Estimated Levels of Growth Hormone, Prolactin, FSH, LH, Iron and Ferritin in Women Infected with Trichomonas Vaginalis In, Iraq

Article · November 2014

CITATIONS
0

READS
104

1 author:

Saleem Khteer Al-Hadraawy
University Of Kufa
51 PUBLICATIONS **29** CITATIONS

SEE PROFILE

International Journal of Advance Pharmaceutical Science & Innovative Research Development

ISSN 2343-2370
Vol.03,Issue.01,
November-2014,
Pages:0026-0029

www.semargroup.org,
www.ijapsird.org

Estimated Levels of Growth Hormone, Prolactin, FSH, LH, Iron and Ferritin in Women Infected with Trichomonas Vaginalis In, Iraq

DR. MOHAMMAD E. AL-QRABY[1], DR. SALEEM K. AL-HADRAAWY MAYSOON[2], K. A. AL-HADRAAWY[3]
Medical laboratory, Dept of Technical Institute, College of Sciences / University of Kufa.

Abstract: The research was conducted on 30 out patients and 20 healthy women to determine the influences of infected with T. vaginal is on levels of iron, ferritin and growth hormone in women infected with T. vaginal is in compared with healthy group. Who have visited Al-Sadder medical city and Al-Zahra Hospital in Al- Najaf Province during the period from January till August 2013. Diagnosis infection with this parasite by using the wet amount microscope for vaginal secretion. The results showed significant decrease ($P<0.05$) in iron, ferritin, FSH, LH and growth hormone in T. vaginalis infection patients in compared to control group.

Keywords: FSH, LH, T. Vaginalis.

I. INTRODUCTION

Trichomonas vaginalis is one of the commonest sexually transmitted pathogens in the world, with an estimated 170 million cases occurring each year, the epidemiology of the disease is still poorly understood and some practitioners continue to question its importance, It is one of the most common causes of non-viral sexually transmitted diseases in the world (Weinstock, 2004). T.vaginalis infection typically elicits aggressive local cellular immune responses with inflammation of the vaginal epithelium and exocervix in women and the urethra of men(Shafir, 2009). The parasite principally infects the squamous epithelium of genital tract but can be recovered from the urethra and has been found in the fallopian tubes and the pelvis. In males; T. vaginalis causes urethritis and prostatitis. Respiratory infections are acquired perennially from infected mothers. (Upcroft,2001). The molecular biology based diagnostic methods such as hybridization assay and PCR have been employed in the diagnosis of T. vaginalis in different sittings (Ojuromi, 2007). T. vaginalis is an obligate parasite, which cannot live without close association with the vaginal, urethral or prostatic tissues (Arora, 2005).

Pathogenesis of Trichomoniasis: Although trichomoniasis is the most common non-viral sexually transmitted disease, the pathogenicity of T. vaginalis is not thoroughly understood. Trichomonads participate in a host-parasites relationship, causing them to adhere to epithelial cells. The ability of trichomonads to adhere is affected by time, temperature, and pH level. T. vaginalis grows best in an anaerobic environment with a pH 6.1 (Rook, 2007).

This increase in pH creates a better environment for the growth of the parasite (Goode, 1994). Additionally, certain factors contributing to pathogenicity, such as cell-detaching factor, are inactivated at a pH of less than 5. Cell-detaching factor activity had also been found to be inhibited by estrogen, although it is unknown whether hormones have any other effect on the parasite (Garber, 1991). The roles played by pH and hormones in trichomoniasis may explain the observation that symptoms of the disease are often worse during menstruation (Heine, 1993). Menstrual blood creates a rich milieu for T. vaginalis reproduction at a higher pH than is normally found in the vagina. Additionally, the blood provides increased amounts of iron, which enhances the ability the of T. vaginalis to attach to the vaginal epithelium (Lehker, 1991).

II. SUBJECTS AND METHODS

A. Specimens

From January till August, 2013, 30 samples were collected from patients and 20 healthy who attended the clinics in AL-Sadder Teaching Hospital and AL-Zahra Hospital in AL-Najaf province,vaginal swabs were collected, from women patients and blood samples were also drawn from the same patients by vein-puncture into specimen tubes and remains for 30 minutes at room temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes (Backman/counter, Germany**)** to separate the serum and collected in other sterile tubes, each sample of serum was divided into three parts; each of them was kept in deep freeze at -20C^{o} till used for the determination of iron, ferritin and growth hormone.

B. Wet mount Examination

Immediately, 1 drop from one of the tubes was applied to a glass slide, covered with a cover slip, and examined under the microscope by using the high power objective (X40) for the presence of T.vaginalis. The wet mounts mere examined for at least 10 minutes (12). Positive results were defined as the presence of one or more Trichomonads with characteristic motility (jerky movement) and morphology

(Demoeet al., 1996). The Trichomonads may be inactive and non-motile as in chronic or asymptomatic condition (13). The wet amount is also used to demonstrate the presence of clue cells in vaginal secretions, these cells were epithelial cells covered by masses of bacteria of varying morphology (14).

C. Serum Iron (Colorimetric Test)

The colorimetric test method was used to estimate the serum of iron via Randox reagents, code HB012. (Randox Kit, U.K) by cypress diagnostics biochemistry analyser.

1. Procedure

1. The solution was brought at room temperature for 10 minutes.
2. The standard and a reagent blank were prepared for each series of determination into identified test tube as follow
3. Mix and wait 5 minutes at 37C° or 10 minutes at room temperature.
4. Read absorbance of standard and sample against standard / sample blank. The colour is stable for at least 30 min.
5. The final absorbance was red at 590 nm.

	Standard blank	Standard	Sample blank	ample
Standard Sample Working reagent R3	200 ml 1.0 ml	200 ml 1.0 ml 1 drop	100 ml 1.0 ml	200 ml 1.0 ml 1 drop

2. Calculation of Results Iron (mg /dl)

Serum Iron concentration =

$$\frac{Abs.sample - Abs.samleblank}{Abs.standrad - Ab\ standard\ blank} X100(\text{Stand. Con.}) \qquad (1)$$

D. Ferritin ELISA

This test was intended to quantify the serum levels of ferritin through the immunoezymatic technique Enzyme-Linked Immunosorbent Assay (ELISA) using bio Elisa reader ELx 800 (bio kit, U.S.A.) .The human Accu Bind ferritin ELISA kit was achieved according to the manufacturing company (Monobind Inc , U.S.A.) .

1. Procedure

1. The components of the kit were equilibrated at the room temperature before use.
2. 25 µ l of standard, controls and sample was added per well.
3. 100 µ l of Biotinylated ferritin Antibody was added to each well. Wells were covered with a sealing tape and incubated for 30 minutes. The timer was started after the last sample addition.
4. The micro plate was washed six times with 300 ml of wash buffer using bioeliser washer ELx 50 (bio kit, U.S.A.).
5. 100 µ l of ferritin Enzyme Reagent was added per well and incubated for 30 minutes. The bio Elisa reader ELx 800 (bio kit, U.S.A.) was turned on and set up the program in advance.
6. The micro plate was washed as described above.
7. 100 µ l of working substrate solution was added per well and incubated for about 15 minutes or until the optimal blue colour density develops.
8. 50 µ l of stop solution was added to each well. The colour will change from blue to yellow.
9. The absorbance on bio Elisa reader EL x 800 was read at a wave length of 450 nm immediately. Results were provided within 1 minute on the LCD display and printed out.

III. SERUM GROWTH HORMONE ESTIMATION

This assay executed with specific kit for Enzyme Immunoassay for the Quantitative Determination of Human Growth Hormone (HGH) Concentration in Human Serum, supplied by (Diagnostic Automation, INC, Cat # 1901Z).

1. Secure the desired number of coated wells in the holder.
2. Dispense 50ml of standard, specimens, and controls into appropriate wells.
3. Dispense 100ml of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-22oC) for 60 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100ml of TMB substrate into each well. Gently mix for 5 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100ml of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read optical density at 450nm with a microtiter reader within 30 minutes.

A. Estimation of FSH hormone

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

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 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. 0.100 ml (100µl) of FSH-Enzyme Reagent solution was added to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. 350µl of wash buffer was added (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.
8. 0.100 ml (100µl) of working substrate solution was added to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.
9. Incubate at room temperature for fifteen (15) minutes.
10. 0.050ml (50µl) of stop solution was added to each well and gently mix for 15-20 seconds). 11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should beread within thirty (30) minutes of adding the stop solution.

B. Serum LH hormone Estimation

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 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. 0.100 ml (100µl) of LH-Enzyme Reagent was added to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. The contents of the microplate were discarding by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. 350µl of wash buffer was added (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.
8. 0.100 ml (100µl) of working substrate solution was added to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.
9. Incubate at room temperature for fifteen (15) minutes.
10. 0.050ml (50µl) of stop solution was added to each well and gently mix for 15-20 seconds). Always add reagents in the same order to minimize reaction time differences between wells.
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

IV. RESULTS

The results of research revealed that the levels of serum iron, ferritin and growth hormone in women infection with T.vaginalis were significant decrease ($P< 0.05$) in compared to control group, as seen in figure (1). Also the results showed the levels of FSH, LH and prolactin hormone were significant decrease ($P< 0.05$) in compared to control, as seen in figure (2).

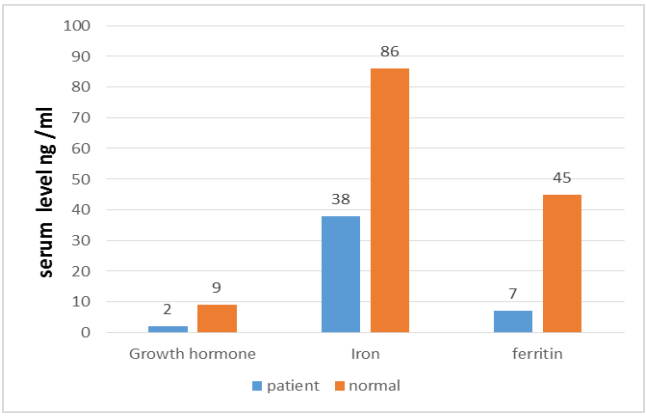

Figure 1: Comparison between growth hormone, ferritin and iron levels in women infected with Trichomonas vaginalis and control group.

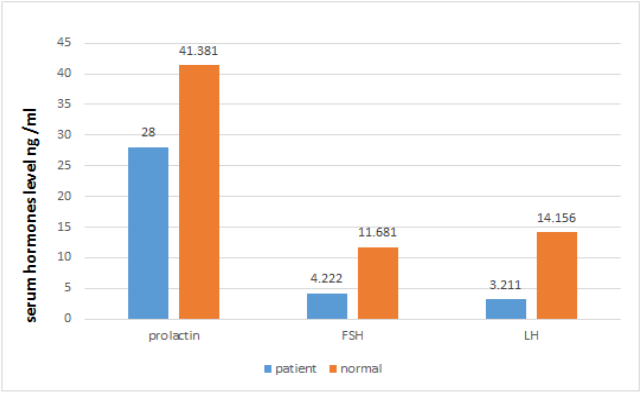

Figure 2: Comparison between prolactin, FSH and LH levels in women infected with Trichomonas vaginalis and control group.

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

V. DISCUSSION

The results revealed that the serum growth hormone, iron and ferritin significantly decrease in Trichomonas vaginalis infection patients compared to control group. Also serum prolactin, FSH and LH were significantly decreasing in T.

vaginalis infection patients compared to control group. The decrease in iron level in patients with T. vaginalis maybe due to the pathogenicity of this parasite dependent on the relationship between iron concentration and adhesion of parasite on epithelial cell (Garcia et al. 2003).Whereas the source of iron maybe from the hemolysis of red blood cells from lesion occur by the parasite or from blood of menstrual which increase by infection (Al-Hadraawy, 2013). The consuming of iron by T. vaginalis may cause a decrease in the iron levels. The decrease in ferritin levels maybe due to an increase in consuming iron by this parasite and this leads to decrease in the storage of iron as ferritin or increased utilized by parasite whereas some studies describing T. vaginalis as an iron source (Lehker &Alderete, 1992 and Al-Hadraawy, 2013). As confirmed by the data from Fernando et al. (2009) that T. vaginalis use the ferritin as an iron source; therefore, ferritin decreased when an infectious process occur.

The current study agrees with the study of Weinberg (1999) who recorded that the T. vaginalis uses the ferritin as source of iron and these lead to decrease in the ferritin in serum of men infected with T. vaginalis compared to control group. The present results have revealed that the serum prolactin,FSH and LH significantly decrease in T. vaginalis infection patients compared to control group. The decrease in prolactin level in patients with T. vaginalis maybe due to the Phagocytosis plays an important role in T. vaginalis pathogenicity, making it an object of investigations.Iron deficiency inT. vaginalis lead to increased dopamine release that inhibits prolactin secretion(Barbaraet al.,2006)also dopamine inhibits the release of FSH and LH from gonadotrophs(Kim et al., 2001;Levavi etal.,2006). insulin like growth factor-I inhibits GH release by a negative feedback mechanism thereforedecreased of growth hormones may be due to increased level of insulin like growth factor-I with infection of Trichomonas vaginalis whereas indicates that the parasite genes share identities with mammalian homologues, including insulin-like growth factor (Hend etal., 2007;Kuoetal.,2012).

VI. REFERENCE

[1] Weinstock H, Berman S, Cates W, JR.(2004). Sexually transmitted disease among American youth: incidence and prevalence estimates, 2000. Perspect Sex Report Health; 36(1):6-10.

[2] Shafir, SC.; Sorvillo, FJ. and Smith. L. (2009). Current issues and considerations regarding trichomoniasis and human immunodeficiency virus in African-Americans. Clin Microbiol Rev; 22: 37-45.

[3] Upcroft P. (2001): Drug Targets and Mechanism of Resistance in the Anaerobic Protozoa .Clin . Micro. Rev . 14:150-164

[4] Ojuromi, O.; Oyibo, W. and Tayo, D. (2007). Reliance on microscopy in Trichomonas vaginalis diagnosis and its prevalence in females presenting with vaginal discharge in Lagos , Nigeria , J. infect. Devel, Coun. 1: 210-213.

[5] Arora, D. R., and Arora, B. (2005). Medical Parasitology, 2nd edn., CBS Publishers. Ejeizie, G. C. (1981). The parasitic disease of school children in Lagos, Nigeria. Acta Trop. Med., 38: 78-84.

[6] Rook, G. A. W. (2007). The hygiene hypothesis and the increasing prevalence of chronic inflammatory disorders. Royal Soc. Trop. Med. Hyg., 101: 1072-1074.

[7] Goode, M. A., K. Grauer, and J. G. Gums. (1994). Infectious vaginitis. Selecting therapy and preventing recurrence. Postgrad. Med. 96:85-98.

[8] Garber, G. E., L. T. Lemchuk-Favel, and G. Rousseau. (1991). Effect of β-estradiol on production of the cell detaching factor of Trichomonas vaginalis. J. Clin. Microbiol. 29:1847-1849.

[9] Lehker, M. W., R. Arroyo, and J. F. Aldrete. (1991). The regulation by iron of the synthesis of adhesins and cytoadherence levels in the protozoan parasite Trichomonas vaginalis. J. Exp. Med.171:2165-2170.

[10] Heine, P., and J. A. MacGregor. (1993). Trichomonas vaginalis: a re-emerging pathogen. Clin. Obstet. Gynecol. 36:137-144.

[11] Al-Hadraawy S. K (2013).Molecular and Immunological Study of women Infected with Trichomonasvaginalis Parasite in an- Najaf Province, Ph.D. thesis, collage of science, university of Kufa, 155.

[12] Barbara, f.; Elias, J. Julia, S. Agustin, C.; Niko, K.; Gloria, W. and Betsy, L. (2006). Iron Deficiency in Infancy Predicts Altered Serum Prolactin Response 10 Years Later, NIH-PA Author Manuscript; 60(5): 513–517.

[13] Kim, J.D., Suzuki, Y. and Aida, K. (2011).The Control Mechanism of Gonadotropin-Releasing Hormone and Dopamine on Gonadotropin Release from Cultured Pituitary Cells of Rainbow Trout Oncorhynchusmykiss at Different Reproductive Stages. Fish AquatSci 14: 379-388.

[14] Levavi-Sivan, B.;Biran, J. and Fireman, E. (2006). Sex Steroids Are Involved in the Regulation of Gonadotropin-Releasing Hormone and Dopamine D2 Receptors in Female Tilapia Pituitary. Biology of Reproduction 75:642–650.

[15] Hend, A.;Sahar, A.;Abou H.; Eman,A.; Elabd2andAmel, Y. (2007). levels of growth hormone, albumin andferritin in human fascioliasis and schistosomiasis. Parasitol., 37 (2): 659 – 670.

[16] Kuo-Yang, H.; Po-Jung, H.; Fu-Man, K.; Rose Lin, A.; John, F.;Alderete,D. and Petrus T. (2012). Comparative Transcriptomic and Proteomic Analyses of Trichomonasvaginalis following Adherence to Fibronectin. Volume 80 Number 11: 3900–3911.