

Assessment of Changes in the Hormonal Levels in Women Infected with *Trichomonas vaginalis* in Kirkuk, Iraq

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Abstract

Background: *Trichomonas vaginalis*, a flagellated protozoan, is the cause of the sexually transmitted disease known as vaginal trichomoniasis. **Objectives:** The study was carried out to assess the level of some sexual hormones in the serum of women suffering from trichomoniasis. **Materials and Methods:** From 1020 women, blood was collected to separate serum for detection of the level of sex hormones (estrogen [E2], progesterone [Pro.], luteinizing hormone [LH], and follicle-stimulating hormone [FSH]) by the Cobas e411 method using kits for each hormone according to manufacturer's instructions. **Results:** The results recorded a significant decrease in the levels of estrogen, progesterone, and FSH, (45.8 ± 7.7 pg/mL), (0.338 ± 0.483 ng/mL), and (5.83 ± 1.37 mIU/mL), respectively, in the serum of female patients as compared with the healthy control women (71.3 ± 6.8 pg/mL), (0.743 ± 0.338 ng/mL), and (7.65 ± 2.51 mIU/mL), respectively. However, no significant differences were observed in the level of LH between infected (5.53 ± 2.56 mIU/mL) and noninfected women (5.82 ± 2.15 mIU/mL). **Conclusion:** A significant drop in the level of certain sexual hormones has an adverse effect on the incidence of trichomoniasis in women in the Kirkuk province.

Keywords: Infected women, sexual hormones, *Trichomonas vaginalis*, trichomoniasis

INTRODUCTION

The sexually transmitted parasite *Trichomonas vaginalis* has been linked to a number of unfavorable outcomes in women, including pelvic inflammatory illness, increased HIV acquisition, transmission, and preterm birth.^[1,2] In the middle of the twentieth century, decades before *Chlamydia trachomatis* infection would be recognized as such, *T. vaginalis* infection was initially characterized as a venereal illness.^[3,4] Simple microscopy can be used to observe the movable protozoan that causes trichomoniasis in women. However, wet preparation microscopy is particularly sensitive because these creatures are big, mobile, and obvious.^[5,6]

The clinical presentation of *T. vaginalis* in women can include an unpleasant odor, discharge, dysuria, genital irritation or itching, and pain during sexual activity; in men, symptoms including dysuria, urethral itch, and discharge may be present. Approximately 80% of cases of *T. vaginalis* are asymptomatic.^[7,8] The disease has a high

rate of reinfection from women and their partners who are asymptomatic.^[9]

Natural hormonal fluctuations that can cause epithelial shedding in the female urogenital tract, excretion that can affect salinity, pH, and toxicity of the environment, and flux in commensal and infectious microbe communities are examples of such changes.^[8,10] Hormonal variations can affect bacterial adhesion by changing epithelial cell quantity and morphology, influencing receptor site production on epithelial cells, and changing the pH or mucus composition in the surrounding area.^[11,12]

The presence of specific androgen and estrogen receptors in *T. vaginalis* suggests that steroid hormones may directly affect *T. vaginalis*, and the presence of hormone-binding

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proteins in a variety of microorganisms, including protozoa, supports the hypothesis that hormones may directly affect microorganism virulence.^[12,13] The proper quantity of these hormones ensures that the vaginal mucosa receives adequate blood supply and that the ideal level of lubrication is maintained.^[14]

Therefore, in this study, we attempt to evaluate the level of some sexual hormones in the serum of women suffering from *T. vaginalis*.

MATERIALS AND METHODS

Study participants

A total of 1020 women were examined during their visits to the gynecological consultants in Azadi Teaching Hospital, private clinics, midwifery, and childbearing hospitals for the period of December 2021 to June 2022.

Specimen collection

Vaginal swab and urine collection

High vaginal swabs were collected by using the sterilized speculum. Patients' discharges were gathered using cotton swabs from the posterior vaginal fornix, and specimens were immediately sent to the laboratory for further testing.^[15] All patients were asked to provide 20–40 mL of urine, which was centrifuged at $1000 \times g^{-1}$, $500 \times g$ for 5 min.^[16] This initial centrifugation was performed at a low speed to help maintain the viability of the *Trichomonads*.

Sample collection

Blood samples were taken from 20 women infected with *T. vaginalis* and 30 healthy women (control). The blood was collected from all patients at the follicular phase, except for menopausal patients who did not have a cycle. After collecting the whole blood, it was left at room temperature for 15–30 min to allow it to clot. The clot was removed by centrifuging at 3000 rpm for 5 min.^[17] The separated serum was preserved in labeled Eppendorf tubes at a deep freeze (-20°C) till hormonal testing.^[18,19]

Assessment of clinical isolates

Wet mount

The samples were used for direct microscopy as soon as they arrived at the laboratory. Swabs were smeared on the slide before being covered with a coverslip. A microscope slide was also used to transfer two drops of aliquot urine.^[20] It was then inspected under a microscope for motile *Trichomonas*. Light microscopy at $40\times$ was used to rapidly evaluate vaginal discharge or urine wet preparations (wet preps) for approximately 10 min. The peculiar jerky motions of *T. vaginalis* allow it to be distinguished. Flagellar movement is occasionally observed. For examination under the microscope and to determine the components, stains such as Giemsa or Leishman stain were used.^[21]

Blood tests

Four hormones (estrogen, progesterone, luteinizing hormone [LH], and follicle-stimulating hormone [FSH]) were analyzed simultaneously by Cobas e411,^[22] with their kits according to manufacturer's instructions. Each hormone had a unique reagent, which they loaded into the machine and began scanning. Then, the sample ID and position were selected, and the frozen serum was allowed at room temperature till completely melted. After 20 μL of the serum was added to the cuvette and inserted into the device, the four hormones were selected. All results were automatically calculated and recorded.^[23]

Statistical analysis

The results were statistically analyzed by applying the mini-tab statistical program and by the *t* test. The arithmetic means were compared with determining significant differences according to the Duncan's test at the probability levels of 0.05 and 0.01.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with the patient's verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to document number 3657 (including the number and the date on 11/11/2021) to get this approval.

RESULTS

Prevalence of *Trichomonas vaginalis*

The prevalence of *T. vaginalis* was studied in 1020 women in Kirkuk province using a wet mount from a vaginal swab or urine. Displays that 20 (1.96%) individuals had *T. vaginalis* and 1000 (98.04%) were negative.

Light microscope examination of *T. vaginalis* and the detection of some organelles such as the nucleus, undulating membrane, anterior flagella, and posterior flagella using Giemsa stain is depicted in Figure 1.

Changes in the hormonal levels in women infected with *Trichomonas vaginalis*

Estrogen

Estrogen levels in trichomoniasis were significantly reduced ($P \leq 0.05$) in infected women to (45.8 ± 7.7) pg/mL of follicular phase and (47.73 ± 7.46) pg/mL of menopausal, as compared with the control group's (71.3 ± 6.8) pg/mL of follicular phase and (60.07 ± 4.47) pg/mL of menopausal. The E2 hormone values are displayed in Table 1.

Progesterone

The concentrations of progesterone in trichomoniasis infected women significantly ($P \leq 0.01$) decreased



Figure 1: *Trichomonas vaginalis* wet mount preparation under a light microscope: (A) 40×, (B) 100×, and (C) Giemsa stain with 40× magnification

Table 1: Level of estrogen hormone in patients infected with *Trichomonas* and healthy women

Groups	No. of samples in follicular phase	E2 (pg/ mL) Mean $\pm$ SD	No. of samples in menopausal phase	E2 (pg/mL) Mean $\pm$ SD
Infected	16	45.8 $\pm$ 7.7*	4	47.73 $\pm$ 7.46*
Healthy	20	71.3 $\pm$ 6.8	10	60.07 $\pm$ 4.47
Statistical analysis	t Test of difference = 0 (vs. $\neq$): t = 2.53 and P = 0.047		t Test of difference = 0 (vs. $\neq$): t = 3.09 and P = 0.054	

SD = standard deviation, E2 = estrogen, Pg/mL = Pico gram/milliliter, No. = number

*Significant

Table 2: Level of progesterone hormone in patients infected with *Trichomonas* and healthy women

Groups	No. of samples in follicular phase	Progesterone (ng/mL) Mean $\pm$ SD	No. of samples in menopausal phase	Progesterone (ng/mL) Mean $\pm$ SD
Infected	16	0.338 $\pm$ 0.483*	4	0.164 $\pm$ 0.115**
Healthy	20	0.743 $\pm$ 0.338	10	0.578 $\pm$ 0.188
Statistical analysis	t Test of difference = 0 (vs. $\neq$): t = 2.41 and P = 0.047		t Test of difference = 0 (vs. $\neq$): t = 5.02 and P = 0.001	

SD = standard deviation, ng/mL = nano gram/milliliter, No. = number

*Significant

**Highly significant

Table 3: Level of LH hormone in patients infected with *Trichomonas* and healthy women

Groups	No. of samples in follicular phase	LH (MIU/mL) Mean $\pm$ SD	No. of samples in menopausal phase.	LH (MIU/mL) Mean $\pm$ SD
Infected	16	5.53 $\pm$ 2.56	4	34.4 $\pm$ 7.73
Healthy	20	5.82 $\pm$ 2.15	10	34.6 $\pm$ 7.80
Statistical analysis	t Test of difference = 0 (vs. $\neq$): t = 0.26 and P = 0.799		t Test of difference = 0 (vs. $\neq$): t = 0.02 and P = 0.984	

SD = standard deviation, MIU/mL = milli-international units/milliliter, No. = number

to (0.338 $\pm$ 0.483) pg/mL of follicular phase and (0.164 $\pm$ 0.115) pg/mL of menopausal, compared with the control group's (0.743 $\pm$ 0.338) pg/mL of follicular phase and (0.578 $\pm$ 0.188) pg/mL of menopausal. The progesterone hormone values and statistical analysis are shown in Table 2.

Luteinizing hormone

No significant difference ($P \geq 0.05$) was observed between healthy and infected women concerning LH concentration, as shown in Table 3.

Follicle-stimulating hormone

As shown in Table 4, FSH levels significantly differed ($P \leq 0.05$) during the follicular phase (7.65 $\pm$ 2.51 decreased

to 5.83 $\pm$ 1.37), but not during menopause (85.1 $\pm$ 14.9 in healthy women and 76.2 $\pm$ 21.6 in patients).

DISCUSSION

The current incidence rate of *T. vaginalis* in this study was lower than that recorded in a study conducted in Baghdad, AL-Liqaa Hospital, where trichomoniasis was found in 18/250 (7.2%) of the women who revised the family unit's approach to care.^[24] Also, the prevalence of trichomoniasis among women visiting the medical centers in the governorate of Samarra, Iraq, the rate of confirmed infection (14.76%).^[25] In 65 articles between 2002 and 2020 about *T. vaginalis* for testing the infection in the general Turkish population, they found that (2.87%) of women were infected.^[26] Also, *T. vaginalis* has a frequency of 2.1%

Table 4: Concentration of FSH hormone in patients infected by *Trichomonas* and healthy women

Groups	No. of samples in follicular phase	FSH (MIU/mL) Mean $\pm$ SD	No. of samples in menopausal phase	FSH (MIU/mL) Mean $\pm$ SD
Infected	16	5.83 $\pm$ 1.37*	4	76.2 $\pm$ 21.6
Healthy	20	7.65 $\pm$ 2.51	10	85.1 $\pm$ 14.9
Statistical analysis	t Test of difference = 0 (vs $\neq$): t = 2.26 and P = 0.050		t Test of difference = 0 (vs $\neq$): t = -0.76 and P = 0.492	

SD = standard deviation, MIU/mL = milli-international units/milliliter, No. = number

*Significant

in the United States, approximately the same result as in our study.

Our study was compatible with Aed Asaad Al-Tikrity *et al.*^[27] hormonal changes that may cause some types of vaginal infections. Lower estrogen levels can cause vaginal irritation and inflammation when a woman's estrogen levels change over the course of her lifetime. Tonay *et al.*^[13] discovered that the common genital infection, *T. vaginalis*, possesses both particular estrogen and particular androgen receptors. AL-HADRAAWY's study discovered a link between estrogen and *T. vaginalis*, with estrogen decreasing *T. vaginalis* pathogenicity but estrogen-mammalian cell interactions increasing infection spread.^[28] However, this result was incompatible with^[29] in Babylon/Iraq, the relationship of *T. vaginalis* with sex hormones for women in Babylon/Iraq was estrogen and progesterone rates increased in the infected women compared with non-infected.

The present results study was incompatible with the study by Al-Qraby *et al.*^[30] investigation in Kufa. They found that LH was decreased in trichomoniasis patients and agreed with decreasing FSH. These findings were consistent with the findings of Al-Samarrai^[25] who reported that *T. vaginalis* in men in the Tikrit governorate/Iraq with a therapeutic attempt using medicinal herbs resulted in a moderate decrease in LH and FSH levels in trichomoniasis-infected men, although the outcome of our study was significant for FSH but not for LH. In addition, our study was compatible with the study conducted at Al-Qadisiyah University, Iraq regarding the effects of vaginal trichomoniasis on women suffering from delayed pregnancy. LH and FSH levels were significantly lower ($P \leq 0.05$) in the patient group (17.313 $\pm$ 11.221) MUI/mL and (8.011 $\pm$ 7.001) MUI/mL, respectively, compared with the control group (21.623 $\pm$ 17.156) MUI/mL and (10.388 $\pm$ 9.835) MUI/mL, respectively.^[31] The hormonal imbalance was reported in patients that were infected with some bacterial contamination of the vagina.^[32]

CONCLUSION

We concluded from the study that the sexual hormones, mainly the titer of estrogen and progesterone hormones, declined in the serum of infected women, which enhanced the severity of the disease.

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Not applicable.

Conflicts of interest

There are no conflicts of interest.

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