

Immune Profile in Aborted Iraqi Women with Toxoplasmosis

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Abstract

Background: Toxoplasmosis is one of the most important causes for abortion in women. The immune responses have a role in the outcome of such infection in gestated women. **Aim:** The current study was designed to investigate the immune profile in aborted Iraqi women with toxoplasmosis. **Materials and Methods:** Fifty-five aborted women and 29 healthy control women were enrolled in this study. Enzyme-linked immunosorbent assays were used to estimate serum levels to each of interleukin-1 alpha (IL-1 α), granulocyte-monocyte colony-stimulating factor, IL-8, IL-4 IL-10, IL-12, interferon gamma (INF- γ), and IL-6. Single-radial-immunodiffusion assay was used to estimate serum levels of C3, C4, and total immunoglobulin gamma. **Results:** Serum levels of IL-8 showed significant elevation, while IL-6 and INF- γ showed significant dropping in infected women compared to control. Other immune factors showed nonsignificant differences between the two groups of the present study. **Conclusion:** Disturbance of immune response associated with toxoplasmosis may explain the success of parasite in escaping from discrimination and elimination by the immune system then supporting its survival and replication.

Keywords: Abortion, adaptive immune response, innate immune response, single radial immune diffusion, toxoplasmosis

INTRODUCTION

Toxoplasmosis, an infectious disease caused by *Toxoplasma gondii*, is one of the most prevalent causes of abortion and congenital aberrations in infected women.^[1] The global annual incidence of congenital toxoplasmosis was estimated to be 190,100 cases.^[2] This was equivalent to a burden of 1.20 million Disability Adjusted Life Years (DALYs). Indeed, *T. gondii* can be transmitted either horizontally, by ingestion of tissue cysts or oocysts in contaminated food or water, or vertically, from mother to fetus. Primary infection of the immunologically naive mother might result in abortion, hydrocephalus, as well as neurological and ocular disease of the newborn. In general, toxoplasmosis is asymptomatic in the immune competent individuals but may develop serious disease under immunocompromised condition.^[3]

Immunity against *T. gondii* results from a complex cell-mediated immune response involving inflammatory cell, lymphocyte, macrophages, and cytokines.^[4] Granulocyte-monocytes colony-stimulating factor (GM-CSF) is a pro-inflammatory hematopoietic growth factor produced by many cell types, including T-cells. GM-CSF plays an important role in many cells such as dendritic cell activation, granulocyte survival,

and enhancement of macrophage and microglial function.^[5] Studies using murine model with toxoplasmosis reported a detrimental role for GM-CSF by increasing parasitic burden in the peritoneal macrophage. However, regulation of GM-CSF expression, particularly by T-cells, is not well understood and is a critical gap in our knowledge of GM-CSF biology.^[6,7]

Interleukin (IL)-8 is a chemokine produced by macrophages and other cell types such as epithelial cells. Indeed, IL-8 induces chemotaxis of neutrophils, hence other granulocytes to migrate toward the site of infection.^[8]

Complement 3 and 4 (C3, C4) components are important proteins in the complement system which play a role in phagocytosis process by C3b and Fc receptor on macrophages leading to increasing macrophages activation.^[9]

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Adaptive and innate immunity play decisive role in control of toxoplasmosis. Elimination of such parasite requires production of immune modulators including interferon gamma (IFN- γ) that activates various cell-intrinsic antiparasitic defense pathways within infected cells. Production of IFN- γ in *T. gondii* infection is dependent on secretion of IL-12. Several cells have been proposed to be important sources of IL-12 during *T. gondii* infection, including neutrophils, macrophages, plasmacytoid dendritic cell, and the subset of conventional dendritic cells expressing CD8 α .^[10] Both innate immune cells such as NKs and adaptive immune cells such as Th1 as well as CD8 T-cells are responsible for INF- γ augmentation then supporting elimination of such infection. In the absence of the IL-12 p40 gene or the IL-12-receptor-associated signal transducers Tyk2 and transcription factor STAT-4, IFN- γ production is severely impaired resulting in susceptibility to acute *Toxoplasma* infection.^[11]

Pathogenesis of toxoplasmosis may affect by Th2 cytokines (IL-4, IL-5, IL-6, IL-10, IL-13, and IL-14), which play a major role in the pathogenesis of parasitic diseases. In fact, IL-5 is the major cytokine responsible for the increase in the eosinophil population in parasitic infection, while IL-6 enhances antibodies production and exerts a pro-inflammatory effect by stimulating the generation of acute phase protein, and IL-10 and IL-12 control the type of the immune response.^[12] IL-10 inhibits cytokine synthesis, and by blocking the production of IL-6 and TNF- α , it causes an advantage of the response occurring with Th2 involvement and B-cell activation. However, IL-12 facilitates formation of a Th1 type responses.^[13]

The immunoglobulin gamma (IgG) in toxoplasmosis have diagnostic value since the parasite is intracellular.^[14] Because there is no immunological study to full immune profile in Iraqi aborted women with toxoplasmosis, this study was designed to do so.

MATERIALS AND METHODS

The current study included 55 women with recurrent abortion proved with toxoplasmosis (mean of ages is 27.43, ranged from 18 to 42 years) at Consulting Clinic of Al-Emamain Al-Khadhumain Teaching hospital, Baghdad, over the period from September to April 2014. Samples of control group were collected from 29 family-unrelated apparently healthy women (ages mean is 28 ranged from 17 to 44 years). From each participant, venous blood (5 ml) was collected in plan tubes. Separated serum were divided into many aliquots in Eppendorf tubes and kept at -20°C till the tests of immune factors were performed.

Enzyme-linked immunosorbent assays (ELISA) were used to evaluate serum levels to each of IL-1 α , GM-CSF, IL-8 (Immunotech, France), IL-4 (eBioscience, England), IL-10, IL-12, and IL-6 (Biosource, Belgium). Single-radial-immunodiffusion assays were used to evaluate serum levels of C3, C4, and IgG (Binding site, England). The current study was approved by Research Ethical Committee

of College of Medicine/AL-Nahrain University. All the participants were provided with written informed consent to enroll in this study.

Statistical analysis

Raw data were analyzed using software of SPSS program (version 20). Student's *t*-test was used to compare means of immune factors between cases and control. Levels of measured factors expressed as (Mean $\pm$ Standard error). Significance in all tests was set at 0.05 ($P \leq 0.05$).

RESULTS

Statistical analysis of the obtained results showed important output for immune response accompanying to toxoplasmosis. Regarding innate immunity cytokines, serum levels of GM-CSF showed nonsignificant dropping (21.16 ± 1.02 pg/ml, $P = 0.07$) compared to control group (36.55 ± 3.06 pg/ml). Moreover, IL-1 α showed nonsignificant elevation (42.16 ± 15.30 pg/ml, $P = 0.08$) compared to control group (16.00 ± 10.37 pg/ml). On the other hand, levels of IL-8 showed significant elevation (2102.10 ± 287.72 pg/ml, $P = 0.005$) in infected women compared to control (222.50 ± 81.01 pg/ml) group [Table 1].

Regarding adaptive cellular immunity cytokines, levels of IL-12 showed nonsignificant elevation (33.38 ± 6.50 pg/ml, $P = 0.83$) compared to control group (27.90 ± 9.79 pg/ml). In contrast, IFN- γ showed significant dropping (562.33 ± 51.19 pg/ml, $P = 0.02$) compared to control group (671.80 ± 58.47 pg/ml). For adaptive humoral immunity cytokines, serum levels of IL-4 and IL-10 showed nonsignificant elevation (25.76 ± 1.52 pg/ml, $P = 0.57$) and dropping (9.55 ± 4.13 pg/ml, $P = 0.31$), respectively, compared to control groups (20.30 ± 2.01 pg/ml) and (10.10 ± 2.06 pg/ml) for levels of IL-4 and IL-10, respectively. On the other hand, levels of IL-6 showed significant dropping (101.40 ± 21.21 pg/ml, $P = 0.01$) compared to control group (165.40 ± 6.02 pg/ml) [Table 1].

Total levels of IgG showed nonsignificant decrease (1203.66 ± 104.01 pg/ml, $P = 0.08$) compared to control group (1684.0 ± 193.3 pg/ml). In relation to complements, both of

Table 1: Cytokines levels in infected women compared to control group

Immune factor	Mean $\pm$ SE (pg/ml)		P
	Control	Toxo	
GM-CSF	36.55 $\pm$ 3.06	21.16 $\pm$ 1.02	0.07
IL-1 α	16.00 $\pm$ 10.37	42.16 $\pm$ 15.30	0.08
IL-8	222.50 $\pm$ 81.01	2102.10 $\pm$ 287.72	0.005
IL-12	27.90 $\pm$ 9.79	33.38 $\pm$ 6.50	0.83
INF- γ	671.80 $\pm$ 58.47	562.33 $\pm$ 51.19	0.02
IL-6	165.40 $\pm$ 6.02	101.40 $\pm$ 21.21	0.01
IL-4	20.30 $\pm$ 2.01	25.76 $\pm$ 1.52	0.57
IL-10	10.10 $\pm$ 2.06	9.55 $\pm$ 4.13	0.31

GM-CSF: Granulocyte-macrophage colony-stimulating factor, INF- γ : Interferon gamma, IL: Interleukin, SE: Standard error

Table 2: Immunoglobulin gamma and complement 3, complement 4 complement levels in infected women compared to control group

Immune factor	Mean±SE (mg/dl)		P
	Control	Toxo	
IgG	1684.0±193.3	1203.66±104.01	0.80
C3	1464.3±176.84	1471.83±100.41	0.84
C4	25.69±3.90	25.23±1.86	0.17

IgG: Immunoglobulin gamma, C3: Complement 3, C4: Complement 4, SE: Standard error

C3 and C4 levels showed nonsignificant increase in infected women compared to control groups [Table 2].

DISCUSSION

The present study revealed an important output in immunological profile among aborted Iraqi women with toxoplasmosis. Regarding innate immunity, nonsignificant dropping of GM-CSF which have a role in the beginning of infection by activation and chemotaxis of neutrophils as well as activation of macrophages, elevation of GM-CSF levels is associated with prolonged inflammatory process and increased expression of adhesion molecules.^[15] Thus, low levels of GM-CSF level in aborted women may be due to resolving of inflammatory process after abortion and changes in the endometrium of the uterus. These results are in accordance with that of Moldenhauer *et al.* who reported a fluctuation in GM-CSF level with ovarian cycle and during ovulation in addition to preparation endothelial cell lining reproductive tract in female to tolerate pregnancy.^[16] Other study by Soren *et al.* reported that levels of GM-CSF were increased in women with previous miscarriage.^[17] These disparities between these results may be due to type as well as timing of sampling. In the current study, the samples were clinical while samples of other studies were based on a tissue culture or murine model.

Elevation of IL-8 levels in the current study revealed increasing of inflammatory process in aborted women and attraction of lymphocyte and neutrophil to endometrium. This result agrees with Zicari *et al.* who reported that inflammatory cytokines such as IL-8 might plays a vital role in the mechanism of protease-induced neurogenic inflammation leading to labor or abortions by enrolling neutrophils and lymphocytes in the endometrium.^[18] Furthermore, Madhappan *et al.* proposed that IL-8 levels in fetal tissue samples from cases of miscarriage were elevated compared to those from an elective abortion group.^[19] On the other hand, Koumantaki *et al.* mentioned that women with spontaneous abortions had pointedly decreased plasma level of IL-8 compared to those with normal pregnancies.^[20] Moreover, Soriano *et al.* reported that no difference in serum levels between prospectively enrolled women with normal pregnancies and those with miscarriage.^[21] Results variations among these studies may attribute to size of collected data or genetic and ethnic background of the study population.

The nonsignificant elevation of IL-1 α levels in the current study may indicate an increase in its secretion by macrophages, neutrophil, epithelial cell, and endothelial cells. Thus, there is a recruitment of inflammatory cell to site of inflammations. Hence, levels of IL-1 α were elevated during course of abortion, but these levels were dropped shortly after abortion as reported by Hunter who explain that elevated levels of IL-1 α may be considered as starting point for inflammation and consequently abortion in pregnant women with active toxoplasmosis infection.^[22] Dimitriadis *et al.* reported that IL-1 has a role in an early implantation and reproduction.^[23] On the other hand, Mohit *et al.* explained that IL-1 α constitutively synthesis by epithelial cells, which means that this cytokine is found in a significant level in both infected and healthy subjects.^[24]

Regarding adaptive immunity cytokines, IL-12 is a master regulator of immune response, especially against intracellular pathogen including *T. gondii* by stimulation of naïve T-cells to differentiate to Th1 cells then stimulating the production of IFN- γ by such cells as well as NK cells.^[25] The current study showed nonsignificant differences in IL-12 levels between cases and controls which may suggest poor recognition of immune system to toxoplasma with an eventual enhancement of infection.

Significant dropping of INF- γ levels in the current study may indicate an escaping mechanism of such intracellular parasite to deviate from cell-mediated immunity which is augmented by INF- γ . In fact, normal pregnancy is associated with an enhancement of Th2 immune response and with suppression of Th1 immune responses to maintain fetus viability, while abortion associated with shifting toward Th1 responses which lead to loss of fetus due to increase in IL-12 levels leading to stimulation of NK cells to produce of INF- γ then TNF- α by macrophages and T-lymphocyte as mentioned by Marie-Pierre *et al.* who also reported that Th2 cytokines inhibit Th1 responses. Thus, it promotes allograft tolerance and therefore may improve pregnancy success.^[26] Hence, the recognition of *T. gondii* danger signals stimulates a cascade of innate cellular and humoral responses. Robust NK cell activation, dendritic cell maturation, macrophage activation, and production of IFN- γ , IL-12, TNF- α , and iNOS which together limit parasite tachyzoite replication. Consequently, these nonsignificant differences in IL-12 and dropping in INF- γ may be due to timing of blood sampling from aborted women, samples collected after different period from abortion at minimal after 4 weeks. Kaňková *et al.* showed that IL-12 levels have increased while IL-10 levels have decreased in murine model infected with *T. gondii*.^[27] Variations in research findings may be due to genetic variations as well as variations in the immune system's robustness and ability to overcome such infectious agents.

Regarding Th2 cytokines, IL-4 stimulates naïve T-lymphocyte to differentiate into Th2 then skewing of immune response toward humoral immunity. In the current study, IL-4 levels showed nonsignificant elevation, while IL-10 levels showed

almost similar results in cases and control. A slight decrease in IL-4 and slight elevation of IL-10 levels were normal outcome after fetal loss as they are no longer required for maintaining the fetus's life. That is because IL-4 and IL-10 have a role in maintaining fetus during pregnancy through suppression of Th1 cytokines production by such cells. Wilson *et al.* reported that IL-10 has anti-inflammatory properties by inhibition INF- γ production and persistence of *T. gondii* in tachyzoite stage and increase IL-4 to stimulate B-cell class switching to produce IgE antibody, the main antibody against parasitic infection. However, the explanation for nonsignificant differences in IL-4 and IL-10 levels after abortion may be due to parasite camouflage from immune system as one of escaping mechanisms from the immune system.^[28] Butcher *et al.* mentioned that M ϕ infection by *T. gondii* induces rapid and sustained STAT3 phosphorylation, independently of host IL-10. Another study by reported that STAT3 is crucial for the effective tachyzoite-mediated suppression of endotoxin-induced IL-12 and TNF- α responses. These results defined a molecular mechanism underlying the parasite's ability to sabotage M ϕ pro-inflammatory cytokine production.^[29]

IL-6 is an important cytokine produced by a variety of cells such as macrophage, endothelial cells, and Th2 cells. This cytokine is responsible for the production of acute phase proteins, increase cytotoxicity of NK cells and cytotoxic T-lymphocytes, and it enhances differentiation of B-cells to plasma cells and increases antibody production. In the current study, IL-6 levels showed significant decrease in case than controls. This result may occur after abortion in women with toxoplasmosis. Shifting toward Th1 immune response in aborted women leads to dropping of IL-6 levels which elevated during pregnancy. Makhseed *et al.* reported that after abortion the immune system will begin to shift toward Th1 immune response after Th2 immune response during pregnancy.^[30] On the other hand, Mousa and Bakhiet mentioned that IL-6 levels have increased during the course of infection by *T. gondii* in aborted women.^[31] Differences in results among these studies may be attributed to timing of samples collection after abortion and to immune competent of volunteers subjects as well as to size of data included in the study.

Nonsignificant differences in total IgG levels in the present study is comparable with Eskandarian *et al.* who mentioned that there is nonsignificant difference between infected and noninfected participants with toxoplasmosis.^[32] This result may reflect the high exposure to this parasite in community. Uttah *et al.* reported different modes of infection with *T. gondii* as contaminated food, water, undercooked meat, and raw meat. Consequently, there is highly exposure level in community for this parasite.^[33]

Levels of C3 and C4 complement components in the current study showed no significant difference between cases and controls. These results agree with Ad'hiah *et al.* did not found differences in C3 and C4 levels between aborted and

healthy participants.^[34] However, our findings disagree with Fadhil *et al.* who reported significant difference in level of C3 and C4 complement between aborted women positive for *T. gondii* and healthy volunteers.^[35] This means that complements components function may not be affected by toxoplasmosis infection.

CONCLUSION

Significant and nonsignificant differences in levels of studied factors were found associated with toxoplasmosis may play an important role in modifying the innate and adaptive immune response as well as a role in modulating both of cellular and humoral immune components. Disturbance of immune response associated with toxoplasmosis may explain the success of parasite in escaping from discrimination and elimination by the immune system and supporting its survival and replication.

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Conflicts of interest

There are no conflicts of interest.

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