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The role of cytokines, TNF-α, IL-6 and pregnancy associated hormones in *Toxoplasma gondii* induced abortion

Waleed K. El-Hashimi[a], Hutham I. Khalil[a], Qasim S. Al-Mayah[b] and Nada M. Al-Bashier[b]

[a]*Department of Microbiology, College of Medicine/Al-Mustansiriya University, Iraq*
[b]*Medical Research Unit, College of Medicine, Al-Nahrain University, Iraq*

ABSTRACT

Toxoplasma gondii is a protozoan that can cross the placenta of the infected mother and causes severe fetal damage or abortion. This study aimed to investigate the role of two cytokines and sex hormones in the T. gondii-induced abortion. A total of 135 placentas from spontaneously aborted women and 13 from women with induced abortion were underwent immunohistochemical test for T. gondii antigen. Out of the 135 women, 34 gave positive result. Accordingly, the study population were divided into three groups: aborted women positive for T. gondii, aborted women negative for T. gondii, and women with induced abortion. TNF-α and IL-6 intensity in placental sections were determined by IHC. Enzyme-linked immunosorbent assay (ELISA) was used to estimate serum level of progesterone and estradiol. Aborted women positive for T. gondii had significantly higher percentage of trophoblasts expressing TNF-α and IL-6 than both aborted women negative for T. gondii and women with induced abortion. Whereas, women with induced abortion demonstrated higher serum level of estradiol and progesterone than aborted women positive or negative for T. gondii.

Keywords: Toxoplasma gondii; abortion; trophoblast; TNF-α; IL-6; pregnancy associated hormones

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INTRODUCTION

Toxoplasma gondii, a typical coccidian parasite related to the phylum sporozoa, is a ubiquitous parasite that infects human [1]. Congenital toxoplasmosis is acquired via vertical transmission to the fetus by transplacental transfer from the mother. If such transmission occurs in early pregnancy, it is usually escorted by severe damage to the fetus or abortion [2]. It is obvious that trophoblast damage induced by *T. gondii* is central to the process of in utero transmission to the fetus [3]. However, the exact mechanism of this damage remains unclear.

The maternal immune system plays a critical role in the establishment and maintenance of pregnancy and successful birth [4]. This role is primarily accomplished through many hormonal changes as well as alteration in the cytokines concentrations both locally (within the uterus) and systematically. Generally, pregnancy favors the survival of many parasites that require Th1 (CMI) response to control them [5]. It appears that pregnancy supports the humoral immunity at the expense of CMI through the production of sex hormones by the uterus and placenta [6]. El-fadaly et al. [7] stated that toxoplasmosis was more prevalent in pregnant women in Egypt, and they attributed that to the sharp elevation in progesterone and estradiol levels in those women.

Many cytokines have beneficial roles in several normal physiologic processes in the placenta. The most important of these roles are trophoblastic invasion and placental proliferation and angiogenesis [8]. However, the deleterious effects of these cytokines cannot be excluded. As a rule of thumb is that Th1 cytokines including TNF-α are abortive factors [9], and Th2 cytokines (IL-4, IL-5, IL-6 and IL-10) are associated with normal pregnancy [10].

Tumor necrosis factor-α is a Th1 response cytokine produced by macrophage, T-lymphocyte, basophils and monocytes. In toxoplasmosis, TNF-α appears to be essential for macrophage activation and inhibition of parasite replication. Many studies have documented the role of this cytokine in the resistance to acute [11] and chronic [12] infection with *T. gondii*. However, during pregnancy, the dark side of this cytokine could be encountered which render some authors to state that whenever the level of TNF-α increase, abortion occurs [13,14]. In a recent study Er [15] found that azithromycine treatment significantly decreased the abortion rate in pregnant rats treated with lipopolysaccharide (a robust inducer of TNF-α). This effect was associated with decreased serum levels of TNF-α. IL-6 has important role during experimental toxoplasmosis. Mice deficient in IL-6 develop high cyst burdens and succumb to severe encephalitis associated with a failure to control parasite replication [16]. Again, the increased level of this cytokine during pregnancy is nocuous. Increased plasma levels of IL-6 were reported in sporadic miscarriage [17]. Furthermore, elevated levels of IL-6 in the placenta, amniotic cells, and deciduous have been demonstrated in pregnancies complicated by preterm premature rupture of the membranes, intrauterine infection, and prematurity [18]. This study aimed to investigate the overlapping roles of TNF-α, IL-6, estradiol and progesterone in *T. gondii*-induced aborton.

MATERIALS AND METHODS

Subjects:
A total of 135 women attending the Obstetrics and Gynecology Department at Al-Yarmouk Teaching Hospital/Baghdad and admitted for evacuation of spontaneous abortion were recruited for this study. Other 13 women who chose the termination of their pregnancy due to maternal cardiac diseases were considered as control group. The study was approved by the ethical committee of College of Medicine/ Al-Mustansiriya University, and a consent forms were obtained from each participant.

Samples

Blood samples

Five mL of venous blood were collected from each women and placed in plane tube where the serum was separated and stored at -20C until be used.

Placenta

The placentas of aborted women were collected from curettage and placed in a 10% formaldehyde solution. Two paraffin embedded blocks were prepared from each placenta.

Immunohistochemistry for the detedction of *Toxoplasma* Ag, TNF-α, and IL-6

Immunohistochemistry was carried out on 5µm thick sections obtained from paraffin-embedded blocks according to Casciaro et al. [19]. Hematoxyline and eosin staining was used to decide which block can be further used in the study (only sections that contain trophoplastic tissue were included in the study). For detection of *Toxoplasma* Ag, TNF-α, and IL-6 monoclonal rabbit antihuman Abs (USBiological/USA) in 1:25-1:50 dilution, mouse antihuman TNF-α Abs (USBiological/USA) in 1:20-1:40 dilution, and rabbit antihuman IL-6 Abs (Seritec/UK) respectively were used. The reaction was developed by the streptavidin-biotin technique using diaminobenzidine tetrahudrochloride as chromagen.

Evaluation of the Immunostaining

Evaluation of the immunostaining was carried out with the assistant of histopathologist. The observer was blind to the clinical diagnosis at the time of assessment, and tissue sections were independently assessed by two observers.
The expression of *Toxoplasma* Ags, TNF-α, and IL-6 was measured by scoring system. The extend of the IHC was determined in 10 microscopic fields at 400X magnification. The number of stained cells were counted.

Statistical Analysis

The Statistical Package for the Social sciences (SPSS, version 14.0) was used for statistical analysis. Independent samples t-test was used for two-group comparison in case of quantitative data, while Chi-square test was performed for the comparison of qualitative data. A p-value < 0.05was considered statistically significant.

RESULTS AND DISCUSSION

***Toxoplasma* Ag Scoring**

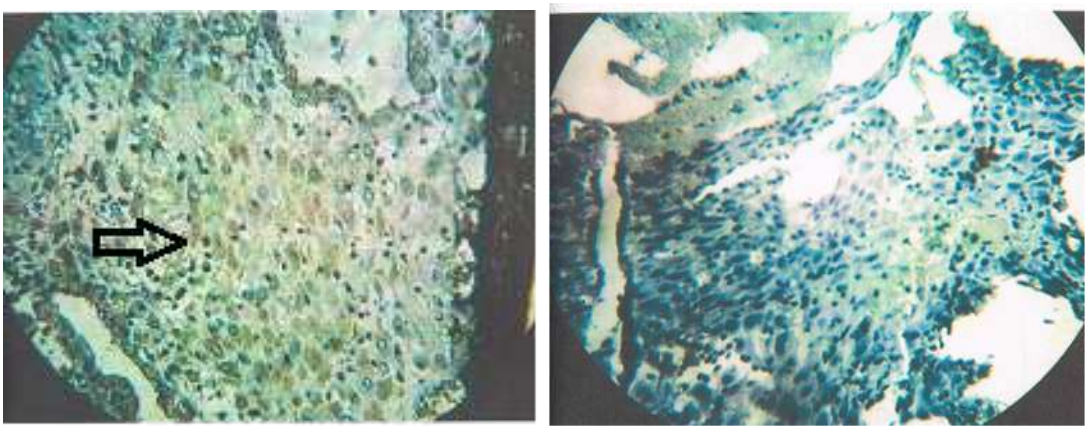
Out of 135 aborted women, 34 (25.2%) were found to be positive for *Toxoplasma* Ag in trophoblast (figure 1). Accordingly, the study population was divided into three groups:

Group A: Aborted women positive for *Toxoplasma* infection (34 women).

Group B: Aborted women negative for *Toxoplasma* infection (36 women) randomly chosen from the rest 101 aborted women.

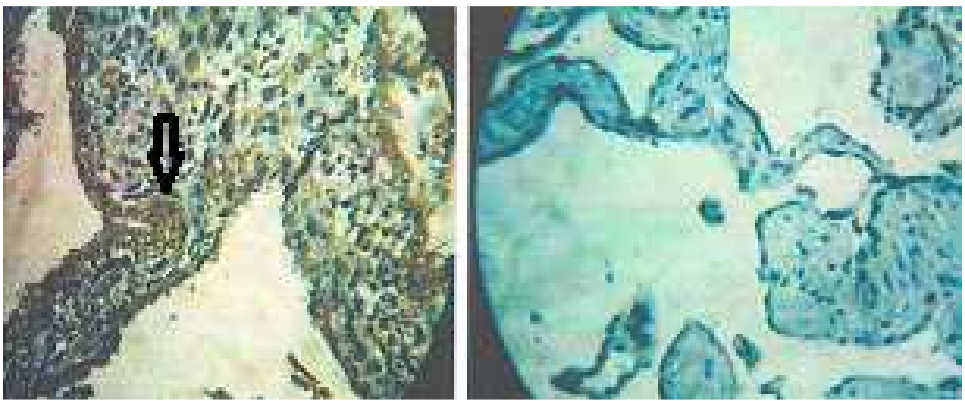
Group C: Women with induced abortion without *Toxoplasma* infection (normal pregnancy) (13 women).

The intensity of IHC expression was graded into three grades: grade 1 (≤ 25% of trophoblasts express IHC), grade 2 (26-75% of them), and grade 3 (≥ 76%). The results showed that 7 (20.6%) of the women in group A (which is the only group positive for *Toxoplasma* Ag) expressed grade 1, and 22 (64.7%) expressed grade 2, while 5 (14.7%) expressed grade 3.



(A) **(B)**

Figure 1: Immunohistochemistry staining of *T. gondii* Antigen in trophoblasts; A: infected cells (arrow), B: uninfected cells (40x)



(A) **(B)**

Figure 2: Immunohistochemistry staining of TNF-α in trophoblasts. A: TNF-α secretary cells (arrow), B: Non secretary cells

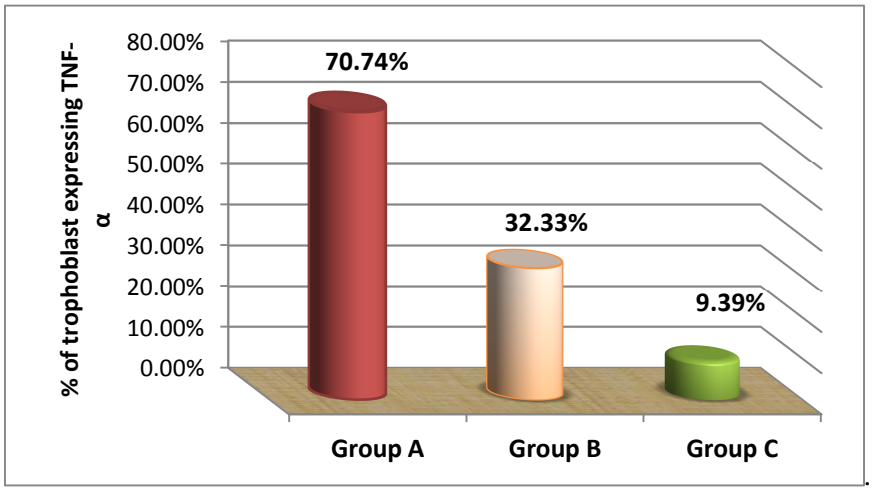


Figure 3: Mean percentage of trophoblasts expressing TNF-α in different groups

Table 1: Intensity of TNF-α expression scored by IHC in different groups

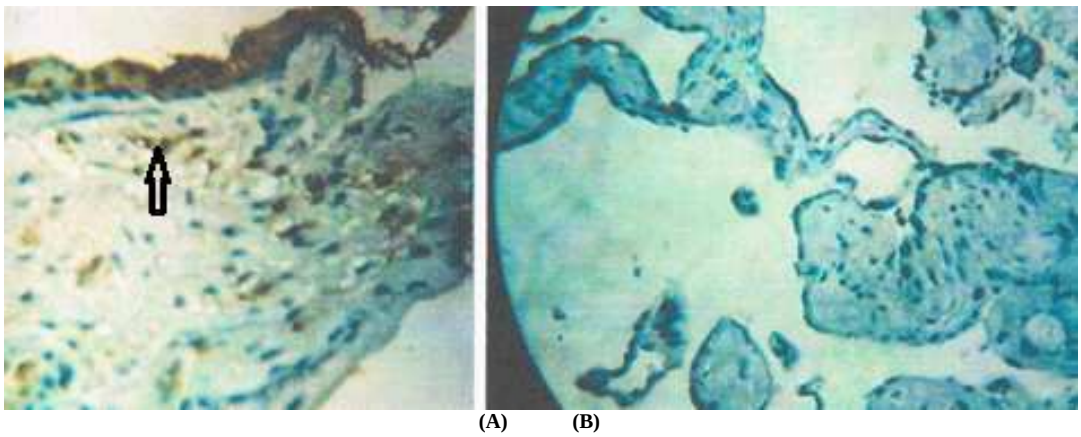
Groups	Grade 1		Grade 2		Grade 3	
	No	%	No	%	No	%
A: Aborted women infected with *T. gondii* (34)	1	2.9	14	41.2	19	55.9
B: Aborted women negative for *T. gondii* (36)	18	50	14	38.9	4	11.1
C: Women with induced abortion (15)	13	100	0	0	0	0

TNF-α Scoring

Mean percentages of TNF-α produced by trophoblasts as evaluated by IHC were 70.47±21.11, 32.33±19.56 and 9.39±4.15 in group A, B, and C respectively, with significant differences among the three groups (figure 2, 3). More than a half of women in group A (19 women, 55.9%) demonstrated an intensive level of TNF-α (garde 1), while 14 women (41.2%) had grade 2, and only 1 woman (2.9%) expressed grade 1. On the other hand, exactly 50% of women in group B, and all women in group C had low expression of TNF-α (table 1).

IL-6 Scoring

Group A showed higher IL-6 mean percentage (65±22.51) than either group B (29.67±20.41), or group C (11.15±4.65) with significant difference (figure 4,5). The intensity of IL-6 production from trophoblast of different groups is shown in table 2. Twenty-two (44.1%) of group A had grade 3 expression compared to 1 women (2.8%), and no woman in group C, with significant difference.



(A) (B)

Figure 4: Immunohistochemistry staining of IL-6 in trophoblasts. A: IL-6 secretary cells (arrow), B: Non secretary cells

Table 2: Intensity of IL-6 expression scored by IHC in different groups

Groups	Grade 1		Grade 2		Grade 3	
	No	%	No	%	No	%
A: Aborted women infected with *T. gondii* (34)	2	5.9	17	50	15	44.1
B: Aborted women negative for *T. gondii* (36)	22	61.1	13	36.1	1	2.8
C: Women with induced abortion (15)	13	100	0	0	0	0

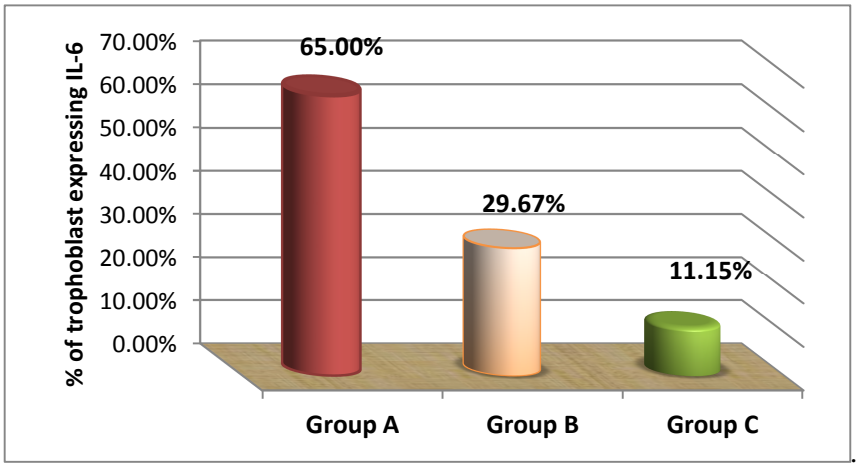


Figure 5: Mean percentage of trophoblasts expressing IL-6 in different groups

Pregnancy is a unique state which represents an extreme challenge for the immune system. For successful pregnancy, it is necessary for immune system of the pregnant female to shift away from inflammatory responses (Th1 responses that contribute in fetal rejection) toward anti-inflammatory reposes (Th2 responses that facilitate the passive transfer of antibodies to the developing fetus) [20]. In fact, there are many factors that induce this shifting, among which, that related to pregnancy hormones, is the elevated levels of prostaglandin during pregnancy. High levels of this hormone stimulate the synthesis of progesterone-induced binding factor (PIBF) by lymphocytes [21], which in turn promotes the differentiation of CD4+ T cells [22]. If, for any cause, this anti-infammatory bias is disrupted, this can result in preterm labor or abortion [23]. On the other hand, this shifting can enhance the pathogenesis of many intracellular pathogens, such as *T. gondii*, which required Th2 response to be overcome. This hypothesis was confirmed in clinical studies. Avelino et al. [24] studied prospectively the incidence of seroconversion for *T. gondii* among 3564 women of childbearing age in Brazil. Among them, 1114 were seronegative upon screening. After 6-12months, the authors reported 66% of women who seroconverted were pregnant. Thus, the reciprocal effect of pregnancy and immune system has two important consequences on the parasitic infection: first, pregnancy will favor the survival of many parasites that require a Th1 response to control them, and second is that the parasitic infection which induces Th1 response will adversely affect pregnancy. Both of these scenarios may explain the events that lead to the termination of pregnancy in the current study.

For TNF-α, there is almost agreement about its adverse role in pregnancy, and whenever the level of this cytokine increases, abortion occurs. TNF-α and other proinflammatory cytokines can stimulate uterine activity and cervical ripening by inducing the production of prostaglandin [25] and cortisol [26]. The current result in this regard is in consistent with many previous works [13,14]. However, this agreement does not fit the results of IL-6. Although some authors reported a sporadic miscarriage associated with increased serum level of IL-6 [18], the general rule is that Th2 cytokines including IL-6 favor the normal pregnancy. It seems that increased or decreased levels of this cytokine either in serum or in gestational tissues have adverse effects on pregnancy. One proposed mechanism is that excessive IL-6 potentially inhibits the generation of CD4+Tregulator cells which are required for pregnancy tolerance [27].

Serum Level of Progesterone

Maternal serum progesterone was higher in group C (36.59±21.64 ng/ml) than either group B (6.85±7.0 ng/ml) or group A (4.33±4.45 ng/ml) with significant difference (figure 6).

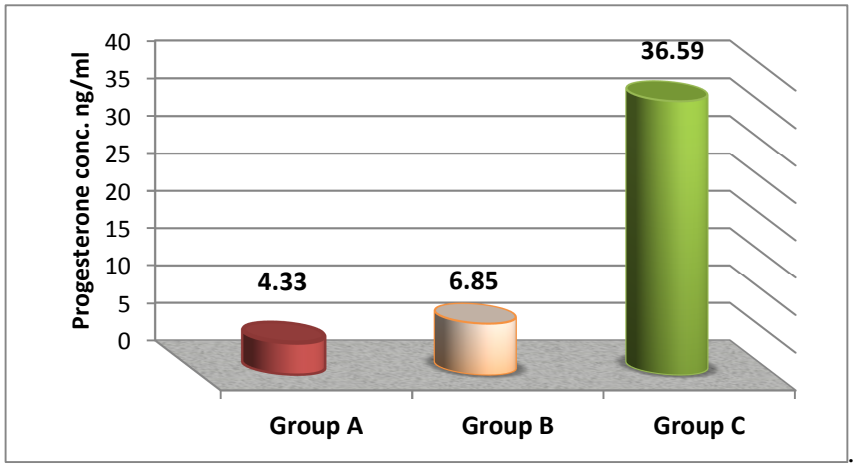


Figure 6: Mean serum levels of progesterone in different groups

Serum Level of Estradiol

Similarly, group C had non-significant higher serum level of estradiol (515.9±527.8 pg/ml) than either group B (341.48±773.42 pg/ml) or group A (107.21±99.33 pg/ml) (figure 7).

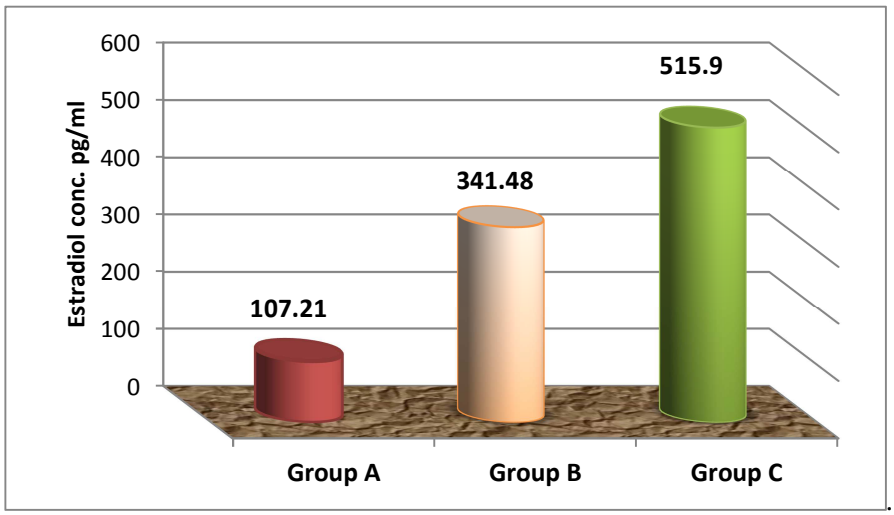


Figure 7: Mean serum levels of estradiol in different groups

Estradiol and progesterone effect several aspects of immune system. It was recorded that low doses of this hormone enhanced expression of TNF-α, IL-6 and IL-1 in monocytes, but high doses reduced the production of these cytokines [28]. Similarly, elevated concentration of progesterone during pregnancy inhibits the development of Th1 and promotes the production of Th2 [29]. Thus, it is reasonable to postulate that low concentration of estradiol and progesterone promote Th1 response while high concentration of the hormone promtes Th2 response [30]. This can clearly explain the low levels of serum estradiol and progesterone in aborted women positive for *Toxoplasma* infection compared to other groups. That implicates reverse effects of certain cytokines on the production of these hormones.

Inflammatory cytokines are thought to inhibit gonadotropin production at the level of hypothalamus and pituitary gland, and to inhibit progesterone synthesis by corpus luteum and promote luteal regression. TNF-α has previously been shown to inhibit luteal steroidogenesis in utero [31], and stimulate apoptosis of human primary villous trophoblast cells [32]. Elevated levels of IL-6 in the placenta, aminiotic cells and deciduas have been demonstrated

in pregnancies complicated by preterm premature rupture of membrane, intrauterine infection and prematurity [19,33].

CONCLUSION

From the results of the current study, it can be concluded that *T. gondii* causes pregnancy failure by at least two independent but superimposed mechanisms: systemic inhibition of estradiol and progesterone production, and local induction of inflammatory process within implantation site represented by high levels of TNF-α and IL-6.

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