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RESEARCH ARTICLE
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The Correlation between Some Immunological Markers and Hormonal Changes among Patients with Toxoplasmosis in Tikrit city

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ABSTRACT

A cross sectional study was carried out in Kirkuk city from 9th of February 2017 to 11th of February 2018. These women attend to Salahaldeen Teaching Hospital, primary health care centers of Tikrit city and some private medical laboratories. The aim of this study was to correlate between some immunological markers and hormonal changes among pregnant women with Toxoplasmosis in Tikrit city. The number of pregnant women understudy was 380 whose ages between 18-42 years old. The rates for Toxo IgM (-)/IgG (+) was 25.0%, Toxo IgM (+)/IgG (-) was 7.90%, Toxo IgM(+)/IgG(+) was 7.37%.,in compare to control groups the rates of toxoplasmosis among pregnant women were higher than non-pregnant women. The results was statistically highly significant relation ($P<0.01$).Regarding the relation of some hormones to the seropervalence of Toxoplasma antibodies , the high rates of serum progesterone and oxytocin levels were increased ,while the levels of serum estrogen were decreased in pregnant women whose had toxoplasma antibodies . So we concluded that the toxoplasmosis has effects on the sex hormones of pregnant women. This due to effect of T. gondii on production and proliferation of some hormones by their effect on endocrine and sex-hormones or other parts in the body. The study concludes that there was a significant correlation between immunological markers and hormonal changes among pregnant women with Toxoplasmosis.

Keywords: *Study, Production, Body, High, Serum*

INTRODUCTION

Toxoplasmosis a disease caused by intracellular protozoan parasite apicomplexan of worldwide distribution named Toxoplasma gondii (T. gondii) and can infect humans as well as virtually all warm-blooded animals, including mammals and birds (1). Humans acquire the parasite by the oral route through the ingestion of cysts in the tissue of undercooked or uncooked meat, vegetables and fruits, or water contaminated with oocysts from infected cat feces (2). Other means of transmission are organ transplantation, blood transfusion, and congenital transmission (1).

Most infections are asymptomatic but in some individuals, especially if immune-compromised, the parasite can become widely disseminated causing severe clinical signs including encephalitis(3).

Primary infection during pregnancy can result in severe damage to the fetus manifested as mental retardation, seizures, blindness, or even death(4).

The sexual hormones as estrogen and progesterone, affect immune cells both quantitatively and qualitatively modulating their coordinated responses. Sex steroids alter immune cells performance through the binding to specific receptors, both nuclear and non-nuclear(5).

Progesterone is known to be essential for implantation mammalian embryo into the wall of the uterus by stimulating production of enzymes responsible for lysis of the zonapellucid in addition the essential progesterone-mediated events are yet to be in the initiation of implantation (6). Progesterone has the capacity to influence the maternal immune system via the progesterone-induced blocking factor (PIBF). (7).

The synthesis and release of estrogens are centrally regulated by the hypothalamus pituitary-gonadal axis. Estrogens are important stimulators of humoral immunity and they have binding sites in both bone-marrow and peripheral B cells (8). In general, the fact that estradiol affects the invasion of T. gondii is a common phenomenon, regardless of the cell type and parasite strain. Estradiol can bind to the host Estrogen receptor (Er) and induce a conformational change in the receptor that allows the Er to bind DNA and alter the expression of other transcriptional regulatory proteins. In this way, estradiol could control the infection of pathogens (9).

Oxytocin (OT) is a nine amino acid neuropeptide synthesized by the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus. Oxytocin is also synthesized in such peripheral tissues as the uterus, corpus luteum, placenta, amnion, and testis, exerting central and peripheral actions, plays an essential role in the mechanisms of parturition and lactation. It is released into the circulation by exocytosis from the posterior pituitary and nerve terminals in response to various stimuli(10) .

Oxytocin, involved in numerous physiological and pathological processes, exerts a variety of actions, including the regulation of the hypothalamo-pituitary-adrenal axis in response to stress, cell proliferation, pregnancy, luteal function, maternal behavior, erectile function, and ejaculation (10).During the pregnancy in the placental decidual , as an endocrine organ, which act as a source of many hormones, including prolactin, a marker of decidualization , and the neurohypophysealnonapeptide hormone (11). The aim of this study was to correlate between some immunological markers and hormonal changes among pregnant women with Toxoplasmosis in Tikrit city.

MATERIALS AND METHODS

Study Population

Patients

A cross sectional study was carried out in Kirkuk city from 9th of February 2017 to 11th of February 2018. The number of pregnant women understudy was 380 whose ages between 18-42 years old. These women attend to Salahaldeen Teaching Hospital, primary health care centers of Tikrit city and some private medical laboratories. An interview was carried out with these pregnant women excluding any pregnant women with hypertension, Diabetes Mellitus, preeclampsia, Rubella and other infection using questionnaire form designed by investigator including pregnancy trimester and history of abortion..etc.

Control Groups

For comparison the Toxoplasma-antibodies rates

This group who are matched their Toxoplasma antibodies rates to the pregnant women studied and consisted of 180 non pregnant women were apparently have at least one successful pregnancy or more with no history of abortion whose age between 18-42 years old. These women presented in same previously mentioned hospitals and care centers.

For comparison of hormonal levels

They classified into two groups were; Toxoplasma antibodies seronegative pregnant women and non-pregnant women who are

matched hormonal levels with seropositive pregnant women.

Sampling (veins blood)

Seven and half ml of blood was collected by vein puncture using 10 ml disposable syringe from each women enrolled in this study. Blood samples were placed into sterile test tubes and left for 30 minutes at 37C° then centrifuged at 3000 rpm for 15 minutes then the clot was removed and the remaining was re- centrifuged at 3000 rpm for 10 minutes twice time and the obtained sera (1-2.5 ml) were then aspirated using automatic micropipette and transferred into clean test tube. Label was fixed on each test tube which then stored in deep freeze at -20 C° for late serological testing for:

Detecting specific Toxoplasma-IgM and Toxoplasma-IgG by using ELISA technique
Estimation the levels of the estrogen, progesterone and oxytocin.
Serological Tests for Toxoplasma antibodies detection
Enzyme Linked Immunosorbent Assay ELISA
1.A.ELISA for T. gondii–IgM
From Bio-Check, Inc323 Vintage Park Dr. Foster City, CA 94404

Principle

Purified T. gondii antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the T. gondii IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. Horseradish peroxidase (HRP)-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of Tetra-methyl-benzidine (TMB) Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM specific-antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

B. Sample: Serum.
ELISA for T. gondii–IgG
From BioCheck, Inc323 Vintage Park Dr. Foster City, CA 94404
As in ELISA for Toxoplama-IgM with exceptions:
Microtiter wells were coated with purified T. gondii antigen.

Enzyme conjugated with anti-IgG.
Cut-off , Negative control and Positive controls for Toxoplama-IgG

ELISA for human serum Oxytocin (OT)

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD Value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-axis and draw a best fit curve through the points on the graph .In the software interface, a best fitting equation of standard curve will be calculated using OD Value and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD Value of samples. Also can enter the corresponding fitting equation and OD Value of samples into Excel to get the concentration of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

ECLIA for serum Estrogen

From Roche Diagnostic, GmbH, Germany, REF:03000079.122 .

A. Principle

Competition principle. Total duration of assay: 18 minutes.
▪1st incubation: By incubating the sample (35 µL) with two estradiol specific biotinylated antibodies, immunocomplexes are formed, the amount of which is dependent upon the analyte concentration in thesample.

E. Data Analysis

The analyzer automatically calculates the analyte concentration of each sample. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

ECLIA for serum progesterone

From Roche Diagnostic, GmbH, Germany, REF:12145383.122 .

Principle

Principle of competition. Total duration of the test: 18 minutes.

• 1st incubation: 30 µl of the sample - in the presence of an antibody biotinylated anti-progesterone monoclonal antibody and a derived from ruthenium complex labeled progesterone are incubated with danazol for the progesterone release. The progesterone derivative of the sample competes with the progesterone derivative labeled by the antibody binding sites.

E. Data Analysis

The analyzer automatically calculates the analyte concentration of each sample. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Statistical Analysis

Computerized statistically analysis was performed using SPSS (Statistical Package for Science Services).Comparison carried out using; Chi-square (X2) and Probability (P value). The P value $\leq$ 0.05 was considered statistically significant (S) and less than 0.01 considered highly significant (H.S.) and greater than 0.05 considered non-significant (N.S.).

RESULTS

Overall rates of T. gondii antibody among pregnant women

The overall rate of Toxo-IgG among 380 pregnant women was 32.37 %, while the overall Toxo-IgM rate was 15.26% as in Table 1.

TABLE 1. Overall seroprevalence of T. gondii antibody among pregnant women.

Results	Overall seroprevalence of Toxoplasma antibodies			
	IgG		IgM	
	No.	%	No.	%
Positive	123	32.37	58	15.26
Negative	257	67.63	322	84.74
Total	380	100	380	100
X2 = 30.640	P=0.00011			H.S.

Seroprevalence of T. gondii antibodies type among pregnant women and control group

Table 4.2. shows that Toxo-IgM (-) / IgG (+) was 25.00 %, Toxo-IgM (+)/IgG (-) was 7.90% , Toxo-IgM (+) / IgG (+) at the same time was 7.37% and Toxo-IgM (-)/ IgG (-) was 59.73% in pregnant women. Regardind the control group (non pregnant women), the rate of Toxo-IgM(-) / IgG (+) was 6.66% , IgM (+) / IgG (-) was 2.23%, and both IgM (+) / IgG (+) at the same time was 1.11%.The results were highly significant.

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TABLE 2: Seroprevalence of T. gondii antibodies type among pregnant women and control group (non -pregnant women).

T. Gondii antibodies	Examined groups			
	Pregnant women		Control (Non -pregnant women)	
	No.	%	No.	%
IgM (-) / IgG (+)	95	25.00	12	6.66
IgM (+) / IgG (-)	30	7.90	4	2.23
IgM (+) / IgG (+)	28	7.37	2	1.11
IgM (-) / IgG (-)	227	59.73	162	90.00
Total	380	100	180	100
X2 = 52.990 P =0.00021 H.S.				

Relation of Toxoplasma antibodies with serum estrogen level

Table 3 shows the relation of serum estrogen level with Toxoplasma antibodies in pregnant women. The highest rate of decreased serum estrogen was 66.66% seen within pregnant women with Toxo-IgM (+)/IgG (-), while the highest rate 53.68% of normal serum estrogen level seen within pregnant women with Toxo-IgM (-) / IgG (+).

TABLE 3: Relation of Toxoplasma antibodies with serum estrogen level.

T. Gondii Antibodies type Seropositive	Serum estrogen level							
	Normal		Increased		Decreased		Total	
	No.	%	No.	%	No.	%	No.	%
Toxo-IgM (-) / IgG (+)	51	53.68	5	5.26	39	41.06	95	100
Toxo-IgM (+) / IgG (-)	8	26.67	2	6.66	20	66.66	30	100
Toxo-IgM (+) / IgG (+)	10	35.72	2	7.14	16	57.14	28	100
Total	69	45.09	9	5.88	75	49.01	153	100
X2 = 8.041 P =0.09 N.S.								

Relation of Toxoplasma antibodies with serum progesterone level

Table 4shows the relation of serum progesterone level with Toxoplasma antibodies in pregnant women. The highest rate of increased serum progesterone was 63.15% and seen in pregnant women with Toxo-IgM (-) / IgG (+), while the highest rate 3.57% of decreased serum progesterone level was seen in those with Toxo-IgM (+) / IgG (+).

TABLE 4 : Relation of Toxoplasma antibodies with serum progesterone level.

T. Gondii antibodies	Serum progesterone level							
	Normal		Increased		Decreased		Total	
	No.	%	No.	%	No.	%	No.	%
Toxo-IgM (-) / IgG (+)	33	34.74	60	63.15	2	2.11	95	100
Toxo-IgM (+) / IgG (-)	11	36.66	18	60.00	1	3.34	30	100
Toxo-IgM (+) / IgG (+)	10	35.71	17	60.71	1	3.57	28	100
Total	54	35.29	95	62.09	4	2.61	153	100
X2 =0.324 P =0.988 N.S.								

Relation of Toxoplasma antibodies with serum oxytocin level

Table 5 shows the relation of serum oxytocin level with Toxoplasma antibodies in pregnant women. The highest rate of increased serum oxytocin was 67.85% and seen in pregnant women with Toxo-IgM (+) / IgG (+).

TABLE 5: Relation of Toxoplasma antibodies with serum oxytocin level.

T. Gondii antibodies seropositive	Total serum Oxytocin level							
	Normal		Increased		Decreased		Total	
	No.	%	No.	%	No.	%	No.	%
Toxo-IgM (-) / IgG (+)	43	45.26	51	53.68	1	1.06	95	100
Toxo-IgM (+) / IgG (-)	11	36.66	18	60.00	1	3.34	30	100
Toxo-IgM (+) / IgG (+)	8	28.57	19	67.85	1	3.57	28	100
Total	62	40.52	88	57.51	3	1.97	153	100
X2 = 3.477 P = 0.481 N.S.								

Level of the hormones in the study group

Table 6 shows the serum hormonal (estrogen, progesterone and oxytocin) levels of 100 seronegative pregnant women (control group 1) and 100 seronegative non pregnant married women (control group 2) in comparison to seropositive pregnant women for Toxoplasma antibodies. The highest rates of women with normal hormonal levels were found in both control groups. The lowest rates of women with increased level of estrogen, progesterone and oxytocin were found in control group2.

TABLE 6: Level of the hormones in the study group.

Serum estrogen level result	Examined groups					
	Seropositive pregnant women		Control (Group 1)		Control (Group 2)	
	No.	%	No.	%	No.	%
Normal	69	45.09	84	84	97	97
Increased	9	5.88	15	15	1	1
Decreased	75	49.03	1	1	2	2
Total	153	100	100	100	100	100
X2 = 129.421 P =0.00017 H.S.						
Serum progesterone level result	Examined groups					
	Seropositive pregnant women		Control (Group 1)		Control (Group 2)	
	No.	%	No.	%	No.	%
Normal	54	35.29	82	82	98	98
Increased	95	62.09	16	16	1	1
Decreased	4	2.62	2	2	1	1
Total	153	100	100	100	100	100
X2 = 123.876 P =0.00018 H.S.						
Serum oxytocin level result	Examined groups					
	Seropositive pregnant women		Control (Group 1)		Control (Group 2)	
	No.	%	No.	%	No.	%
Normal	62	40.52	85	85	98	98
Increased	88	57.51	14	14	1	1
Decreased	3	1.97	1	1	0	0
Total	153	100	100	100	100	100
X2 = 111.456 P =0.00021 H.S.						

DISCUSSION

Toxoplasma gondii is an obligate intracellular parasitic protozoan widely distributed around the world. In addition to humans, it can also infect more than 200 species of animals and causes toxoplasmosis in them that appears to have broad host specificity and the ability to penetrate various host cells and rarely causes severe symptoms in healthy humans and most other hosts. (1).

The sexual hormones as estrogen and progesterone, affect immune cells both quantitatively and qualitatively modulating their coordinated responses. Sex steroids alter immune cells performance through the binding to specific receptors, both nuclear and non-nuclear(5).

Relation of serum estrogen levels with toxoplasmosis.

The present study revealed the rates of serum estrogen level were decreased among most cases of toxoplasmosis in pregnancy. The highest rate of women with decreased level of estrogen (66.66%) had Toxo-IgM (+)/IgG (-). The result was statistically non-significant relation P>0.05. This finding may be due to the effect of Toxoplsama in modulation the immune response and reducing the estrogen hormone levels which lead to stimulation to IFN-γ production. A previous study revealed a significant decrease in serum estrogen, that may explain why toxoplasmosis was more prevalent in pregnant women who can be through a fact that estradiol levels defects in pregnant women improve susceptibility to T. gondii, mainly through suppressing host immune endocrine network (IEN) and progressing parasite re-conversion and latency(12).

Relation of serum progesterone level with toxoplasmosis.

The progesterone hormone is a potent immune modulator as demonstrated during the menstrual cycle and pregnancy act in a number of immunological pathways including the blocking of mitogen-stimulated T cell proliferation, alteration of cytokine secretion of T-cell clones and modulation antibody production (8).

Considering the relation of serum progesterone level with Toxoplasma antibodies among pregnant women, the present study revealed that the rates of serum progesterone were increased among most cases of toxoplasmosis in pregnancy. The highest rate (63.15%) of increased level of progesterone was found in women with Toxo-IgM (-)/IgG (+). The result was statistically significant P>0.05.

This finding may be due to that the increased level of serum progesterone during the pregnancy enhanced the T. gondii infection and modulation the immune response.

During pregnancy, maternal hormones alter the immune responses of the mother in the presence of fetal antigens. The increases in the susceptibility to infection and a diminished pro-inflammatory response have critical anti-parasitic properties that cause an un-favorable development of toxoplasmosis. In the second and third trimester of gestation, there is a significant increase of progesterone levels and during this period, the rate of Toxoplasma infection increases (13) .

Relation of serum Oxytocin level with the seroprevalence of toxoplasmosis.

Oxytocin a hormone exerting central and peripheral actions, involved in numerous physiological and pathological processes, exerts a variety of actions, including the regulation of the hypothalamo-pituitary-adrenal axis in response to stress, cell proliferation, pregnancy, luteal function, maternal behavior, erectile function, and ejaculation that plays an essential role in the mechanisms of parturition and lactation. It acts through its receptors, the number of which increases in the uterus towards labor, thus augmenting the uterotonic effect(10).

Regarding the relation of serum oxytocin level with Toxoplasma antibodies, the present study revealed the rates of increased serum oxytocin level were higher than the rates of decreased oxytocin levels among seropositive pregnant women. The highest rate of increased serum oxytocin level (67.85%) was found in pregnant women with Toxo-IgM (+) / IgG (+), the result was statistically significant P>0.05. This finding

may be due to the effect of T. gondii on the stimulation and increasing the level of oxytocin although in pregnant women, may be increased the oxytocin during preterm labors. (10)

Because oxytocin induces contraction in the myometrium, both the activation and the inhibition of its receptor have long been targets in the management of dysfunctional and preterm labors, respectively(14). Oxytocin pulsatile changes occur in pregnant women at term. Apart from in the pituitary, oxytocin is also produced locally, and, in fact, placental oxytocin acting in a paracrine fashion may be more important than circulating oxytocin for the mechanism of labor. Oxytocin receptors are also up-regulated at the end of gestation and sensitivity to oxytocin-induced contractions is greatly increased compared to the no pregnant uterus. A significant increase in the number of oxytocin receptors in the myometrium and decidua is observed in women with both term and preterm labor (14).

Regarding the hormonal levels, the rates of normal levels were higher in control groups as shown in compare to seropositive pregnant women. The difference between two groups was statistically highly significant $P<0.01$.

The study concludes that there was a significant correlation between immunological markers and hormonal changes among pregnant women with Toxoplasmosis.

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