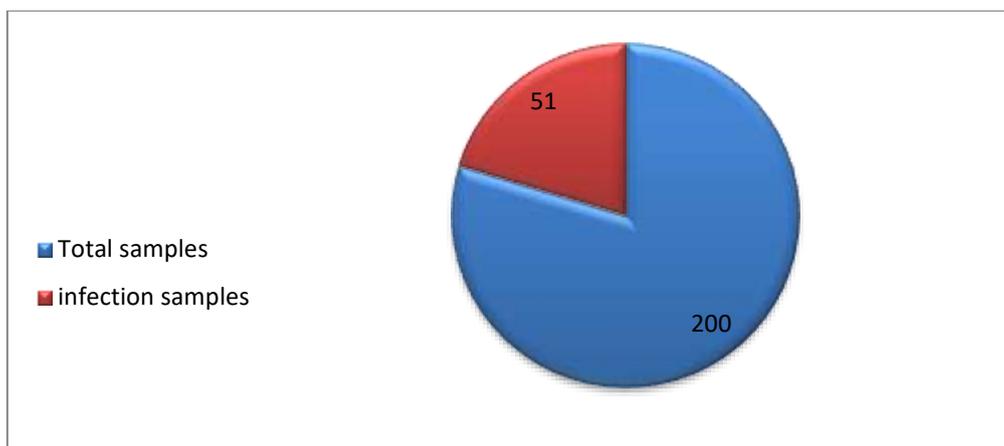


Fig. 1: Positive and negative rates for Trichomoniasis infection

2- The results of R-time PCR method shows that the percentage of infection with *T. vaginalis* is 29.5% (59/200) as shown a figure (2). molecular methods are newer and more accurate in diagnosing microorganisms depending on the very small amount of DNA, this ratio(29.5%)is large, a clear indication of the relationship between the prevalence of parasitic infection in women who had a normal pregnancy Previously but they now suffering from delayed pregnancy, and its consistent with what researchers (Hillier,2013; El-Shazly ,2001) reported in their study that the parasite has the potential to cause infertility and cervical cancer in some cases, as well as the *T. vaginalis* was found to be able to pass through Fallopian tubes and transmit many pathogens that cause inflammation and infertility. It is worth mentioning that the parasite analyzes the red blood cells to obtain the bioparticles due to having a virulence factor (Haemolysin) so the parasite plays a role in the destruction of the vaginal cells (Lehker et al.,1990) as well as that the parasite own the cell detaching factor, which affects the adhesion of the parasite to the cells

of the vagina and leads to separate the cells and aggregation in the middle leading to the dissection of the lining of the vagina (Garber et al.,1987), the ratio 29.5% (59/200 which record in our study more than 20/1478 (1.3%)that recorded in a study (Leli et al.,2016) on childbearing women using the R-time PCR , and more than results of a study (Shahnazi, 2017), whom recorded positive rate infection(11.1%) of patients were infected with Trichomoniasis by used PCR technique, also more than(10.4%)that recorded by (RYU and MIN, 2006) between women in the republic of Korea when they using (PCR) technique for diagnostic depending on primers based on the repetitive sequence cloned from *T. vaginalis* (TV-E650), and a study of (Brotman et al.,2012), which detected of *T. vaginalis* and recorded (2.8%) 11 / 394 by PCR based the 18S rRNA with β -tubulin genes, the differences of percentages that mention in the our study and with the other studies because of the difference in the size of samples collected and the uncontrolled conditions in Lab. that affect on the PCR (AI-Abodi,2018).

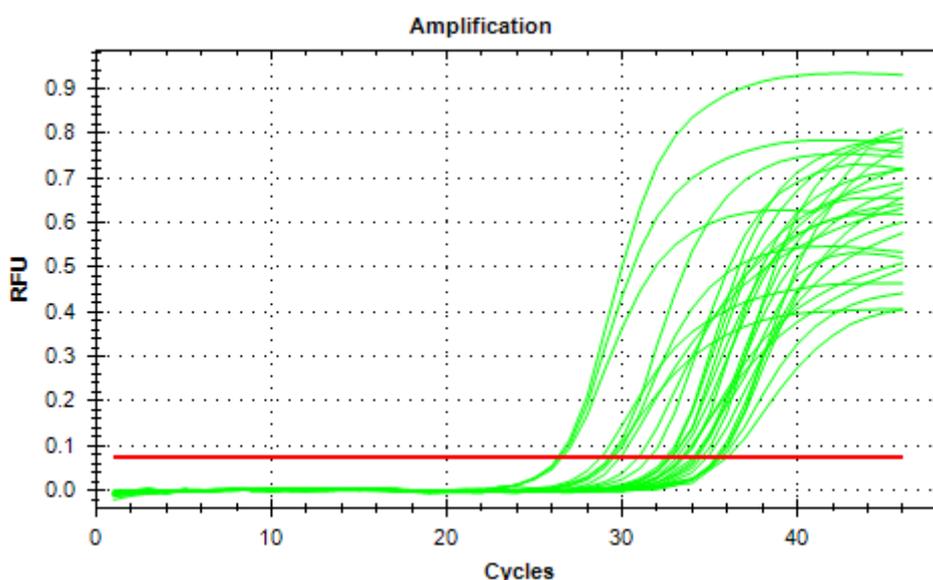


Fig. 2: Amplification plot for Real-Time PCR for positive infection samples with Trichomoniasis

3- Determination the level of the sex hormones associated with pregnancy

The results of the present work showed a decrease in the level of LH hormone between the group of infected women with

parasite and the healthy control group at a probability level ($p \leq 0.05$), where the concentration of this hormone in the infected women was (17.313 ± 11.221) MUI/ml compared

with the control group (21.623 ± 17.156) MUI/ml, a table (1) shows the values of hormone (LH)

Table 1: The concentration of stimulating hormone (LH) MUI/ml for infected women and a control group

Group(non-infected)	Ex. No.	LH (MUI / ml) Average \pm standard error
Control	20	21.623 ± 17.156
Patients	59	$17.313 \pm 11.221^*$

* Significant ($P \leq 0.05$).

The data of the present work noticed a decrease in the concentration of LH hormone between the infected women **with parasite and the healthy control group at ($p \leq 0.05$)**, where the level of this hormone in the infected women was

(17.313 ± 11.221) MUI/ml compared with the non-infected women (21.623 ± 17.156) MUI/ml, a table (1) shows the values of hormone (LH)

Table 2: The concentration of FSH and (FSH) for infected women compared with a control group

Groups	Ex. No.	FSH(MUI / ml) Average \pm standard error
Control (non-infected)	20	10.388 ± 9.835
Patients	59	$8.011 \pm 7.001^*$

* Significant ($P \leq 0.05$).

The data of the present study showed a significant reduction for the level of LH, FSH in the sera of the group of patients compared with the non-infected group, the concentration of these hormones in patients was (17.313 ± 11.221) MUI / ml and (8.011 ± 7.001) MUI / ml respectively, while its concentration (21.623 ± 17.156) MUI /ml and (10.388 ± 9.835) in the control groups, these results disagree with [8] when targeted in his study the *T. vaginalis* in men in Tikrit governorate/ Iraq with a therapeutic attempt it using medicinal plants, and pointed to there are a slight decrease in the level of LH and FSH in men with Trichomoniasis, has indicated that the infection with *T. vaginalis* is not a major reason to prevent the sperm from fertilizing the ovum and does not directly affect the vitality of sperms because its lack of acute impact on the most important sex hormones control of the vitality of reproductive sex cells, which are LH and FSH, and the researcher has supported the results of the study (Khalaf et al.,2010) which indicated that (1.9%) only of men with Trichomoniasis suffer at the same time of infertility, as well as in the study (Ozdemir et al.,2011) recorded a rate of infection slightly higher than the previous study (2.5%), and both studies indicated that the incidence of Trichomoniasis is a rare cause of infertility in men except for chronic and acute cases because they will affect in the long term on the regulation of the glands attached to the male reproductive system and the maintenance of the function of the reproductive canal and regulate the process of production of sperm (Andrews et al.,2009), Low levels of LH and FSH refer to uterine and ovarian disorders (Thackeray et al.,2010 ; Schubert et al.,2003), low values of the hormone level in the patients under study indicate that the *T. vaginalis* parasite can cause disorders beyond the vagina and reach of the female genital tract thus affect the uterus and ovary and hormonal imbalance during the period of infection is the

reduction of the level of the hormone LH leads to the disruption of the process of ovulation, and low level of hormone FSH of the body and thus adversely affect the growth of ovum and maturity, leading to prevent pregnancy because of unavailability ovum can be zygote specifications by sperms.

CONCLUSION

Summarize the results that the prevalence of Trichomoniasis in women is one of the important reasons to prevent pregnancy, even if there is a normal pregnancy in advance, as the infection *T. vaginalis* affect the location of the settlement of the parasite (vagina), which is the portal of sperms to the female reproductive system and reach the egg and beyond the impact of the parasite to the rest of the parts of the reproductive system of female is the cervix ,uterus and ovaries resulting in a hormonal imbalance represented by the low level of feeder hormones (LH and FSH) in the blood of patients infected with parasite.

ACKNOWLEDGMENT

We thank the specialist doctor at the women's and children's hospital in Al-Qadisiyah governorate for his help in obtaining samples from women with Trichomoniasis.

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Cite this article: Khadeeja Abees Hmood Al – Khaidy. 2020. Effect of Vaginal Trichomoniasis on Women who Suffer from Delayed Pregnancy. *European Journal of Molecular & Clinical Medicine*, 7(1), pp. 45 – 50, DOI: <https://doi.org/10.5334/ejmcm.278>