Relationship Between Toxoplasmosis Infection and Levels of Progesterone and Estrogen Hormones in Aborted Women In Wasit Province, Iraq

Shifaa Ali Abdulmohsin Al-Hachami [1]*, May Naji Al-Khanak [2], Jalal AbdulRazzaq Tofah Al-Azzawi [3]

[1, 2] Department of Biology, College of Science, University of Wasit, Wasit, Iraq

[3] Ministry of health, Wasit Directorate of health, Wasit, Iraq

Emails:shafaa9091@gmail.com[1], mayalkhanaq@uowasit.edu.iq[2], jalaltofah42@jmail.com[3]

** Corresponding author*

Abstract

The current study aimed to determine relationship between *Toxoplasma gondii* infection and levels of Progesterone and Estrogen in aborted women and normal delivery women in Wasit province. The study included two main groups: patients and controls of Iraqi women. The control was included 50 women (non-infected normal delivery women), whilst the patient group were included 146 Toxo-seropositive serum samples. Serum samples from patients and control were collected and detected the levels of Progesterone and Estrogen by using ELISA technique. The results also showed the total rate of Progesterone in 1st trimester, 2nd trimester and 3rd trimester were 146/300 (4.50 ± 1.77, 7.72 ± 4.19 and 30.46 ± 11.93) ng/ml, respectively in *T. gondii* seropositive patients serum samples, whereas 50/50 (21.69 ± 7.89, 41.63 ± 15.56 and 126.72 ± 39.71) respectively. In control serum samples by using ELISA, statistical analysis results indicated high significant differences between the patients and control women. Also, the results showed the total rate of Estrogen in 1st trimester, 2nd trimester and 3rd trimester were 146/300 (91.41 ± 65.80, 853.19 ± 331.40 and 1003.18 ± 530.02) ng/ml, respectively in *T. gondii* seropositive patients serum samples, whereas 50/50 (1042.86 ± 501.69, 4327.09 ± 1294.66 and 4972.67 ± 1010.16) respectively. In control serum samples by using ELISA, statistical analysis results indicated high significant differences between patients and control women.

Keywords: Toxoplasmosis, ELISA, Progesterone, Estrogen

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Introduction

Toxoplasmosis is one of the major common diseases parasites caused by *Toxoplasma gondii*, a coccidian protozoan, (Shojaee *et al.,* 2018). *T. gondii*is an obligate parasite intracellular with a complicated cycle of life (Çakmak *et al.*, 2017), warm-blooded animals most infected by the parasite, the felid (cat) family is the primary host, in addition, the human (Al-Shaeli et al., 2020). Animal infection caused by eating infected meat, or by ingestion of the feces of a cat which has infected lately (Raza *et al.,* 2019).

The primary pathway of human infection involves the intake of drinking water, eating fruit or vegetables, or coming into contact with soil that is contaminated with oocysts of *T. gondii* (Freppel *et al.*, 2019). The other path of human infection is the transmission of tachyzoites (rapidly dividing) from the mother pregnant to the growing fetus. This path of infection maybe leads to abortion, chorioretinitis or severe developmental disorders (Shojaee *et al.,* 2018).

Toxoplasmosis spread is global and several statistics show that parasite infection (*T. gondii*) is estimated is between 15-85% in populations, and this relies primarily on the sex, age and immune status of persons, As well as the existence of an important factor is the extent to which it is important to realize that the infection might not always cause noticeable symptoms requiring the attention of the person for medical advice (Sasai *et al.,* 2018).

The most common route of disease transmission byConsumption of *T. gondii* -contaminated food, vegetable, water, and muscle cysts present in undercooked meat and also congenital infection, Infection by this parasite in pregnancy can lead to mental disorders and deafness, abortion, and vision disturbances after birth (Soleymani *et al.*, 2020).

An important outcome of congenital toxoplasmosis is spontaneous abortion (Alvarado-Esquivel *et al.*, 2014). Spontaneous abortion is the greatest common adverse pregnancy outcome From etiological perspective, It may perhaps happen as a result of genetic factors environmental, toxins infectious agents such as virus or bacteria and other maternal or paternal effects (chronic disease) (Zheng *et al*., 2017). Most of females undergo abortion or birth with congenital malformations due to one, two or all the following important causatives *T. gondii*, Rubella virus and Cytomegalovirus (Majeed *et al*., 2020).

In addition, the ability of sex-hormones and pregnancy-associated hormones to influence the severity of *T. gondii* infection is of particular public health interest due to the ability of this parasite to cause congenital disease if infection occurs during pregnancy. There is currently considerable evidence that steroid hormones affect the course of toxoplasmosis in humans and mice (Al-Warid and –Qadhi, 2012).

Progesterone is a natural hormone secreted by the corpus luteum of women (Henderson, 2018). If the levels of progesterone could not be properly maintained, threatened miscarriage or even miscarriage will eventually occur. Feng *et al.* (2017) pointed out that if pregnant women cannot maintain appropriate progesterone levels, threatened abortion or even miscarriage would eventually occur. Therefore, proper levels of progesterone and estrogen can ensure and maintain women's normal pregnancy as well as safe and smooth development of embryos (Li *et al.*, 2022).

Estrogens are produced primarily in ovaries, also in the liver, adipose tissue, heart, and, most importantly, the brain (Denley *et al*., 2018). Corpus luteum and the placenta responsible for creating Estradiol hormone during the pregnancy, any abnormalities or deficiencies in the Corpus luteum or the placenta in the manufacture of Estradiol hormone may cause miscarriage (Ibadi, 2016).

The present study was aimed to determine the relationship between *T. gondii* infection and levels of Progesterone and Estrogen hormone in aborted women and normal delivery women by using ELISA test in Wasit province.

Materials and methods

Experimental Design

The study is carried out in collaboration Al-Zahra teaching hospital and Alkut Hospital for Gynecology, Obstetric and Pediatrics. Total 196 serum samples are collected from January to November (2021) for which include two different women's groups are used 50 samples from normal delivery women act as Control groups and 146 Toxo-seropositive Serum samples act as patients group.

Samples collection

Five ml of venous blood is collected from infected and control samples placed in a gel tube and put half an hour in a water bath at 37°C until blood is coagulated, After that centrifugation at 3500 rpm for 10 min to obtain a serum which is kept in the frozen at -20°C in sterile and clean plastic tubes until it is used (Hussein *et al.,* 2022).

Detection the levels of Progesterone and Estrogen hormones

Toxo-seropositive serum samples of 146 patients and 50 control were tested for Progesterone and Estrogen by using ELIZA technique, a test kits from Elabscience®company (USA) was used , Progesterone and Estrogen assays were performed following the manufacturer’s instructions (Al-Qurabey, 2018).

StatisticalAnalysis

The Data were analyzed by using SPSS computersoftware for findings mean ± SD. Independent t-test was used to determine whether there is a statistically significant difference between the means of two study groups ,Probability values less than 0.05 were considered significantly different (Gharban and Yousif, 2020, 2021).

Results and Dissection

Detection levels Progesterone in *T. gondii* seropositive patients and control by using ELISA

The results of current study observed that the total rate of Progesterone in *T. gondii* seropositive patients and control serum samples in 1st trimester by using by using ELISA were 146/300 (4.50 ± 1.77 ng/ml) in *T. gondii* seropositive patients whereas, whereas 50/50 (21.69 ±7.89 ng/ml) in control serum samples, statistical analysis results indicated high significant differences at ($p<0.05$) between the prevalence of the infected patients and control (Table 1, Figure 1).

The results of current study observed that the total rate of Progesterone in *T. gondii* seropositive patients and control serum samples in 3nd trimester by using by using ELISA were 146/300 (7.72 ± 4.19 ng/ml) in *T. gondii* seropositive patients whereas, 50/50 (41.63 ± 15.56 ng/ml) in control serum samples, statistical analysis results indicated high significant differences at ($p<0.05$) between the prevalence of the infected patients and control (Table 1, Figure 1).

The results of current study observed that the total rate of Progesterone in *T. gondii* seropositive patients and control serum samples in 3rd trimester by using by using ELISA were 146/300 (30.46 ± 11.93 ng/ml) in *T. gondii* seropositive patients whereas, whereas 50/50 (126.72 ± 39.71 ng/ml) in control serum samples, statistical analysis results indicated high significant differences ($p<0.05$) between the prevalence of the infected patients and control (Table 1, Figure 1).

Table (1): Relationship between Stage of pregnancy and Levels of Progesterone Hormone in Patients and Control

Time	Patient		Control		
	Mean ± SD(ng/ml)	S.E	Mean ± SD(ng/ml)	S.E	P-value¥
1st trimester	4.50±1.77	0.39	21.69±7.89	1.72	0.001**
2ndtrimester	7.72±4.19	0.94	41.63±15.56	3.479	0.001**
3rd trimester	30.46±11.93	2.67	126.72±39.71	8.88	0.001**

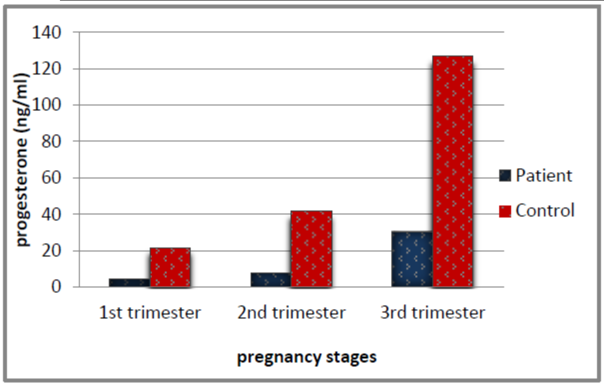

Figure (1): Relationship between Stage of pregnancy and Levels of Progesterone Hormone in Patients and Control

The results of the present study agreement with The results by El-Hashimi *et al.* (2014) in Baghdad recorded progesterone levels were 34/135(4.33±4.45 ng/ml) in spontaneous abortion women and 13/13 (36.59 ± 21.64 ng/ml) in control by using ELISA. As well, the results of the present study agreement with results of AL-Gharrawi *et al.* (2012) in Baghdad Province, recorded Progesterone hormone concentration in the aborted women in 1st trimester 52/70 (10.28 ± 3.99) ng/ mL and 2nd trimester 18/70 (26.90 ± 9.68) ng/mL compared with normally delivered pregnancies 20/20

(219.79 ± 5.11) ng/mL. There is a high significant difference between aborted women groups and normally delivered pregnancies using ELISA. Also, the results of the present study agreement with results of Al-Taei and Jasim (2021) in Tikrit Province recorded the progesterone levels were 75/100 (0.465 ± 0.012 pg/ml) in patient compared with 25/100 (0.875 ± 0.115 pg/ml) in control group, there was a higher significant difference using ELISA. The results of the present study agreement with results of Mohammed *et al.* (2021) in Ramadi city / Anbar Province showed that the mean concentration of Progesterone hormones 44/66 (5.64 ng\ml) in seropositive-Toxoplasmosis women, while estimate 22/66 (22.87 ng\ml) in non-infected pregnant women.

The results of the present study disagreement with Zamil *et al*. (2017) in Diyala, recorded the progesterone level was 41/84 (59.5 ng/ml) in patient and 19/84 (7.22 ng/ml) in control group, there is no significant difference in toxoplasma group than control group using ELISA. Also, the results of the present study disagreement with results of the Al-Warid and Al-Qadhi (2012), the study results showed that there were no significant differences in progesterone levels between infected and non-infected pregnant women with *T. gondii*, although high progesterone level were noticed in non-infectedpregnant women compared with low level in women infected with *T. gondii* (18.30 ± 9.84 ng/ml) and (11.19 ± 9.76 ng/ml) respectively, by using the VIDAS.

The hormones of the human body change during pregnancy and thus affect the immune response of women, and this increases the chance of infection due to diminished initial inflammatory response of the body, especially the antibodies to the parasite. Also, the progesterone hormone has a different effect on toxoplasmosis disease, as it leads to exacerbation of infection or to limit the reproduction of the parasite since progesterone is produced in the ovaries, uterus, and brain (Al-Taei and Jasim, 2021). The decrease in the concentration of progesterone during Abortion comes in response to the mother's immune system to reject the fetus by suppresses modulatos uterine contraction, and the decrease in the concentration of progesterone is associated with abortion during the first three months From pregnancy (Naji and Tahrr, 2015).

Significant differences in levels of studied hormones, because hormone is made by the Corpus lutein and is the main source of hormone secretion in the first stage of pregnancy by making Pregnenolone compound, as initial to the hormone progesterone Whereas in the second and third stages of pregnancy, the placenta is the main source of its production and a lower level from the endometrium and adrenal cortex by the hydroxylation of cholesterol in the low-density lipoprotein (LDL) to form the Pregnenolone compound from which the progesterone is made (Mohammed *et al.*, 2021).

Detection levels of Estradiol in *T.gondii* seropositive patients and control by using ELISA

The results of current study observed that the total rate of Estradiol in *T. gondii* seropositive patients and control serum samples in 1st trimester by using by using ELISA were 146/300 (6.12 ± 0.38 ng/ml) in *T. gondii* seropositive patients whereas, whereas 50/50 (12.29 ±1.26 ng/ml) in control serum samples, statistical analysis results indicated high significant differences at ($p<0.05$) between the prevalence of the infected patients and control (Table 2, Figure 2).

The results of current study observed that the total rate of Estradiol in *T. gondii* seropositive patients and control serum samples in 3nd trimester by using by using ELISA were 146/300 (3.91 ± 2.17 ng/ml) in *T. gondii* seropositive patients whereas, whereas 50/50 (12.29 ±1.26 ng/ml) in control serum samples, statistical analysis results indicated high significant differences at ($p<0.05$) between the prevalence of the infected patients and control (Table 2, Figure 2).

The results of current study observed that the total rate of Estradiol in *T. gondii* seropositive patients and control serum samples in 3rd trimester by using by using ELISA were 146/300 (1003.18 ± 530.02 ng/ml) in *T. gondii* seropositive patients whereas, whereas 50/50 (4972.67 ± 1010.16 ng/ml) in control serum samples, statistical analysis results indicated high significant differences at ($p<0.05$) between the prevalence of the infected patients and control (Table 2, Figure 2).

Table (2): Relationship between Stage of pregnancy and Levels of Estrogen Hormone in Patients and Control

Stage of pregnancy	Patient		Control		
	Mean ± SD(ng/ml)	S.E	Mean ± SD(ng/ml)	S.E	P-value¥
1st trimester	91.41±65.80	20.81	1042.86±501.69	158.65	0.001**
2nd trimester	853.19±331.40	104.79	4327.09±1294.66	431.55	0.001**
3rd trimester	1003.18±530.02	167.61	4972.67±1010.16	336.72	0.001**

Data presented as Mean ± SD, ¥: Independent t-test was used to test between groups. ** Highly significant (P<0.01)

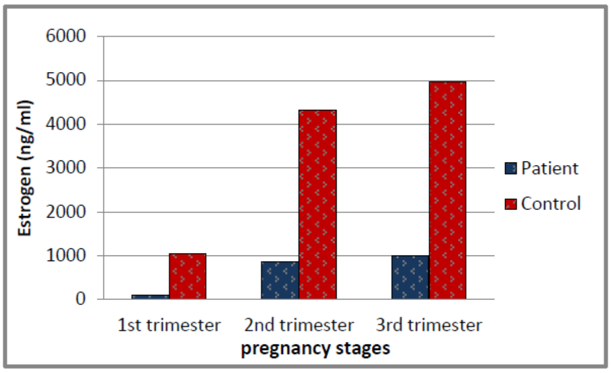

Figure (2): Relationship between Stage of pregnancy and Levels of Estrogen Hormone in Patients and Control

The results of the present study agreement with results of El-Hashimi *et al*. (2014) in Baghdad recorded estradiol levels were 34/135(107.21±99.33 pg/ml) in patient 13/13(515.9±527.8 pg/ml) in control, there was no a significant difference using ELISA.As well, The results of the present study agreement with The results by AL-Gharrawi *et al.* (2012) in Baghdad Province, recorded Estradiol concentration was significantly lower p=0.0001 in the aborted women in 1st 52/70(110.21 ± 19.22) pg/m and in 2nd 18/70(166.55 ± 39.86) pg/mL than in normally delivered pregnancies 20/20 (3910.74 ± 271.35) pg/mL, There is a high significant difference between aborted women groups and normally delivered pregnancies using ELISA.The results of the present study agreement with results by Mohammed *et al.* (2021) in Ramadi city / Anbar Province showed that Toxoplasmosis also affected on level Estradiol as it reduced 44/66 (2.24 ng\ml) in seropositive-Toxoplasmosis women compared with 22/66 (4.97 ng\ml) in non-infected pregnant womenthere is significant difference in toxoplasma group than control group using ELISA.

The results of the present study disagreement with Zamil *et al.* (2017) in Diyala, recorded the estradiol level was 41/84 (1147 pg/ml) in patient and 19/84 (50.4 pg/ml) in control group, there is no significant difference in toxoplasma group than control group using ELISA

Significant differences in levels of studied hormones, because Corpus luteum responsible for creating Estradiol hormone during the first trimester of the pregnancy and the placenta during the second and third trimesters of pregnancy, Any abnormalities or deficiencies in the Corpus luteum or the placenta in the manufacture of Estradiol hormone may cause abortion (Ibadi, 2016).

Conclusions

T. gondii was the most widespread species in women and therefore the current study detect the relationship between *Toxoplasma gondii* infection and the levels of Progesterone and Estrogen hormones in aborted women and normal delivery women by using ELISA. In this study observed an increase in the (Progesterone and Estrogen) in the serum of aborted women compared with normal delivery women, this indicating their role in the immune response against infection of this parasite. It is necessary to establish efficient strategies to control and reduce *T. gondii* transmission in women in Wasit province.

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