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Estimation of Estrogen, Progesterone Levels in Women Used Contraceptive Pills Infected with Trichomonas Vaginalis Parasite

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Abstract: The aim of this research was to diagnosis of Trichomonas vaginalis by use wet amount microscope and evaluate the level of estrogen, progesterone, in women used pills at contraceptive and do not used with T. vaginalis infection; blood specimens were collected from 85 women whom have visited the department of infertility at Al-Sadder medical city, Al-Zahra Hospital in Najaf Province during the period from June till October, 2013. Twenty healthy looking age matched women taken to serum tubes and serum was separated. Serum was used for evolution of the level of estrogen and progesterone in women infected with T. vaginalis. The level of estrogen and progesterone were evaluated in serum using ELISA technique .T.vaginalis was isolated from 15 women with a prevalence rate 17.64% by using wet amount microscope. The results revealed a significant increase in estrogen and progesterone in women infected with T. vaginalis in compared to control group.

Keywords: Trichomonas Vaginalis, ELISA.

I. INTRODUCTION

Trichomonas vaginalis, a parasitic protozoan, is the causative agent responsible for vaginal trichomoniasis in women. This infection is the most common non-viral sexually transmitted disease and has a predilection for human urogenital tract (Petrin, 1998). T. vaginalis is a sexually transmitted parasitic protozoan known to be responsible for an estimated 180 million new infections per year, making it the most prevalent non-viral sexually transmitted pathogen worldwide. It can also be transmitted to neonates during passage through an infected birth canal, but the infection is usually asymptomatic. T. vaginalis infection is frequently asymptomatic in adults; it can cause urethritis in men. Symptomatic women with trichomoniasis usually complain of vaginal discharge, vulvovaginal soreness, and/or irritation. Complications of trichomonal vaginitis that have been reported include premature rupture of membranes, premature labour, low birth weight (Uneke, 2006, and Cox, 2005).

The conventional methods for diagnosis involve the direct microscopic examination of wet mount or culture-based systems for vaginal smears (Hardick, 2001) .The factors associated with high prevalence are the same as those of sexually transmitted disease, poor personal hygiene, multiple sexual partners, and low socio/ economic status (Bowden, 2000). Immune responses including humoral and cell- mediated immunity and evokes lymphocyte function including cytokine production. Cytotoxic effects and antibodies produced after presentation by antigen manufacturing cells (Jae – Hoc, 2004). Relative risk of developing invasive cervical cancer (Yazar, 2002) and six fold higher probability of infection by human

Immunodeficiency virus (HIV) are linked to this disease (Valadkhni, 2004). The aim of this study to estimate the levels of estrogen and progesterone in serum of women used pills at contraceptive and do not used, with T. vaginalis infection.

II. MATERIALS AND METHODS

The study was conducted on 85 women with trichomoniasis disease and 20 of healthy women as control groups, when all these cases were examined and defined as suspected with T.vaginalis by obstetrician when attended to AL-Zahra, maternity and pediatric, AL-Sadder teaching hospital in AL-Najaf province from January to August 2013.

A. Sample collection

Four ml of blood was collected from each clinical suspected women with T.vaginalis infection and non-suspected women (as control group) by disposable syringe , blood samples was drawn in sterile plain tubes and remains for 30 minutes at room temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes (Back man/counter, Germany**)** to separate the serum and collected in another sterile tube.

B. Estimation of estrogen

This assay executed with specific kit for Enzyme Immunoassay for the Quantitative Determination of Human

estrogen Hormone Concentration in Human Serum, supplied by (Monobind, INC, Cat # 4925-300).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.025 ml (25 µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the Estradiol Biotin Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 30 minutes at room temperature.
6. Add 0.050 ml (50µl) of Estradiol Enzyme Reagent to all wells. Add directly on top the reagents dispensed in the wells.
7. Swirl the microplate gently for 20-30 seconds to mix.
8. Cover and incubate for 90 minutes at room temperature.
9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
10. Add 350µl of wash buffer (see Reagent Preparation Section),decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
11. Add 0.100 ml (100µl) of substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.
12. Incubate at room temperature for twenty (20) minutes.
13. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm.The results should be read within thirty (30) minutes of adding the stop solution.

C. Estimation of Progesterone

This assay executed with specific kit for Enzyme Immunoassay for the Quantitative Determination of Human Progesterone Hormone Concentration in Human Serum, supplied by (Monobind, INC, Cat # 4825-300).

1. Format the microplate' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.025 ml (25 µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of Progesterone Enzyme Reagent to all wells.
4. Swirl the microplate gently for 10-20 seconds to mix.
5. Add 0.050 ml (50µl) Progesterone Biotin Reagent to all wells.
6. Swirl the microplate gently for 10-20 seconds to mix.
7. Cover and incubate for 60 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
10. Add 0.100 ml (100µl) of Substrate solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells.
11. Incubate at room temperature for twenty (20) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. The results should be read within thirty (30) minutes of adding the stop solution.

III. RESULTS

The results of research revealed that the levels of serum estrogen, progesterone in women used pills at contraceptive and women do not used any type of contraceptive in women infection with T.vaginalis were different significant ($P < 0.05$) in compared to control group, as seen in figure (1), (2) and (3).

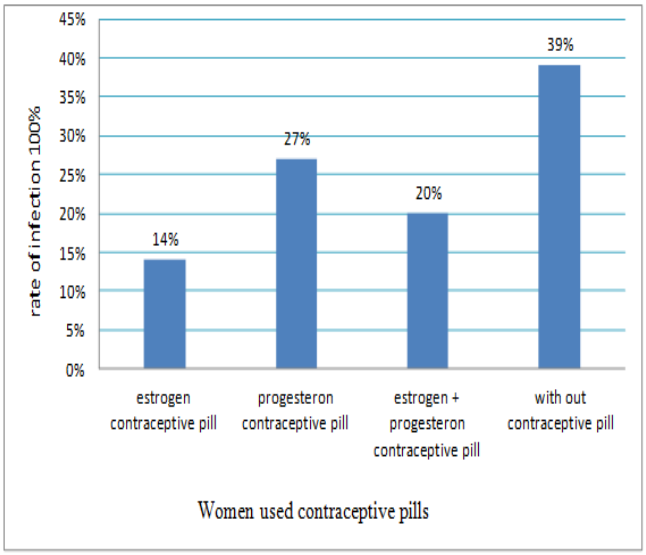

Figure1: showed the rate of infection % with Trichomonas vaginalis in women used contraceptive pills and in women without contraceptive pills.

Significant difference (P<0.05) between control group and patients**.**

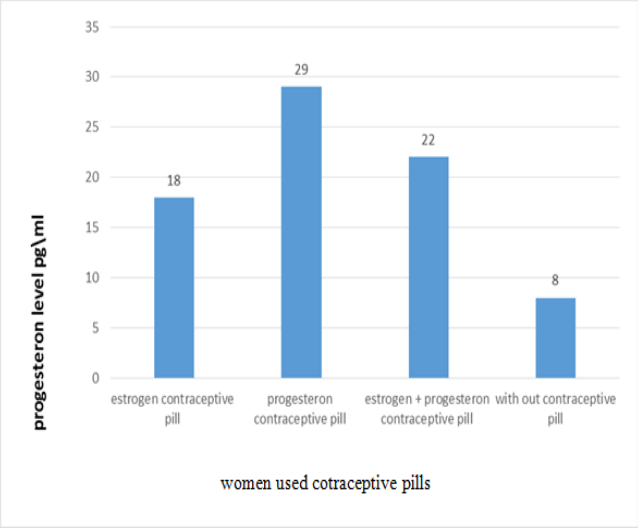

Figure2: Comparison progesterone hormone, levels in women used contraceptive pills with Trichomonas vaginalis infection.

Significant difference (P<0.05) between control group and patients

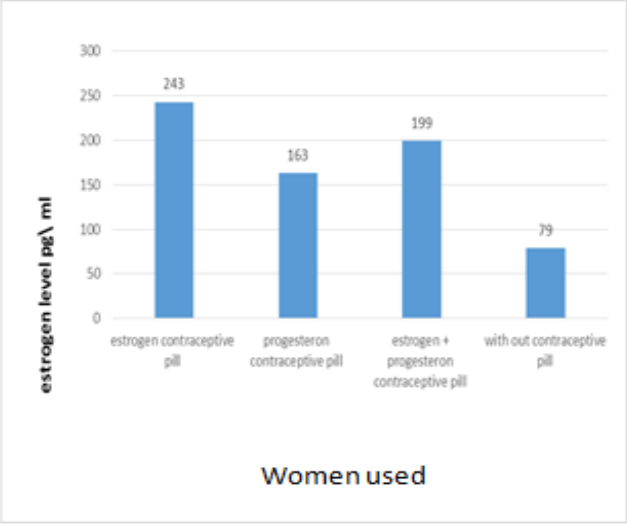

Figure3: Comparison estrogen hormone, levels in women used contraceptive pills with Trichomonas vaginalis infection.

Significant difference (P<0.05) between control group and patients.

IV. DISCUSSION

These results revealed the effect of estrogen and progesterone as used contraceptive pills which can enhance or suppress the growth of vaginal flora and influence transmission of T.vaginalis. This results found that infection increased in women not using contraceptive pills due to increase Glycogen which was stored in vagina mucous membrane leading to production of Lactic acid and decrease PH of vagina which were a good environment for growth of T.vaginalis (Miteb, 2000)). The oral contraceptives provide a favorable pH and add the necessary glycogen for nourishment and because of the progestin component of the pills will increase cervical mucosal thickness and make it hostile to spermatozoa and pathogens (Galask &Lareson, 1986). These results were similar to a study done by (Al-Hadraawy, 2013) who recorded that the highest rate of infection was in women who did not used any type of contraceptives.

Other study indicate that estrogen hormones may depress cell-mediated immunity, impair the activity of natural killer cells, and suppress some aspects of neutrophil function lead to increased incidence of parasite infection (Styrt and Sugarman.,1991), The hormonal milieu has been shown to alter mammalian host susceptibility to numerous infections such as oestrogens that alter susceptibility to infection by oral contraceptives in different way of mechanisms whereas it alter the host immune responses,alter in other defences such as cervical mucus and oestrogen mediated alterations of mammalian cell structure. (Sugarman and Mummaw.,1990). For progesterone contraceptive pills The mechanism for a protective effect of progesterone-only contraceptives is not clear, but it has been suggested that exogenous hormones may interfere with binding to androgen and estrogen receptors that are present on T. vaginalis (Scott McClelland etal., 2007).

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