

# The Significance of CXCL10 and CXCL16 in Serum and Placenta of Toxoplasmosis - Induced Abortion

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**Abstract:** The level of CXCL10 and CXCL16 was determined in sera and placenta homogenate of toxoplasmosis-induced abortion (25 cases), in addition to 25 cases of accidental abortion. Twenty-five healthy control women were also included for comparison of serum level. CXCL10 level was significantly increased in serum ( $39.99 \pm 1.96$  pg/ml) and placenta homogenate ( $36.35 \pm 1.27$  pg/ml) of toxoplasmosis-induced abortion patients, as well as accidental abortion patients ( $42.27 \pm 0.98$  and  $35.54 \pm 1.40$  pg/ml, respectively) compared to control serum level ( $29.66 \pm 2.03$  pg/ml), while the increases can be more pronounced in serum. CXCL16 showed a similar pattern of increased level. These results suggest that systemic production of CXCL10, CXCL16 play a significant role in inducing of abortion in toxoplasmosis patients.

**Keywords:** Toxoplasmosis, Abortion, Chemokines

## 1. Introduction

Infection with the parasite *Toxoplasma gondii* typically results in mild or asymptomatic disease in adults, but can become severe in pregnant females (1). Congenital transmission is documented in humans and rodent models (2), infection during early pregnancy, however, can result in excessive IFN- $\gamma$  production, apoptosis of placental cells, and fetal desorption (3).

A number of chemokines and their receptors have been identified at the maternal-fetal interface recently. The plasma levels of CXCL8 (IL-8), CCL2 (MCP-1), CXCL10 (IP-10), and CXCL12 (SDF-1) were elevated in severe preeclamptic women compared with those in normal pregnant women. Chemokines may promote or suppress first-trimester trophoblast proliferation, migration and invasion, such CXCL12, CXCL14, CXCL16, CX3CL1, CCL14 and CCL4 (4; 5).

The action of cells for innate immune system are mediated by chemokines, which are essentially involved in regulating cell trafficking, and represented by a group of cytokines that have the ability to induce chemotaxis (6).

CXCL-10 (also known as IFN- $\gamma$ -induced protein IP-10), has been shown to be a novel biomarker for severity of *T. gondii* (7). It appears that most of the parasitic diseases of the CNS involve significant alterations of CXCL10 expression in peripheral blood of infected host (8).

CXCL-16 is expressed by trophoblastic cells in the first trimester of the pregnancy and induced their invasion and proliferation, it suggest that CXCL-16 is play roles in placentation (9).

Hence, this study aimed to investigate the level of CXCL-10 and CXCL-16 proteins in serum and extraction of placental tissue in patient (aborted women) complaining spontaneous miscarriage, and were *T. gondii* positive.

## 2. Materials and Methods

A total number of 50 aborted pregnant women (16-42 years old) in karbala province, Iraq were included in this study; they were admitted to Hospital Teaching of Karbala for spontaneous miscarriages for evacuation, According to the results of the latex Agglutination, Dip Stick and ELISA for the detection of anti *T.gondii* IgG and IgM antibodies analysis for the detection of *T.gondii* in sera and placental extract, the patients were divided into groups:

1- 25 aborted women positive for *T.gondii*.

2- 25 aborted women negative for *T.gondii*,

and included serum only from 25 women as control, those who were revised to the Gynecology Theater in the hospitals.

**Blood Collection:** Five ml of venous blood were collected and sera were then aspirated using a Pasteur pipette and dispensed into sterile eppendorf tubes (100  $\mu$ l in each) and stored at -20  $^{\circ}$ C until used. Enzyme Linked Immunosorbent Assay (ELISA) for the detection of IgM and IgG antibodies for *T.gondii* in serum. Materials that were provided with the kit: (ACON, USA).

**Placenta extract:** Added to 2 ml of Diluent buffer solution to 2g of pieces of the placenta for the purpose of obtaining the extract and stored 100  $\mu$ l in each eppendorf tube at -20  $^{\circ}$ C until used. The CXCL-10 and CXCL-16 level in serum and placenta extract in each group was carried out following the instructions in the kit's leaflet of PeproTech, USA Mini ELISA.

The sample results were calculated by interpolation from a standard curve that was performed in the same assay as that for the samples by using standard curve fitting equations for CXCL-10 and CXCL-16.

### 3. Statistical Analysis

The level of cytokines was analyzed using the computer programme SPSS (Statistical Package for Social Sciences) version 13. Their data were given as mean  $\pm$  standard error (S.E.), and differences between means were assessed by ANOVA (Analysis of Variance).

### 4. Results

Table (1) shows that there was no statistical difference ( $P > 0.05$ ) in the mean level of CXCL10 serum and placental extract between aborted groups with positive and negative Toxoplasmosis. While there was a significant difference ( $p < 0.05$ ) in the mean percent of CXCL-10 between groups and with control.

Table 2 shows a significant difference in the serum level of CXCL16 between aborted groups. And there was no statistical difference ( $P > 0.05$ ) in the mean level in placenta extract.

**Table 1: Mean level of CXCL10 in study groups**

Groups		No.	Pgml( CXCL10)				Denken Test *
			Mean	SD. E.	Lower Value	Upper Value	
Abortion with <i>T. gondii</i>	Serum	25	39.99	1.96	16.07	52.66	AB
	Placenta	25	36.35	1.27	22.95	46.78	B
Abortion without <i>T. gondii</i>	Serum	25	42.27	0.98	29.47	52.26	A
	Placenta	25	35.54	1.40	16.51	47.89	B
Control		25	29.66	2.03	11.72	46.64	C

Similar letters refer to the non-significant differences at ( $P < 0.05$ ).

**Table 2: Mean level of CXCL16 in study groups**

Groups		No.	) Pgml( CXCL16				Denken Test *
			Mean	SD. E.	Lower Value	Upper Value	
Abortion with <i>T. gondii</i>	Serum	25	14.96	0.63	9.63	22.15	A
	Placenta	25	7.21	0.86	1.07	18.00	C
Abortion without <i>T. gondii</i>	Serum	25	10.05	0.92	4.21	21.94	B
	Placenta	25	7.74	0.71	2.19	16.42	C
Control		25	4.95	0.23	3.11	7.15	D

Similar letters refer to the non-significant differences at ( $P < 0.05$ ).

### 5. Discussion

Chemokines play a central role in the regulation of processes that are immunologically essential for T cell function; for instance, T cell migration within the lymphoid system and their targeting of pathogens in sites of inflammation. In addition chemokines are essentially involved in regulating cell trafficking, and represented by a group of cytokines that have the ability to induce chemotaxis (6).

In this study the level of CXCL10 cytokine that showed a significant increase in serum of infected groups is a key mediator of the interferon response referentially attracts activated T-helper1 (Th1) lymphocytes to sites of inflammation, and is an inhibitor of angiogenesis. The present study indicated that IP-10 is critical for survival following *T. gondii* infection and is essential for guiding antigen specific CD41 and CD81 T cells into infected organs. *T. gondii* evokes a strong humeral and cellular immune response in the infected host. The chemokine CXCL10 aspecific chemo attractant for activated T cells(14). These observations suggest that CXCL10 may play a broaderrole in the localization and the function of effector T cells at sites of Th1inflammation.

The T-cell chemokines CXCL10, and CXCL11 are overexpressed in stromal cells, and endothelial cells (10). Importantly, it found that 60% of cases of fetal death had evidence placental macrophages of fetal origin of chronic

chorioamnionitis and high amniotic fluid concentrations of CXCL10, which suggests that some cases of unexplained fetal death may represent an extreme form of maternal anti-fetal rejection ( 11 ; 12). Chronic placental inflammatory lesions can be due to maternal anti-fetal rejection, a process associated with the development of a novel form of fetal systemic inflammatory response. The syndrome is characterized by an elevation of the fetal plasma T-cell chemokine.

Histological abnormalities can be observed in the arteries around which NK cells are localized. By producing the chemokines CXCL8 (IL-8) and CXCL10 (IP-10), decidual NK cells regulate trophoblast invasion (9).

In toxoplasmosis there was an accurate equilibrium between a pivotal Th1 response for the control of parasite reproduction and a Th2 coordinate response to end the pathology due to the trigger harmful Th1 response (13) by regulation of the cytokines and chemokines.

### References

- [1] **Roberts, C.W.**, Walker, W., Alexander, J. (2001). Sex-associated hormones and immunity to protozoan parasites. Clin. Microbiol. Rev., 14:476-488.
- [2] **Pfaff, A.W.**, Abou-Bacar, A., Letscher- Bur, V., Villard, O., Senegas, A., Mousli, M. & Candolfi, E. (2007). Cellular and molecular phsiopathology of

- congenital toxoplasmosis: The dual role of INF- $\gamma$ . *Parasitology*, 134:1895-1909.
- [3] **Senegas**, A., Villard, O., Neuville, A., Marcellin, L., Pfaff, A.W., Steinmetz T, Mousli M, Klein JP, Candolfi E.(2009). *Toxoplasma gondii*-induced fetal desorption in mice involves interferon-gamma-induced apoptosis and spiral artery dilation at the maternofetal interface. *Int. J. Parasitol.*, 39:481–487.
- [4] **Jovanovic**, M., Stefanoska, I., Radojicic, L., Vicovac, L. (2010). Interleukin-8 (CXCL8) stimulates trophoblast cell migration and invasion by increasing levels of matrix metalloproteinase (MMP)2 and MMP9 and integrins alpha5 and beta1. *Reproduction*, 139: 789–798.
- [5] **Li**, M.Q., Tang, C.L., Du, M.R., Fan, D.X., Zhao, H.B. & Xu, B. (2011). CXCL12 controls over-invasion of trophoblasts via up regulating CD82 expression in DSCs at maternal–fetal interface of human early pregnancy in a paracrine manner. *Int. J. Clin. Exp. Pathol.*, 4:276–286.
- [6] **Rezende Oliveira**, K., Silva, N.M., Mineo, J.R.&Junior, V.R.(2012). Cytokines and chemokines production by mononuclear cells from parturient women after stimulation with live *Toxoplasma gondii*. *Placenta*, 33(9):682-687.
- [7] **Vasquez**, R.E., Xin, L. & Song, L. (2008). Effect of CXCL10 on dendritic cell and CD4+ T-cell functions during *Leishmania amazonensis* infection. *Infect. Immun.*, 76:161-9.
- [8] **Michlmayr**, D. & Mckimmie, C.S. (2014). Role of CXCL10 in central nervous system inflammation. *Int. J. Inter. Cyt. Med. Res.*, 18-61.
- [9] **Huang**, Y., Zhu, X., Du, M., Wu, X. Wang, M. & Li, D. (2006). Chemokine CXCL16, a scavenger receptor, induces proliferation and invasion of first-trimester human trophoblast cells in an autocrine manner. *Human Repro.*, 21:1083-1091.
- [10] **Kim**, M.J., Romero, R., Kim, C., Tarea, A.L., Chhauy, S. & Lajeunesse . (2009) Villitis of unknown etiology is associated with a distinct pattern of chemokine up-regulation in the feta-maternal and placental compartments : implications for conjoint maternal allograft rejection and maternal anti-fetal graft-versus-host disease. *J. Immunol.*, 182: 3912-3927.
- [11] **Lee**, J., Romero, R., Xu, Y., Kim, J.S., Topping, V., Yoo, W.(2011). A signature of maternal anti-fetal rejection in spontaneous preterm birth: chronic chorioamnionitis, anti-human leukocyte antigens, and c4d. *PlosOne*. 6(2): e16806.
- [12] **Lannaman**, K., Romero, R., Chaemsaitong, P., Ahmed, A. Yeo, L., Hassan, S. (2015). Fetal death: an extreme form of maternal anti-fetal rejection. *Am. J. Obste. Gynecol.*, (Supplement 1) : s251.
- [13] **Robert-Gangneux**, F., Darde, M. L. (2010). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin. Microbiol. Rev.*, 25:264-296.
- [14] **Ali**, W.R. (2016). The role of some Cytokines and trace elements in pregnant women with acute toxoplasmosis. *IbnAl-Haitham J. for Pure & Appl. Sci.*, 29: -23-30.