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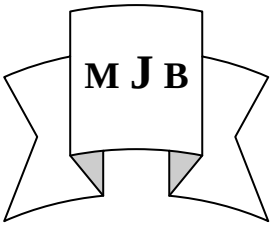

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Abstract

The present investigation dealt with studying the influence of chronic infection by protazoan parasite *Toxoplasma gondii* on levels testosterone, progesterone and prolactin hormones in pregnant women through trimesters of pregnancy. A total number of 55 pregnant women with chronic toxoplasmosis(Seropositive IgG) and 51 healthy pregnant (Seronegative IgG) were used. The results revealed that chronic infection by *T. gondii* exhibited significant increased of testosterone serum levels and significant decreased of prolactin serum levels in all trimesters. we found no significant difference in progesterone levels in overall seropositive IgG pregnant women, We have detected fluctuation in levels of progesterone from trimesters to other, in first trimester progesterone levels did not show significant variation, significant decrease in progesterone levels in seropositve IgG in second trimester while a significant increase occurred to progesterone in seropositive IgG pregnant women when compared with those of the control group during third trimester. Testosterone , progesterone and prolactin concentrations were highest in the 3rd trimester. We can conclude that chronic infection by *T. gondii* in pregnant women associated with variations in levels of testosterone, progesterone and prolactin hormones and these variations may be influences the probability of the *Toxoplasma* infection or that infection changes the concentration of hormones in infected host as adaptive stratagem enhance parasite survival in host.

Keywords: Testosterone; Progesterone; Prolactin ; chronic toxoplasmosis

التغيرات في مستويات هرمون الشحمون الخصوي وهرمون الحمل وهرمون الحليب في النساء الحوامل المصابات بداء المقوسات المزمن

الخلاصة

تناول البحث الحالي دراسة تأثير الإصابة المزمنة بالطفيلي الابتدائي *Toxoplasma gondii* في النساء الحوامل على مستويات هرمون الشحمون الخصوي وهرمون الحمل وهرمون الحليب خلال فصول الحمل. العدد الكلي المستخدم للنساء الحوامل المصابات (موجب مصليا للكلوبلين IgG) كان ٥٥ و ٥١ نساء حوامل سليمات (سالب مصليا للكلوبلين IgG). أظهرت النتائج أن الإصابة المزمنة بالطفيلي أظهرت زيادة معنوية في مستوى هرمون الشحمون الخصوي المصلي ونقصان معنوي في مستوى هرمون الحليب في جميع فصول الحمل. ولم نجد اختلاف معنوي في مستوى هرمون الحمل في النساء الحوامل الكلي وقد حدد تذبذبا في مستويات هرمون الحمل من فصل إلى أخر، ففي الفصل الأول للحمل لم تلاحظ فروق معنوية في مستويات الهرمون، في حين هنالك انخفاض معنوي في مستواه في الفصل الثاني وزيادة معنوية في الفصل الثالث عند النساء الحوامل المصابات مقارنة بالنساء الحوامل في مجموعة السيطرة. كانت تراكيز جميع الهرمونات هي الأعلى في الفصل الثالث للحمل مقارنة ببقية الفصول. نستطيع أن نستنتج بأن الإصابة المزمنة بالـ *T. gondii* في النساء الحوامل ترتبط بتغايرات في مستويات هرمون الشحمون الخصوي وهرمون الحمل

وهرمون الحليب وهذه التغايرات أما أن تؤثر في احتمالية الإصابة بالطفيلي أو إن الإصابة بالطفيلي هي التي تغير تراكيز الهرمونات كحيلة تكيفية تساعد على بقاء الطفيلي في المضيف.

كلمات مفتاحية: هرمون الشحمون الخصوي ،هرمون الحمل ،هرمون الحليب ،داء المقوسات المزمن

Introduction

Latent toxoplasmosis is clinically asymptomatic, but usually life-long infection, characterized by the presence of *Toxoplasma* bradyzoite cysts, typically in the nervous and muscular tissues, and by lifelong protective (both humoral and cellular) immunity to reinfection, manifested by the presence of low levels of anti-*Toxoplasma* IgG in the serum of infected individuals [1]. Between 20% and 80% of the population in various countries have life-long "asymptomatic" latent toxoplasmosis. Congenital toxoplasmosis occurs in infants that are infected during gestation, following a primary challenge of the mother [2].The foetus is only at risk of congenital disease when acute infection occurs during pregnancy but congenital infection has also been reported from a chronically infected immunocompromised mother with a reactivation of toxoplasmosis [3].

Numerous epidemiological and clinical studies have noted differences in the incidence and severity of parasitic diseases between males and females. Although in some instances this may be due to gender-associated differences in behavior, there is overwhelming evidence that sex-associated hormones can also modulate immune responses and consequently directly influence the outcome of parasitic infection [4]. Several field and laboratory studies link sex differences in immune function with circulating steroid hormones, not only can host hormones affect responses to infection, but parasites can both produce and alter hormone concentrations in their hosts [5]. James [6] hypothesizes that many parasites and pathogens change the concentration of steroid hormones, and he suggested that testosterone and oestrogen, of infected hosts which often results in a shift in the sex ratio, namely in the increase of the proportion of males in the offspring. Kaňková *et al*. [7] indicated that *Toxoplasma* infection changes the concentration of serum testosterone in mice and human rather than changed concentration of testosterone influences the probability of the *Toxoplasma* infection.

Lim *et al.* [8] demonstrated that *Toxoplasma gondii* infection enhances expression of genes involved in facilitating synthesis of testosterone, resulting in greater testicular testosterone production in male rats, but their results do not confirm a statistically significant increase in blood testosterone.

Progesterone has been shown to inhibit T cell, macrophage, and NK cell activity [9]. Progesterone has also been shown to decrease production of NO and nitrite by macrophages [10].

Gay-Andrieu *et al.*[11] showed that progesterone does not modulate *T. gondii* (RH strain) replication in the murine macrophage cell line (RAW 264·7), either in non-activated or IFN-γ/LPS-activated cells, despite its effect on NO production.

Acute decreases in PRL levels in both rodent models and humans result in decreased immunoeffectiveness [12]. It has been demonstrated in experimental studies that exogenous prolactin has antiparasitic activity in microglial cells as a reaction against the *T. gondii* infection [13]. Therefore, to protect

against *Toxoplasma* infection, a group of cells in the pituitary gland may proliferate to produce prolactin and thereby activate the microglial cells, and it is possible that pituitary adenoma evolves from this type of prolactin producing cell hyperplasia [14].

In present study we have attempted to find if there was a correlation between chronic infection by *T. gondii* and levels of testosterone, progesterone and prolactin hormones in the study groups.

Materials and Methods

Samples:

Sera of 55 (19 first trimester, 17 second trimester and 19 third trimester) seropositive IgG anti-*Toxoplasma* antibodies pregnant women(previously identified by Eliza method) and 51 (16 first trimester, 18 second trimester and 17 third trimester) healthy subjects used as a control group were included for the estimation of Testosterone, Progesterone and Prolactin concentrations. these samples were obtained from the typical Al-Mahaweel healthy center in north of Babylon province , this center include pregnant care unit where pregnant women visited monthly. Their ages were 26.53 ± 7.3 with a range of 18-42 years. The pregnant women were divided into 3 groups by gestational age; the first trimester (1 –3 month, n = 95), the second trimester (4–6 month, n = 214) and third trimester (7–9 month, n = 89).

Collection of blood:

Disposable syringes and needles were used for blood collection. Venous blood samples, about 4-5 ml were collected from pregnant women in plane tubes. After allowing the blood to clot at room temperature for 15 min, blood samples were centrifuged at 3000 xg for 15 min. Sera were separated, and store in -40 C° to determine Testosterone, Progesterone and Prolactin levels.

Determination of Testosterone, Progesterone and Prolactin concentrations in serum

For the quantitative determination of total testosterone, Progesterone and Prolactin concentrations in serum of pregnant women used Testosterone, Progesterone and Prolactin EIA(enzyme immunoassay) Test Kits manufactured by Monobind Inc. Lake Forst, USA.

Statistical analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS) version 12 for Windows. Results are expressed as mean ± standard deviation (SD). Statistical significance and difference from control and test values were evaluated by Student's t-test. A probability value of $P<0.05$ indicated a statistically significant difference.

Results

The Overall variations in levels of hormons in pregnant women seropositive IgG *Toxoplasma* antibodies and in controls are presented in Table 1. We have detected higher serum levels of testosterone in patients with chronic toxoplasmosis compared to controls ($p<0.01$). prolactin was significantly lower in chronic toxoplasmosis patients compared to controls ($p<0.01$). The progesteron level did not show significant variation.

Table 1 Testosterone, Progesterone and Prolactin hormones in pregnant women with chronic toxoplasmosis.

Hormones	+ve IgG (test)	SD	-ve IgG (control)	SD	P value
testosterone ng/ml	1.95*	1.37	0.94	0.84	1.8E-5
progesterone ng/ml	54.95	6.96	54.58	6.42	0.780
prolactin ng/ml	58.68*	46.61	96.06	41.20	3.0E-5

* The mean difference is significant at the .05 or .001 level.

Table 2 showing significant ($p<0.05$) increase in testosterone level in seropositve IgG first trimester pregnant women as compared to control group whereas the levels of prolactin showed a significant($p<0.01$) decrease in seropositve IgG patients in comparison to control subjects. The progesterone levels did not show significant variation.

Table 2 Testosterone, Progesterone and Prolactin hormones in pregnant women with chronic toxoplasmosis in first trimester .

Hormones	+ve IgG (test)	SD	-ve IgG (control)	SD	P value
testosterone ng/ml	0.91*	0.92	0.35	0.52	0.041
Progesterone ng/ml	48.81	4.35	49.82	4.24	0.496
prolactin ng/ml	16.35*	12.58	49.04	23.88	1.0E-05

* The mean difference is significant at the .05 or .001 level.

In second trimester pregnant women the results demonstrated significant ($p=0.01$) increase in testosterone levels in seropositve IgG, while a significant ($p<0.05$) and ($p<0.01$) decrease for progesterone and prolactin levels respectively in seropositive IgG pregnant women when compared with those of the control group. (table 3).

Table 3 Testosterone, Progesterone and Prolactin hormones in pregnant women with chronic toxoplasmosis in second trimester .

Hormones	+ve IgG (test)	SD	-ve IgG (control)	SD	P value
testosterone ng/ml	2.06*	1.52	0.95	0.80	0.010
progesterone ng/ml	54.26*	4.7	57.66	4.07	0.028
prolactin ng/ml	40.73*	13.22	100.79	20.83	1.22E-11

* The mean difference is significant at the .05 or .001 level.

Data for third trimester pregnant women are shown in Table 4 There were significant differences in testosterone, progesterone and prolactin concentrations between the seropositive and seronegative IgG pregnant women. However, testosterone and progesterone concentrations demonstrated significant ($p<0.01$) increase. whereas the prolactin concentration showed a significant($p<0.05$) decrease in seropositve IgG patients in comparison to control subjects.

Table 4 Testosterone, Progesterone and Prolactin hormones in pregnant women with chronic toxoplasmosis in third trimester .

Hormones	+ve IgG (test)	SD	-ve IgG (control)	SD	P value
testosterone ng/ml	2.89*	0.80	1.49	0.78	8.6E-06
progesterone ng/ml	61.70*	4.34	55.82	7.72	0.007
prolactin ng/ml	117.06*	20.29	135.30	20.96	0.012

* The mean difference is significant at the .05 or .001 level.

The present study demonstrates statistically significant variations in hormones levels during trimesters of pregnancy in both seropositive IgG pregnant women and seronegative IgG pregnant women (control group) . Testosterone, progesterone and prolactin concentrations were highest in the 3rd trimester (2.89, 61.7 and 117.57 ng/ml respectively) in seropositive IgG pregnant women (Fig.1).In seronegative IgG pregnant women , Testosterone and prolactin concentrations were highest in the 3rd trimester (1.50 and 135.30 ng/ml respectively), while progesterone concentration was highest in 2nd trimester (57.66 ng/ml) (Fig.2).

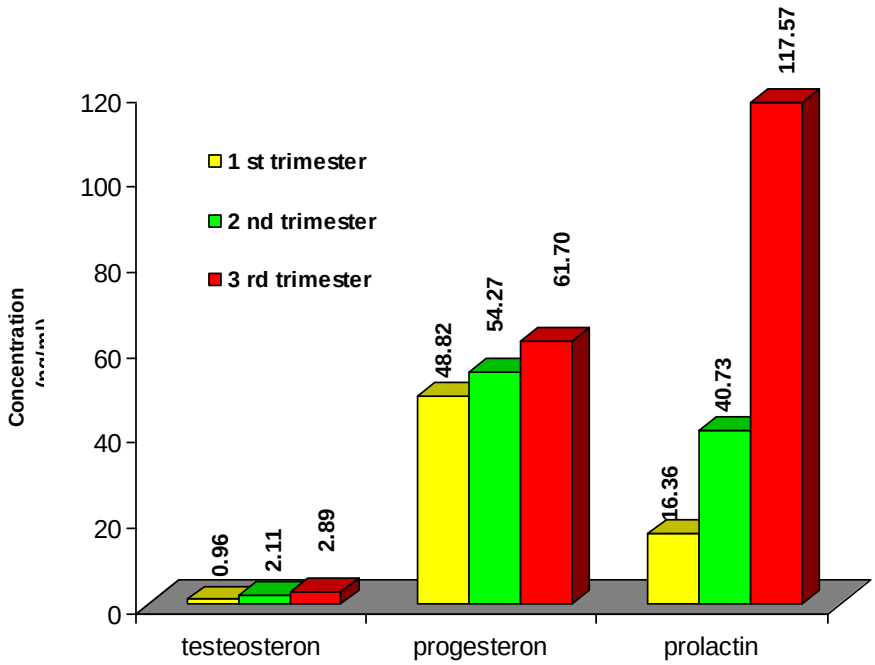

Figure 1 Variations in concentratons of testosterone, progesterone and prolactin during trimesters of pregnancy in pregnant women with chronic toxoplasmosis.(LSD: testosterone 0.51, progesterone 2.04, prolactin 7.27).

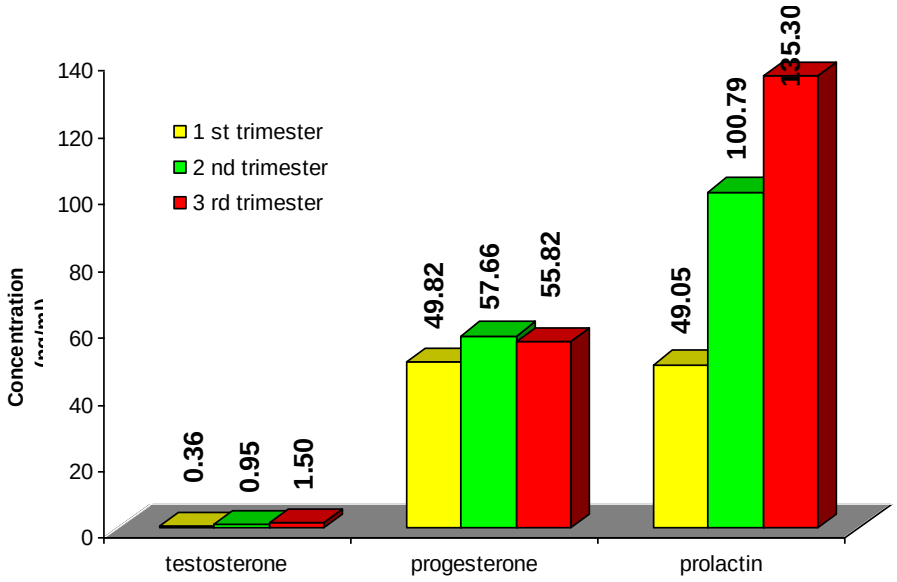

Figure 2 Variations in concentratons of testosterone, progesterone and prolactin during trimesters of pregnancy in pregnant women without toxoplasmosis (control group).(LSD: testosterone 0.35, progesterone 2.73, prolactin 10.66).

Discussion

Not only can host hormones affect responses to infection, but parasites can have pronounced effects on hormone signaling within the host. additional studies suggest that protozoan parasites can alter hormone concentrations in their hosts [5].

One of the most important problems of *T. gondii* infection in humans is congenital toxoplasmosis. Mechanisms which control materno–foetal transmission are poorly understood and pregnancy hormonal

impregnation may play a role [11]. In current study we evaluated three important hormones are testosterone, progestetone and prolactin.

Testosterone

Significant increase in testosterone values were found in the seropositive IgG pregnant women when compared to the seronegative IgG ones in this study (tables 1, 2, 3 and 4). This finding is corroborated by a study done by Shirbazou *et al.*[15] where they found significant increase in the levels of plasma testosterone in women and men with lgG anti-*Toxoplasma* antibody. It has been reported by Flegr *et al.* [16] that infected human males exhibit a statistically nonsignificant increase in salivary testosterone levels. Flegr *et al.* [17] have found *Toxoplasma*-infected men to have a higher concentration of testosterone and *Toxoplasma*-infected women to have a lower concentration of testosterone than *Toxoplasma*-free controls.they attributed the opposite direction of the testosterone shift in men compared to women to gender specificity. Lim *et al*.[8] found greater testosterone synthesis in testes of infected male rats with chronic toxoplasmosis. In mice, the observations by Kankova *et al*.[7] are in contrast with this finding since they observed that there was significant decrease in the serum testosterone values for the male and female in their study, they suggested that the decrease of testosterone concentration could be an adaptive response of infected mice to *Toxoplasma*-induced immunosuppression by decreasing the concentration of testosterone, the infected mice could partly compensate the laten toxoplasmosis-associated down-regulated cellular immunity, namely the observed suppressed reactivity of macrophages and lymphocytes to the antigen in *in vitro* assays [1].

There are two hypothesises explain the relationship between the infection by *Toxoplasma* and changes in testosterone concentration in infected host, the first hypothisis assumes that the changed concentration of testosterone influences the probability of the *Toxoplasma* infection, because of high concentrations of testosterone are known to have immunosuppressive effects [4;18], which could result in a higher probability of acquiring *Toxoplasma* infection. This explanation correspond with the result of present study where we found increase levels of testeosterone hormone in infected groups, on other hand this explanation incompatible with the result of Kankova *et al*.[7] where they found decrease levels of testeosterone hormone in infected mice (male and female). They speculated that the decrease of testosterone concentration could be an adaptive response of infected mice to compensate *Toxoplasma*-induced immunosuppression and Such compensation might increase the probability of the survival of infected mice after contact with various pathogens in their natural environment. It is also possible that the physiological reaction to *Toxoplasma* infection differs qualitatively between mice and humans because mice have short life comparable with the length of life in human.

The second hypothesis assumes that *Toxoplasma* infection changes the concentration of serum testosterone in infected host. There are some parasitic spescies such as *Taenia crassice* can manipulate the level of steroid hormones to increase their chance of surviving in the hostile environment of the host body [19]. James [6] support this and he suggested that the increased proportion of males in the offspring of *Toxoplasma* infected women and

female mice is a direct effect of *toxoplasma*-induced increase of testosterone in infected hosts. Moreover Kankova *et al*. [7] support that *Toxoplasma* infection changes the concentration of serum testosterone in infected host.

Progesterone

Progesterone, play a critical role in reproduction, including the maintenance of pregnancy in mammals, and immune function. progesterone can have both stimulatory and suppressive effects on the immune system, but is typically regarded as immunosuppressive [5]. Progesterone receptors have been identified in epithelial cells, mast cells, granulocytes (e.g. eosinophils), macrophages, and lymphocytes, also it can bind to glucocorticoid receptors, which are more abundant in the immune system than progesterone receptors, and may represent an alternative mechanism for progesterone-induced changes in immune function [20].

In the present study, we found no significant difference in progesterone levels in overall seropositive IgG pregnant women (tables 1). Our results are basically in agreement with those by Al-Warid and Al-Qadhi [21] result of these study showed that there were no significant differences in progesterone levels between infected and non infected pregnant women with *T.gondii*. On other hand when estimated levels of progesterone in pregnant women seropositive IgG *Toxoplasma* antibodies and in controls with regard of gestation age , We have detected fluctuation in levels of progesterone from trimesters to other, in first trimester progesterone levels did not show significant variation (table 2). significant decrease in progesterone levels in seropositive IgG in second trimester (table 3), while a significant increase occurred to progesterone in seropositive IgG pregnant women when compared with those of the control group during third trimester (table 4).this fluctuation in levels of progesterone during trimesteres of pregnancy may be attribute to attempt of parasite to manipulation immune system of their host through hormone fluctuation. Because progesterone can have both stimulatory and suppressive effects on the immune system [5], variations of progesterone by the parasite may further facilitate growth and reproduction of the parasite and inhibit host responses to infection. Other studies indicated that levels of progesterone were high when host infected by some protazoan parasitic species such as *Plasmodium berghei* in female mice [22].

Prolactin

Prolactin (PRL) is one of the most important hormones involved in immunoregulation in host body [23].exogenic prolactin can induce antiparasitic activity in microglial cells as a reaction against the *T. gondii* infection [13]**.**

In current study we observed significant decrease in levels of prolactin in all positive IgG anti-*Toxoplasma* antibody pregnant women groups (total pregnant , 1^{st} ,2^{nd} and 3^{rd} trimesters of pregnancy ; tables1, 2, 3 and 4 respectively) when compare with control groups. The high levels of prolactin hormones in seronegative groups (control) in opposition to seropositive groups (chronic infection) may be indicate to protective action of PRL in a host organism against *Toxoplasma* infection. It has been reported that prolactin hormone increases the production of immune globulins, cytokines and autoantibodies [24].

Mavoungou [25] reported that prolactin concentrations increase during pregnancy, regardless of parity.

He showed that prolactin concentration did not differ according to *P. falciparum* status. Dzitko *et al.* [26] have found increased prevalence of latent toxoplasmosis in women with an aberrant level of prolactin.

Dzitko *et al.* [23] revealed that pre-incubation of the *Toxoplasma* tachyzoites with the recombinant human prolactin (rhPRL) *in vitro* resulted in a significant reduction (up to 36.15%) in the replication abilities of the parasite. They suggested that the inhibition of replication was caused by a limited capacity of the parasites to penetrate host's cells as demonstrated by the reduced number of infected cells in their study.

Dzitko *et al.* [27] suggest that a significant increase in the serum PRL level, during pregnancy for instance, might significantly limit the risk of *Toxoplasma* spreading and could play an important role in natural protection against toxoplasmosis, and they revealed that exogenous human recombinant prolactin (rhPRL) as well as autologous endogenous prolactin present in serum – sPRL from inactivated sera significantly restricted intracellular growth of *Toxoplasma* in peripheral blood mononuclear cells cultures. Moreover, analysis of IL-10 production by PBMC infected with *Toxoplasma* and cultured in the presence of sPRL showed a positive correlation between sPRL concentration and the level of IL-10.

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