



Toxoplasma Gondii Infection Related And Interleukin 10 In Wasit Province Patients

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Abstract

Background: Toxoplasmosis is one of the most common parasitic infections in humans, which is caused by *Toxoplasma gondii*. Study levels of cytokines with prominent proinflammatory IL-10 was significantly elevated in amniotic fluid taken from congenital abortion cases.

Materials and Methods: Two hundred and seventeen blood specimens was collected from pregnant women with previous abortion, Serological investigations were done regarding IgM and IgG in the sera of women. Those specimens were then processed by using real ELISA technique searching for evidence of *Toxoplasma gondii* and CMV virus, in addition to detection of Interleukin 10 in abortion women.

Result: Regarding correlation between IgM and IgG concentrations and Interleukin 10 outcomes indicated that Interleukin 10 was 287.76 pg/mL in cases of *Toxoplasma* IgM and 182.62 pg/mL in *Toxoplasma* IgG, (p value= 0.035). Effect of age on *Toxoplasma* IL-10 concentration in patients and control showed that the concentration of IL-10 in *Toxoplasma* was 248.76 pg/mL in the age group 15-24 (p value= 0.049). Effect of age on both IL-10 concentration in patients and control results showed that the concentration of IL-10 in CMV was 182.62 pg/mL in (15-24) age group, (p value= 0.021). Among collected specimens aged between 15-44 years old was included in order to test the relationship between concentration of IL-10 and age of patients, results showed that the concentration of IL-10 in both the concentration was 184.31 pg/mL in the age group 15-24 (p value= 0.010).

Conclusion: Our results showed that *Toxoplasma gondii* was correlated to Interleukin 10 in abortion cases.

Key words: *Toxoplasma gondii*, abortion, Interleukin 10.

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1-Introduction

Congenital infections are one of the most important causes of prenatal morbidity and mortality, particularly in developing countries (Sharma *et al.*, 2015). The transient immunosuppression occurred in pregnancy increase the vulnerability of pregnant women to various infectious agents. The ability of the fetus to resist infection is limited and the fetal immune system is unable to prevent the dissemination of infectious microorganisms to various tissues (Parlak *et al.*, 2015). Among these pathogens, the prevalence of *Toxoplasma gondii* (*T. gondii*), *Rubella virus* *Cytomegalovirus* (CMV); also known as the members of TORCH complex, are very high and they may cause congenital malformations, multiple abortions, premature deliveries, and stillbirths during pregnancy (Karad and Kharat, 2015).

Toxoplasmosis, an infectious disease caused by *Toxoplasma gondii*, is one of the most prevalent causes of abortion and congenital aberrations in infected women (Paquet *et al.*, 2013; Aldabagh *et al.*, 2018). The global annual incidence of congenital toxoplasmosis was estimated to be 19000 cases. 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 oocytes 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 (Gov *et al.*, 2013).

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 ant parasitic defense pathways within infected cells. Production of IFN- γ in *T. gondii* infection. Cells have been proposed to be important sources during *T. gondii* infection,

including neutrophils, macrophages, plasmacytoid dendritic cell, and the subset of conventional dendritic cells expressing CD8 α (Mashayekhi *et al.*, 2011). Pathogenesis of toxoplasmosis may affect by Th2 cytokines (IL-10), which play a major role in the pathogenesis of parasitic diseases. So IL-10 control the type of the immune response (Gaddi and Yap, 2007).

2-Materials and methods

2-1 Specimens collection

Two hundred and seventeen blood specimens was collected from pregnant women with previous abortion, in addition to twenty specimens as control from healthy women, through a skilled and professional procedure in a gel tube, then blood samples were centrifuged in which supernatant was extracted, extracted samples were preserved in refrigerator -4°C until using later. Samples were taken from patients who were present in Al-Zahra teaching hospital and Al-Kut teaching hospitals. Serological investigations were done in these hospitals according to the instruction of providing company aiming at detection of IgM and IgG in the sera of women with pregnant and spontaneous abortion. Those specimens were then processed by using real ELISA technique searching for evidence of *Toxoplasma gondii* and CMV virus, the lab work was done according to instruction of providing company.

2-2 Procedure of Human Interleukin 10 ELISA Kit

Reagents preparation was done as following: All reagent were brought to room temperature before using. It was reconstituted 120 ul from the original standard (1600 pg/ml) with 120 ul of standard diluent to generate a 800 pg/ml standard stock solution, it was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. It was prepared duplicate standard points by serially diluting the standard stock solution, 1:2 with standard diluent to produce 400 pg/ml, 200 pg/ml, 100 pg/ml and 50 pg/ml solutions. Standard diluent was served as the zero standard (0 pg/ml). any remained solution was frozen at -20°C and used within one month. It has been determined

the number of standard wells. Addition of 50 μ l stop solution to each well was done, the blue color was changed to yellow immediately. It was read the absorbance O.D. at 450nm using a microtiter plate reader. The OD value of the blank control well is set as zero. Assay should be carried out within 10 minutes after adding stop solution.

2-3 Calculation of results for all markers used in current study

Known concentrations of each marker standard and its corresponding reading optical density (OD) was plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of each marker in sample was determined by plotting the samples O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

2-4 Statistical Analysis

Data were entered, coded, and analyzed in SPSS (statistical package for social sciences)

software program version 26. Data analysis were done using different tests. Frequency and percentages were used for the description of categorical variables.

3-Results

3-1 Correlation between IgM and IgG concentrations and Interleukin 10

In this study IgM concentration was correlated the Interleukin 10 concentration, outcomes indicated that Interleukin 10 was 287.76 pg/mL in cases of *Toxoplasma* IgM and 182.62 pg/mL in *Toxoplasma* IgG, regard CMV Interleukin 10 concentration was 174.97 pg/mL in cases of IgM and 167.01 pg/mL in IgG, about both infection cases results recorded 184.31 pg/mL and 189.01 pg/mL of 10 concentration in the cases of IgM and IgG respectively, finally control reported that 10 concentration was 177.18 pg/mL in IgM cases and 130.16 pg/mL in IgG cases. The current results indicated significant differences (p value= 0.035) as shown in table (1) and (2).

Table (1): Correlation between IgM and Interleukin 10 concentration

Concentration of Interleukin-10		
Pathogens	Antibodies classes	Mean of IL-10 concentration pg/mL
<i>Toxoplasma</i>	IgM	248.76a
Cytomegalovirus	IgM	174.97 b
Both	IgM	184.31 b
Mean of Control	IgM	177.18
P value	0.035	

*Similar letter means no significant difference

Each number in concentration represent mean of all tested samples

Table (2): Correlation between IgG and Interleukin 10 concentration

Concentration of Interleukin-10		
Pathogens	Antibodies classes	Mean of IL-10 concentration pg/mL
<i>Toxoplasma</i>	IgG	182.62b
Cytomegalovirus	IgG	167.01 b
Both	IgG	189.01 b
Mean of Control	IgG	130.16
P value	0.035	

*Similar letter means no significant difference

Each number in concentration represent mean of all tested samples

3-2 Effect of age on *Toxoplasma* IL-10 concentration in patients and control

Among collected specimens aged between 15-44 years old was included in order to test the relationship between concentration of IL-10

and age of patients, results showed that the concentration of IL-10 in *Toxoplasma* was 248.76 pg/mL in the age group 15-24, 244.18 pg/mL in the age group 25-34 and 212.20 pg/mL were in the age group 35-44, the present

results indicated significant differences (p value= 0.049) as shown in table (3).

Table (3): Comparison between IL-10 concentration in patients and control according to age

IL-10 concentration according to age		
Antibodies of infected pathogens	Age groups	Concentration of IL-10 pg/mL
<i>Toxoplasma</i>	15-24	248.76a
	25-34	244.18a
	35-<44	212.20b
Control	17-45	177.18 ab
P value	0.049	

*Similar letter means no significant difference
Each number represent mean of all tested samples

3-3 Effect of age on CMV IL-10 concentration in patients and control

Among collected specimens aged between 15-44 years old was included in order to test the relationship between concentration of IL-10 and age of patients, results showed that the concentration of IL-10 in CMV was 182.62

pg/mL in the first age group, the concentration in second age group 25-34 was 170.60 pg/mL and 150.19 pg/mL in the third age group 35-44, the present results indicated significant differences (p value= 0.021) as shown in table (4).

Table (4): Comparison between IL-10 concentration in patients and control according to age

IL-10 concentration according to age		
Antibodies of infected pathogens	Age groups	Concentration of IL-10 pg/mL
Cytomegalovirus	15-24	182.62 ab
	25-34	170.60 ab
	35-<44	150.19 ac
Control	17-45	177.18 ab
P value	0.021	

*Similar letter means no significant difference
Each number represent mean of all tested samples

3-4 Effect of age on both IL-10 concentration in patients and control

Among collected specimens aged between 15-44 years old was included in order to test the relationship between concentration of IL-10 and age of patients, results showed that the concentration of IL-10 in both the

concentration was 184.31 pg/mL in the age group 15-24, 180.38 pg/mL in the second age group and 170.32 pg/mL were in the third age group, the results were compared with control and outcomes were 177.18 pg/mL, the present results indicated significant differences (p value= 0.010) as shown in table (5).

Table (5): Comparison between IL-10 concentration in patients and control according to age

IL-10 concentration according to age		
Antibodies of infected pathogens	Age groups	Concentration of IL-10 pg/mL
Both	15-24	184.31b
	25-34	180.38 b
	35-<44	170.32 b
Control	17-45	177.18 ab
P value	0.010	

*Similar letter means no significant difference
Each number represent mean of all tested samples

4-Discussion

Biesiada et al. (2006) revealed that the parasite after invading the human body multiplies inside the cell, causing damage to the reticulo-endothelial system. Rapid multiplication of the parasite and formation of the so-called pseudocysts are characteristics of the acute phase of invasion, our outcomes was compatible to this study, also Nickdel et al. (2001) showed that in parasitic invasions, an increase is observed in the production of antibodies, especially in helminth infections. This defect results from disturbances in the regulation of antibody production by Th cells, especially IgM in *Toxoplasma* participates in the reaction of antibody-dependent cellular cytotoxicity (ADCC). Cytotoxic activity of eosinophils is increased under the influence of cytokines (TNF- α , IL-5 and IL-10). Moreover the present data were agreed with the study performed in a group of patients infected with *T. gondii*, we evaluated Th2 humoral response (IL-5, IL-6, IL-10). The study objective was to assess the effect of *T. gondii* on chosen indices of the immune response. The study involved 52 women infected with *T. gondii* (aged 18–42 years) the levels of IgE, IL-5, IL-6, IL-10, it was found the level of IL-10 to be fivefold higher in the course of *toxoplasmosis* than in healthy controls (Matowicka-Karna, Dymicka-Piekarska, and Kemonia 2009).

Additionally a study conducted by (Aldabagh et al. 2018) reported that the humoral response in toxoplasmosis involves IL-5 and IL-6 that exert a proinflammatory effect and IL-10 inhibiting the action of IL-6. It has been proved that parasite invasion promotes release of IL-5, IL-8, TNF- α and IL-10 which is accompanied by eosinophilia with an inflammatory state (Young et al. 2020). It was found that the level of IL-10 to be fivefold higher in the course of *toxoplasmosis* than in healthy controls, therefore, we could have expected a decrease in IL-6 production, which was however not found, IL-10 plays an essential role in the inflammatory response during acute *T. gondii* infection (Babaie et al. 2018). IL-10 counteracts the harmful effects of the inflammatory response which is based on the increased production of TNF- α , IFN- γ , and NO associated with intestinal multiplication of *T. gondii* (Mose et al. 2019).

The results of current study were not in accordance with (Abdulla et al. 2022) mentioned that abortion is a condition that occurs due to one of the pathological injuries, often one of the members of the TORCH is the real cause, the study aimed to investigate the impact of toxoplasmosis, infections with abortion, and also, the identification of immunological marker (interleukin-10) that may be associated with age of patients. Anti-*Toxoplasma* IgG, IgM, Herpes simplex virus-2 IgM, human soluble leukocyte antigen class I–G and interleukin- 10 were estimated by ELISA technique, while the expression of IL-10 was investigated. The results showed that among aborted women the rate of anti-*Toxoplasma* and HSV-2 IgM antibodies occurred within the age groups (21–30) years and (31–40) years 32 (100.0%) and 36 (100.0%), respectively. A significant relationship was found between IL-10 and cases with a $P=0.005$ (Cheng and Sharma 2015). In another study it was concluded that there was an increase in interleukin 1 (IL-1) levels and IL-10 levels without relation to age (Sauer et al. 2015). On the other hand (Al-Aaraje and Al-Khilkhali, 2020) study showed that the elevated toxoplasmosis and Herpes-2 IL-10 rate was related to age groups in about 7(18.9 %) with a highly significant difference ($P<0.001$). IL-10 actively suppresses the maternal immune system to avoid rejection of the fetal allograft (Cheng and Sharma 2015). The current study also was agreed with that the aborted women in the age group 30-40 years old carriers high IL-10 concentration than the toxoplasmosis and Herpes-2 had decreased HLA-G (Tersigni et al. 2020)

Alterations in the $\gamma\delta$ T cell compartment have been reported in immunocompromised individuals infected with human CMV (HCMV)-seropositive patients and were higher than in HCMV^{POS} controls, mimicking HCMV reactivation, whereas their serum was PCR-negative for HCMV. Upon restimulation with HEV-infected or uninfected cells and selected cytokines, (Barragué et al. 2021) indicated that these cell lines produced IFN- γ and IL-10 (Pitard et al. 2008). According to our knowledge all present studies mentioned the same fact. These data were agreed to (Munro et

al. 2019) as circulating type 2 helper T-cell (cTh2) is proportional to the number and extension of organ involvement, number of circulating plasmablasts, and serum concentration of IgG4. cTh2 cytokines, such as interleukin (IL)-4 and IL-10 are present in IgG4-RD (Munro *et al.* 2019). Sezgin *et al.* (2010) recorded that CMV produces a homologue IL-10, which helps in evading the immune system and development of retinitis. Perugino and Stone (2020) has been find that there was a presumed CMV retinitis started during IgG4-RD activity. One hypothesis is that elevated serum cTh2 and IL-10 levels lead to the weakening of Th1 cell-mediated immune response especially in older aged patients. Our data were agreed to all mentioned studies above.

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