Possible role of some aspects of immune response in aborted women infected with Toxoplasma gondii

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Abstract
The immune response that was induced by Toxoplasma gondii infection was found to be protective to mother, but it was deleterious to the fetus; i. e. the infection induced an immunopathological state in the fetus. The induction of high levels of TNF – α, IFN – γ, IL - 1α, IL – 6, IL – 8 and IL – 10 due to T. gondii infection may exert an inhibitory effect on serodiagnosis (inhibition the synthesis of progesterone and estradiol) which may explain their possible role in termination of pregnancy. The mechanisms of the other factors may be also induced after T. gondii infection.

Key words: Immune response, T. gondii, abortion, progesterone and estradiol.

Introduction
Toxoplasmosis is caused by intracellular parasite Toxoplasma gondii and may be contracted by consuming contaminated meat or by coming in contact with cat feces containing oocyst (Hökelek, 2005). If a pregnant women contract toxoplasmosis, it may be passed through the placenta to the fetus, resulting in congenital Toxoplasma infection stimulates humoral immune response as antibody production, which includes IgM and IgG, in addition to cell mediated immunity (Darcy and Santoro, 1994). The parasite causes a very strong type-1 response focused on IFN – γ, TNF – α and IL-10 secreted by T- lymphocyte (Filisetti and Candolfi, 2004). The female parent undergoes complicated change during pregnancy. It appears that pregnancy seems to strengthen the humoral immunity and weaken CMI (Hossein, 1995). Indeed, the immunomodulatory effects of hormones are most evident during gestation when they appear to be essential to successful pregnancy (Raghupathy, 1977). This control is largely achieved through the production of estrogen and progesterone, initially by the uterus and then by placenta. The ability of pregnancy to affect the immune system and indeed of the immune system to affect pregnancy has two important consequences for parasites infection. First, pregnancy will favor the survival of many parasites that require a type – 1 response to control them. Second, parasitic infections that induce a strong type – 1 response will be adversely affected. Both of them scenarios have been demonstrated with protozoan parasites T. gondii and Leishmania major (Robert et al., 2001). The current study was conducted to evaluate the role of sex – associated hormones (progesterone and estrogen) in pathogenesis and immunity to toxoplasmosis in aborted women was occurred by measuring the serum levels of progesterone and estrogen (estradiol) and the levels of...
some cytokines (TNF –α, IFN –γ , IL -1α, IL –6, IL –8 and IL –10) secreted by trophoblast cells of placenta by immunohistochemistry (IHC) technique.

**Materials and Methods**

Blood samples were collected during 2-3 hours after abortion from a total of 51 women aged from 15 – 52 years old attending the Al-Zahraa Maternity and Al-Hakeem hospital and the private laboratories in Najaf/ Iraq during the period from October 2010 to March 2011 who were admitted for evacuation of spontaneous abortion. In addition, ten women with selected termination of pregnancy, due to maternal cardiac disease were considered as healthy group for comparison (induced abortion). A questionnaire sheet was filled out for each woman in the study. The gestational age was calculated for each woman from the date of last menstrual period. Sera were obtained according to Stewart and Koepke (1987).

a) Detection of infections.

Infections were done by means of enzyme linked fluorescent assay for detection anti – *Toxoplasma* (IgM and IgG) in serum (VIDAS TOXO IgM and IgG, Biomerieux, France) as recommended by the manufacturer.

b) Determination of progesterone in human sera.

The radioimmunoassay for progesterone is a competition assay. Samples and calibrator are incubated with $^{125}$I – labeled progesterone as tracer, in antibody – coated tubes, after incubation, the content of tubes is aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are determined by interpolation sheet for Immunotech "Beckman Company" 2006 a, as recommended by the manufacturer of the Kit, Immunotech – France.

c) Determination of estradiol in human serum.

The same principle in determination of progesterone was applied according to the manufacturer (Beckman Company, 2006 b, France 2006 b).

d) Detection of anti – *Toxoplasma* Ags, IL -1α, IL – 6, IL –8 IL-10, IFN –γ and TNF –α proteins in paraffin embedded sections.

Collecting of placenta and staining

The placenta of aborted women were collected from curettage and placed in 10 % formaldehyde under consent senior and physician gynecologist. Two paraffin embedded blocks were prepared from each aborted women (51 spontaneously and 10 induced aborted women). Haematoxylin and eosin staining was carried out to detect the suitable block that will be introduce in the study (section containing the trophoblast tissues were chosen). The *Toxoplasma* antigens in infected trophoblasts were detected by IHC analysis after treating the trophoblast with polyclonal primary Abs. The ability of trophoblast cells to produce the cytokines TNF –α, IFN –γ , IL -1α, IL –6, IL – 8 and IL-10 were investigated by IHC analysis. These analyses were performed on group A: (15 aborted women infected with toxoplasmosis, and group C: 10 women with induced abortion). The primary antibody reacts with antigen in the tissues, and then biotin labeled secondary antibody (link antibody) binds to the primary antibody. When the conjugate is added, the biotinylated secondary antibody will form a complex with peroxidase – conjugated streptavidin and by adding the substrate, which contains 3,3’ – diaminobenzidine (DAB) in chromogenic solution, a brown – colored precipitate will form at the antigen site (Thomas, 2001).
Evaluation of the Immunostaining

Evaluation of the immunostaining was done with the assistance of a histopathologist. The positive immunostaining gave cytoplasmic brown granules. In the peroxidase secondary detection system, the presence of a brown reaction product at the site of the target antigen indicated positive reactivity. The expression of Toxoplasma antigens and cytokines protein was measured by the same scoring system, the extent of IHC determined in 10 fields. Entire tissue sections were examined. The numbers of stained cells were counted under high power microscope (40X); only cells with distinct intracellular staining were counted.

Scoring

The expression of Toxoplasma antigens, cytokines were measured by the same scoring system, by counting the number of positive trophoblastic cell that gave a brown cytoplasmic staining system under the light microscope. The extent of IHC single in the villi was determined in 10 fields (100X magnification). In each field the total number of villi was counted and the extent of cytoplasmic staining of the trophoblastic cells in a given villus was determined as a percent. The total staining score was divided by the number of whole villi per field in 10 fields(Hennessy et al., 1999), so the percentage of positivity stained villi in the 10 field was calculated for each case by taking the mean of percentage of the positivity stained villi in 10 fields (Khalil, 2008).

Statistical Analysis

The data were analyzed using the available software package. The results were presented as number, percentage, and mean ± whenever possible. The data were analyzed by using analysis of variance (ANOVA) test taking P < 0.05 as least limit significance. These manipulations were carried out according to statistical analysis system (SAS, 2001).

Results

Scoring system of Toxoplasma IHC

Trophoblasts within placental villi infected with T. gondii were evaluated by IHC technique in which, the infected villi were determined in 10 fields, the total numbers of villi were counted and the extent of cytoplasmic staining of the infected trophoblastic cells in a given villus was determined as percent. The extent of IHC signal positivity was significant in group A with mean percentage 50.15 ± 2.6 (tab. 1).

TNF-α scored by IHC

There was an increase of TNF-α mean percentage in the placenta of aborted women infected with T. gondii (72.56 ± 3.7) compared to that produced by placenta of both aborted women uninfected with T. gondii and induced aborted women, 32.43 ± 4.2 and 10.64 ± 1.8 respectively (P < 0.05) (tab. 2) and (Fig. 1).

IFN- scored by IHC

There was an increase of IFN- mean percentage in the placenta of aborted women infected with T. gondii (125.56 ± 2.4) compared to that produced by placenta of both aborted women uninfected with T. gondii and induced aborted women, 62.43 ± 1.4 and 20.64 ± 1.3 respectively (P < 0.05) (tab. 2) and (Fig. 1).

IL-1α scored by IHC

There was an increase of IL-1α mean percentage in the placenta of aborted women infected with T. gondii (63.43 ± 0.7) compared to that produced by placenta of both other aborted women, 30.26 ± 1.2 and 2.43 ± 0.9 respectively (P < 0.05) (tab. 2) and (Fig. 1).

IL-6 Scored by IHC

There was an increase of IL-6 mean percentage in the placenta of aborted women infected with T. gondii (64.35 ± 1.5) compared to that produced by placenta of both
aborted women uninfected with *T. gondii* and induced aborted women, $28.43 \pm 2.2$ and $10.38 \pm 3.8$ respectively ($P < 0.05$) (tab. 2) and (Fig. 1).

**IL – 8 Scored by IHC**

There was an increase of IL-8 mean percentage in the placenta of aborted women infected with *T. gondii* ($163.97 \pm 5.9$) compared to that produced by placenta of both aborted women uninfected with *T. gondii* and induced aborted women, $79.28 \pm 2.5$ and $10.87 \pm 1.35$ respectively ($P < 0.05$) (tab. 2) and (Fig. 1).

**IL-10 scored by IHC**

There was an increase of IL-10 mean percentage in the placenta of aborted women infected with *T. gondii* ($213.25 \pm 4.2$) compared to that produced by placenta of both aborted women uninfected with *T. gondii* and induced aborted women, $6.43 \pm 2.2$ and $4.38 \pm 1.6$ respectively ($P < 0.05$)(tab. 2) and (Fig. 1).

**Table 1:** Mean percentage ± of infected placental tissue obtained from aborted women by IHC technique.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>*A</td>
<td>15</td>
<td>50.15 ± 2.6</td>
</tr>
<tr>
<td>*B</td>
<td>14</td>
<td>0.0</td>
</tr>
<tr>
<td>*C</td>
<td>10</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*A: Group of aborted women infected with *T. gondii.*
*B: Group of aborted women uninfected with *T. gondii.*
*C: Group of induced abortion (normal pregnancy).*
Fig. 1: Immunohistochemistry staining in trophoblastic tissue from women with abortion, by DAB chromogen. Counter stained with mayer's hematoxylin.

Magnification power (400 X)

a. IHC of TNF – α
b. IHC of IL – 6
c. IHC of IL – 8

d. IHC of IL – 1 α
e. IHC of IL – 10
f. IHC of IFN - γ
Table 2: Mean cytokines percentage ± of placental tissue in aborted women by IHC technique.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>TNF - α</th>
<th>IFN-</th>
<th>IL - 1α</th>
<th>IL - 6</th>
<th>IL - 8</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>*A</td>
<td>15</td>
<td>72.56 ± 3.7</td>
<td>125.56 ± 2.4</td>
<td>63.43 ± 0.7</td>
<td>64.35 ± 1.5</td>
<td>163.97 ± 5.9</td>
<td>213.25 ± 4.2</td>
</tr>
<tr>
<td>*B</td>
<td>14</td>
<td>32.43 ± 4.2</td>
<td>62.43 ± 1.4</td>
<td>30.26 ± 1.2</td>
<td>28.43 ± 2.2</td>
<td>79.28 ± 2.5</td>
<td>6.43 ± 2.2</td>
</tr>
<tr>
<td>*C</td>
<td>10</td>
<td>10.64 ± 1.8</td>
<td>20.64 ± 1.3</td>
<td>2.43 ± 0.9</td>
<td>10.38 ± 3.8</td>
<td>10.87 ± 1.35</td>
<td>4.38 ± 1.6</td>
</tr>
</tbody>
</table>

*A: Group of aborted women infected with *T. gondii*.  
*B: Group of aborted women uninfected with *T. gondii*.  
*C: Group of induced abortion (normal pregnancy).

Table 3: Mean maternal serum level ± SE of progesterone (ng/ ml) and estradiol (pg/ ml) of aborted women by RIA technique.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Progesterone</th>
<th>Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>*A</td>
<td>15</td>
<td>5.22 ± 3.2</td>
<td>108.42 ± 3.2</td>
</tr>
<tr>
<td>*B</td>
<td>14</td>
<td>7.30 ± 2.8</td>
<td>342.50 ± 1.6</td>
</tr>
<tr>
<td>*C</td>
<td>9.0</td>
<td>38.20 ± 4.5</td>
<td>520.82 ± 8.9</td>
</tr>
<tr>
<td>*D</td>
<td>10</td>
<td>2.80 ± 1.8</td>
<td>62.00 ± 1.8</td>
</tr>
</tbody>
</table>

*A: Group of aborted women infected with *T. gondii*.  
*B: Group of aborted women uninfected with *T. gondii*.  
*C: Group of induced abortion (normal pregnancy).  
*D: Group of non-pregnancy healthy women.

**Serum progesterone concentration.**
Maternal serum progesterone was significantly higher in induced aborted women (normal pregnant 38.20 ± 4.5 ng/ml) compared to that in all other groups (P > 0.05) (tab. 3).

**Serum estradiol concentration.**
Serum concentration of estradiol was found to be significantly high in serum of induced aborted women (normal pregnant) (520.82 ± 8.9) compared to that in all other groups (P > 0.05) (tab.3).

**Discussion**
The cytokines TNF-, IFN-and IL-10 play an important role in abortion, as their administration increase the abortion rate and specific antagonists decrease the abortion rate (Chaouat *et al.*, 1990). TNF – α stimulates the programmed death of human
primary villous trophoblastic cells and IFN-γ augments TNF-α-mediated killing of trophoblasts (Yui, 1994; Kilani, 2007). Both these cytokines inhibit the outgrowth of human trophoblastic cells in vitro (Berkowitz et al., 1988). It has been proposed that macrophage-derived TNF-α stimulates NK cells to produce IFN-γ, which further activates the macrophage, as occurs in the early defense response to infection agents. The other potential source of IL-10 and IFN-γ and systemic Th1-type responses may cause abortions via augmenting level of such cytokines. The cytokines (like TNF-α and IFN-γ) are abortogenic via up regulation of fg 12 prothrombinase activity (Clark et al., 1998). It is widely accepted that term as well as preterm labor is associated with elevated uterine production of proinflammatory cytokines, IL-1β, IL-6, IL-8 and TNF-α (Steinborn et al., 1998). These cytokines are thought to stimulate uterine activity, either directly or via an increase in prostaglandin production, attraction of leukocytes, and tissue remodeling. Reducing the inflammatory infiltrate or inhibiting cytokines production in these cells might be effective in the treatment of preterm labor (Young et al., 2002). In the same pathway, these cytokines may cause abortion. The administration of exogenous prostaglandins (intravenously, intra-amniotically, or vaginally) induces abortion in all species and at any stage of gestation. These data suggest that pregnancy is maintained by a mechanism that tonically suppresses uterine prostaglandin synthesis throughout gestation. Moreover, a defect in this inhibitory mechanism may be associated with early pregnancy loss (Jaschevatzky et al., 1983). These cytokines have been show to propagate the local inflammatory cascade and to stimulate increase prostaglandin production. Elevated levels of IL-6 in the placenta, amniotic cells and deciduas have been demonstrated in pregnancies complicated by preterm premature rupture of the membranes, intrauterine infection, and prematurity (Fukuda et al., 2002; Unfried et al., 2003). The possibility that immune activation might cause pregnancy failure by inhibiting the reproductive endocrine system has been largely unexplored, despite indications that immune process regulates reproductive endocrine function in non-pregnant mammals. Inflammatory cytokines are thought to be inhibiting gonadotropin production at the level of the hypothalamus and pituitary in cases of chronic or acute illness and to inhibit progesterone synthesis by the corpus luteum of the ovary and promote luteal regression as part of the normal ovulatory cycle. Cytokines have previously been shown to inhibit luteal steroidogenesis in vitro (Davis and Rueda, 2002). Thus it is possible that some agents cause pregnancy failure by two independent but superimposed mechanisms: systemic inhibition of ovarian function and local induction of inflammatory process within implantation sites (Bornstein, et al., Erlebacher et al., 2004).
Case number: Date:  
Patient name: Age:  
Blood group: Address:  
Consanguinity: Occupation:  
Educational level: 

Date of LMP: Gestational age: 
Number of previous pregnancies: 
Number of previous abortions: 
No. of parity:  
Summary of obstetrical history (Abortions in details):  
G1: G2: G3: 
Repeated abortion consecutive or not

Congenital anomalies:  
1. In patient:  
2. In family:  
3. In fetus:  

Previous diagnosed for toxoplasmosis: 
Contact with animals: 
Preexisting medical diseases: 

Uterine abnormalities: 

Family history of genetic diseases: 

Appendix I: Questionnaire sheet for each patient included in a study.  

References  
Fukuda, H., Masuzaki, H., Ishimaru, T. (2002). Interleukin – 6 and interleukin – 1 receptor antagonist in amniotic fluid and cord blood in patients with preterm,


