

# SEROPREVALENCE of TOXOPLASMA- IGA and its RELATION to SERUM IL-4 LEVEL AMONG PREGNANT WOMEN

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## **Abstract:**

Toxoplasmosis is caused by infection with the parasite *Toxoplasma gondii*. It is one of the most common parasitic infections in humans and is most typically asymptomatic. However infection in a pregnant woman can cause severe and disabling disease in the developing fetus. The presented study aimed to determination the correlation of Toxo-IgA seroprevalence among pregnant women to various *Toxoplasma* antigens with the serum IL-4 level. From the June 2017 to January 2018 ,300 pregnant women enrolled in this study attended to Salahalden General Hospitals . The pregnant women were examined for Toxo-IgA and determine their specificity for various *Toxoplasma* antigens by using line immune assay and the serum IL-4 evaluated by using ELISA technique . The rates of Toxo-IgA seropositive were 22 [7.33 %]. Considering the reactivity of determined Toxo-IgA against various *Toxoplasma* antigens; the rates were 12[54.54%], 13[59.09%], 11[50.00%] ,14[63.63%] ,17[77.27%], 13[59.09%] ,11[50.00%] and 18 [81.81%] seropositive for *Toxoplasma* ROP1C, MIC3, GRA7, GRA8, p30 , MAG1, GRA1 and rSAG1 antigens respectively. Regarding to the total serum IL-4 level ,the highest rate of decreased serum IL-4 level among Toxo-IgA seropositive was 59.09%.In the presented study we concluded the highest rates of decreased serum IL-4 levels was 54.55% seen within Toxo-IgA seropositive for GRA7 and GRA1 antigens , and the rates of increased serum IL-4 level ranged from 5.88% to 9.09% within all seropositive groups to various *Toxoplasma* antigens.

**Keywords:** *Toxoplasma* ; IL-4 ; IgA ;MAG1;GRA1 ;rSAG1

## **INTRODUCTION**

The *Toxoplasma gondii* (*T. gondii*) is one of the most successful parasites in the world due to its ability to infect and persist in most warm-blooded animals [1,2].The infection by toxoplasmosis due to the exposure to the main sources of infection which are: soil, water or food contaminated with feces from infected cats that contain *T. gondii* oocysts; or raw or uncooked meat that contain bradyzoit cysts[3,4] ,and vertically transmission may during pregnancy, blood transfusion, and organ transplantation [5]. The *T. gondii* infection during pregnancy can have devastating consequences in the fetus ranging from miscarriage ,stillbirth, hydrocephalus, and ocular damage[5] and may also lead to late sequelae in the life of the infected newborn[7].

The infection can result in high morbidity and mortality rates. Indeed, primary infection with *T. gondii* acquired during gestation may lead to miscarriage or severe sequelae in the fetus [8]. It is thus important to screen these particular populations for *T. gondii* infection in order to take appropriate measures. In some countries, monthly prenatal serological screening is performed for all pregnant women whether or not they are considered at risk for *T. gondii* infection [9, 10].

The *T. gondii* has particular electron dense secretory organelles specifically micronemes, rhoptries, and dense granules, contain specific proteins, for example, microneme proteins (MICs), rhoptry proteins (ROPs), and thick granule proteins (GRAs). These proteins considered to assume a fundamental part in intracellular parasitism attack of vertebrate cells by the protozoan *T. gondii*. The Binding to the host cell activated apical release of the micronemal protein MIC at the tight connection zone that structures between the parasite and the host cell. In the next step, invagination of the host cell plasma layer was started by release of the ROP to frame a beginning parasitophorous vacuole (PV). ROP is completely released into the vacuole when attack was finished. Rather than the extremely early events, release of the GRA [11-13].

The most serological diagnosis uses including IgM and IgG for differentiate the acute and chronic toxoplasmosis, while serology detection of Toxoplasma IgA status providing additional information regarding acute infection or reactivation. In the case of positive IgA results, there is a high likelihood of acute Toxoplasma infection, whereas in others reactivation is suspected depending on the other toxoplasma antibodies status as IgG and IgM results [14,15]. Interleukin-4 (IL-4), also known as B cell-stimulatory factor-1 as a B-cell stimulating factor [16,17], Th2 cytokine that shows pleiotropic effects during immune responses [18]. It has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of B cells into plasma cells. It is a key regulator in humoral and adaptive immunity[19].

## MATERIALS AND METHODS

A cross sectional study was carried out in Kirkuk governorate from the June 2017 to January 2018 for studying the time of Toxoplasma infection among 300 pregnant women whose age between 18-42 years attended to Salahalden General Hospital. The pregnant women were examined for Toxoplasma-IgA (Toxo-IgA) seroprevalence and examination the specificity of determined Toxo-IgA for specific various *Toxoplasma* antigens (ROP1C, MIC3, GRA7, GRA8, p30 MAG1, GRA1, and rSAG1) separately by using line immune (*RecomLine ; Mikrogen , GmbH , Germany*) assay and the total serum IL-4 evaluated by using ELISA technique (*Diaclone ; France*). Computerized statistically analysis was performed using SPSS(Statistical Package for Science Services) version 17, SPSS Inc.USA. Comparison carried out using ; Chi-square ( $X^2$ ) and Probability ( P value ). The P value  $\leq 0.05$  was considered statistically significant (S) ,and less than 0.01 considered highly significant (HS) and greater than 0.05 considered non significant.

## RESULTS

The presented study revealed the rate of Toxo-IgA among the total 300 examined pregnant women was 22(7.33%) , while the seroprevalence among 120 control [non pregnant] married women was 2 (1.66 %) as shown in (Table 1).

Table 1 : The seroprevalence of specific Toxo-IgA among pregnant women and control.

Results	Toxo-IgA Antibody			
	Pregnant women		Control	
	No .	%	No .	%
Positive	22	7.33	2	1.66
Negative	278	92.67	118	98.34
Total	300	100	120	100

$\chi^2 = 5.11$ $P = 0.024$ $P < 0.05$ Significant
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Considering the specificity of the determined specific Toxo-IgA against the various *Toxoplasma* antigens [ROP1C, MIC3, GRA7, GRA8, p30, MAG1, GRA1, rSAG1,] separately by using line immunoassay, the rates of Toxo-IgA against to these antigens among the total 22 Toxo-IgA seropositive pregnant women were 12(54.54%), 13(59.09%), 11(50.00%), 14(63.63%), 17(77.27%), 13(59.09%), 11(50.00%) and 18(81.81%) seropositive for *Toxoplasma* ROP1C, MIC3, GRA7, GRA8, p30, MAG1, GRA1 and rSAG1 antigens respectively as shown in (Table 2).

Table 2: The rates of specific Toxo-IgA seropositive against various *Toxoplasma* antigens among pregnant women by using Line immunoassay.

<i>T. gondii</i> antigens	Toxo-IgA seropositive					
	Positive		Negative		Total	
	No .	%	No .	%	No .	%
ROP1c	12	54.54	10	45.46	22	100
MIC3	13	59.09	9	40.91	22	100
GRA7	11	50.00	11	50.00	22	100
GRA8	14	63.63	8	36.37	22	100
p30	17	77.27	5	22.73	22	100
MAG1	13	59.09	9	40.91	22	100
GRA1	11	50.00	11	50.00	22	100
rSAG1	18	81.81	4	18.18	22	100
$\chi^2 = 9.23$ $P = 0.237$ $P > 0.05$ Non significant						

The (Table 3) shows the relation of the serum IL-4 level with the 22 seropositive Toxo-IgA among pregnant women and in comparison to 22 seronegative Toxo-IgA pregnant women as control group. The highest rate of decreased serum IL-4 level seen in Toxo-IgA seropositive was 59.09%, while the highest rate 90.09% of normal serum IL-4 level seen within control group.

Table 3 : Comparison the Total serum IL-4 level among Toxo-IgA seropositive with seronegative pregnant women.

Serum IL-4 Level	Pregnant women			
	Toxo-IgA seropositive (pregnant women)		Toxo-IgA seronegative (pregnant women) as control	
	No .	%	No .	%
Normal	8	36.36	20	90.90
Increased	1	4.55	1	4.55
Decreased	13	59.09	1	4.55
Total	22	100	22	100
$\chi^2 = 15.04$ $P = 0.0001$ $P < 0.01$ Highly significant				

Regarding to the correlation the specificity of Toxo-IgA against various Toxo antigens with the serum IL-4 levels, the highest rates of decreased serum IL-4 levels was 54.55% seen within Toxo-IgA seropositive for GRA7 and GRA1 antigens, and the highest rates of increased serum IL-4 level ranged from 5.88% to 9.09% within all seropositive groups to various *Toxoplasma* antigens as shown in (Table 4)

Table 4 : Correlation of Total serum IL-4 level with Toxo-IgA seropositive against various *Toxoplasma* antigens .

<i>T. gondii</i> antigens	Serum IL-4 Level	Toxo-IgA Seropositive		Total
		No .	%	
ROP1c	Normal	5	41.66	12
	Increased	1	8.34	
	Decreased	6	50.00	
MIC3	Normal	6	46.15	13
	Increased	1	7.70	
	Decreased	6	46.15	
GRA7	Normal	4	36.36	11
	Increased	1	9.09	
	Decreased	6	54.55	
GRA8	Normal	6	42.85	14
	Increased	1	7.14	
	Decreased	7	50.01	
p30	Normal	7	41.17	17
	Increased	1	5.88	
	Decreased	9	52.95	
MAG1	Normal	6	46.15	13
	Increased	1	7.70	
	Decreased	6	46.15	
GRA1	Normal	4	36.36	11
	Increased	1	9.09	
	Decreased	6	54.55	
rSAG1	Normal	8	44.44	18
	Increased	1	5.55	
	Decreased	9	50.01	

Normal range of IL-4 : 1-3 pg/ml .

Increased : more than 3 pg/ml .

Decreased : less than 1 pg/ml .

## DISCUSSION

In the presented study the rate of Toxo-IgA seroprevalence was 7.33% among pregnant women in comparison the lower rate was 1.66% among control group with significant relation  $P < 0.05$  as shown in (Table 1) , the different results recorded among these two groups may be due to the immunological change especially Th1 and Th2 switching and hormonal imbalance during pregnancy lead to increasing the rate of *Toxoplasma* infection among pregnant women .

The *T. gondii* promotes the production of antibodies that aid in killing the parasite [20]. These immunoglobulins are essential for diagnosis however play a minor role in eliminating the parasite. They have been found to protect against the parasite by blocking invasion, opsonizing the parasite for phagocytosis as well as activating the complement pathway [21,22]. Studies have proposed that the antibodies hinder invasion by blocking the activity of secretory-excretory substances that enhance host cell penetration. Immunoglobulin IgM is the first antibody to be produced, appearing after one week of infection. IgA appear next and are used for diagnosing the acute phase of the disease[23-38]. IgA are observed in two forms: mucosal IgA appearing in the mucous secretions and serum IgA. In toxoplasmosis, both types of IgA are found both in the digestive tract and serum in humans. In acquired toxoplasmosis, the appearance of IgA is thought to be an early marker [21,24]. IgG appears later and persists throughout the lifetime of the host [25,26].

Regarding the reactivity of determined Toxo-IgA in the presented study revealed that the different rates of Toxo-IgA reactivity with various Toxoplasma antigens were the highest rate of reaction was 81.18% for rSAG antigen and the lowest rate was 50.00% for GRA7 and GRA1 antigens with non significant relation  $p > 0.05$  as shown in (Table 2). The different rates of Toxo-IgA with the various Toxoplasma antigens may be due to the stimulation of immune response to the *T. gondii* depend on the process of *T. gondii* exposure and expression its antigens to the human immune system specially its intracellular protozoan parasite and the strategy of its replication cycle may lead to different rates of antigenic stimulation the humoral immune response, also the stimulation of IgA may detect in acute and reactivation states. So the non significant relation may be due to the rate of the stimulation the Toxo-IgA for most various Toxoplasma antigens is egalitarian or similar.

The IgA immune response may vary a great deal overall. On the one hand, there may be no such response at all, but, on the other hand, the presence of IgA antibodies may substantiate the suspicion of an acute Toxoplasmosis infection [27,28].

The present study revealed that the rates of abnormal total serum IL-4 level within seropositive Toxo-IgA among pregnant women higher than in Toxo-IgA seronegative pregnant women as control group. So the highest rate of decreased serum IL-4 level seen in Toxo-IgA seropositive was 59.09% with highly significant  $P < 0.01$  relation as shown in table 3. The IL-4 is intimately involved in the regulation of antibody isotype expression and function. Depending on the surface proteins expressed by neighboring cells and the cytokine environment, activated B cells and plasma cells will secrete different antibody classes. The B cells switch between antibody classes by recombination of the various antibody gene regions, [16, 28].

The IL-4 induces Th2 differentiation and inhibits Th1 differentiation [30]. They identified a silencer region in the untranslated region of the IL-4 gene. The Th differentiation, the stability of differentiated Th cells is important for the outcome of immune responses against infections or in autoimmune diseases. IL-4 play major roles in mediating Th stability [31]. The stability of differentiated Th cells is also influenced by the relative concentrations of IL-4. Antibody class switch, expression and effector functions The central players in the humoral immune response are antibodies and their cognate Fc receptors [32].

Considering the correlation the specificity of Toxo-IgA against various Toxoplasma antigens with the serum IL-4 levels, the highest rates of decreased serum IL-4 levels was 54.55% seen within Toxo-IgA seropositive for GRA7 and GRA1 antigens and in comparison to the highest rate of increased serum IL-4 level was 9.09% as shown in (Table 4). This finding may be due to the *T. gondii* infection and their exposure to the human immune system with different stage by their different antigens with vary degree of inhibition and prevent IL-4 production. During the early phases of a *T. gondii* infection, when the combination of rapidly dividing tachyzoites plus the immune response, IL-4 plays an ameliorative role and reduces mortality [32-33]. Thus, IL-4 during the course of *T. gondii* infection in an individual host can play both disease-protective and exacerbative roles [32-37].

Because IL-4 has been demonstrated to significantly modulate immune responses [34], in *T. gondii* infection the IL-12 acts synergistically with IL-18 to produce IFN- $\gamma$  by NK cells. IFN- $\gamma$  synergizes with IL-12 to drive the differentiation of T helper precursor (Thp) to Th1 phenotype, express IL-12 receptor on T cells, and inhibit the antagonist IL-4 to prevent the differentiation of Thp towards Th2 phenotype [35]. So these may lead to the increase the risk

to pregnant women and several studies have reported low levels of IL-4, IL-4-producing cells, and Th1 cytokine/IL-4 ratios in women with spontaneous abortions[36].

## CONCLUSIONS

The level of serum IL-4 was decreased in the most cases of Toxo-IgA seropositive pregnant women especially for GRA7 and GRA1 antigens.

## REFERENCES

- [1] Mendez O., Koshy A. ,***Toxoplasma gondii*: Entry, association, and physiological influence on the central nervous system**, PLoS Pathog,13,7,(2017).
- [2] Cosme A., ***Toxoplasma gondii* Infection and Headache: A Matched Case-Control Study in a Public Hospital in Durango City, Mexico**. J Clin Med Res.,10(1),27-31(2018).
- [3] Avelino M., Campos D. , Parada J., Castro A. , **Risk factors for *Toxoplasma gondii* infection in women of childbearing age**, Braz J Infect Dis, 8,164–174(2004).
- [4] Marcos G., Marina C., Ana M. , Da S. ,**"Prevalence of toxoplasmosis in pregnant women and vertical transmission of *Toxoplasma gondii* in patients from basic units of health from Gurupi, Tocantins, Brazil, from 2012 to 2014"**, PLoS One 10(11), (2015).
- [5] Tenter A., Heckeroth A., Weiss L., ***Toxoplasma gondii* from animals to humans**. Int J Parasitol.,30(12- 13),1217-1258(2000).
- [6] Wallon M., François P. ,**"Congenital Toxoplasmosis,A Plea for a Neglected Disease "** Pathogens 7.1, 25(2018).
- [7] Rodrigues I., Costa T., Avelar J., Amaral W., Castro A., Avelino M.,**Assessment of laboratory methods used in the diagnosis of congenital toxoplasmosis after maternal treatment with spiramycin in pregnancy**, BMC Infectious Diseases, 14,349(2014).
- [8] Montoya J., Remington J. , **Management of *Toxoplasma gondii* infection during pregnancy**, Clin Infect Dis .47,554–566(2008).
- [9] Prusa A., Kasper D., Pollak A., Olischar M., Gleiss A., Hayde M. ,**Amniocentesis for the detection of congenital toxoplasmosis: results from the nationwide Austrian prenatal screening program**, Clin Microbiol Infect,21,191(2015).
- [10] Jones J., Dargelas V., Roberts J., Press C, Remington J., Montoya J.,**Risk factors for *Toxoplasma gondii* infection in the United States**. Clin Infect Dis .:49:878–884(2009).
- [11] Carruthers V., Sibley L., **Sequential protein secretion from three distinct organelles of *Toxoplasma gondii* accompanies invasion of human fibroblasts** ,Eur J Cell Biol,73,114–123(1997).
- [12] Gendrin C., Mercier C., Braun L., Musset K., Dubremetz J., Cesbron M. ,**"*Toxoplasma gondii* Uses Unusual Sorting Mechanisms to Deliver Transmembrane Proteins into the Host-Cell Vacuole"** ,Traffic 9,10, 1665-1680(2008).
- [13] Kotresha D. and Rahmah N., **"Recombinant proteins in the diagnosis of toxoplasmosis"**, Apmis 118,8, 529-542(2010).
- [14] Olariu T., Remington J., McLeod R., Alam A., Montoya J., **Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants**, Pediatr Infect Dis J,30,1056–1061(2011).
- [15] Xiaoyang L. ,**"Multiplexed anti-toxoplasma IgG, IgM, and IgA assay on plasmonic gold chips: Towards making mass screening possible with dye test precision"**, Journal of clinical microbiology 54.7 , 1726-1733(2016).

- [16] Paludan S. ,"**Interleukin-4 and Intefferon- : The Quintessence of a Mutual Antagonistic Relationship**", Scandinavian journal of immunology 48 , 459-468(1998).
- [17] Paul W., **Interleukin-4: signalling mechanisms and control of T cell differentiation** , Ciba Found Symp,204,208–216(1997).
- [18] [18] Howard M., Farrar J., Hilfiker M. , **Identification of a T cell derived B cell growth factor distinct from interleukin-2**, J Exp Med,155,914–923(1982).
- [19] Ryan J., McReynolds L., Keegan A. , **Growth and gene expression are predominantly controlled by distinct regions of the human IL-4 receptor**, Immunity,4,123–132(1996).
- [20] Holec L. , **Toxoplasma gondii Recombinant Antigens as Tools for Serodiagnosis of Human Toxoplasmosis : Current Status of Studies**,20(9), 1343–1351(2013).
- [21] Filisetti D., Candolfi E. , **Immune response to Toxoplasma gondii**, Ann Ist Super Sanita, 40(1), 71–80(2004).
- [22] Dupont C., Christian D., Hunter C. , **Immune Response and Immunopathology**
- [23] Kartey A., Worlasi D. ,**Cytokine responses to Toxoplasma Gondii in immune-competent and immune-compromised individuals at Korle-Bu Teaching Hospital**,Ph D thesis, Ghana (2017).
- [24] Pinon J. ,**IgA specific isotypes or IgE in the evaluation of toxoplasmic risks in immunocompromised subjects** , Rev Fr Lab,223,103-107(1991).
- [25] Cheesebrough M. ,**District Laboratory Practice in Tropical Countries** ,(2nd Edition), New York, Cambridge University Press,10-11(2006).
- [26] Luptakova L., Petrovova E., Mazensky D., Valencakova A., Balent, P.,**Toxoplasmosis in Livestock and Pet Animals in Slovakia**, www.intechopen.com.,(2012).
- [27] Harning D., **Recombinant Toxoplasma gondii surface antigen 1 [P30] expressed in Escherichia coli is recognized by human Toxoplasma specific immunoglobulin M [IgM] and IgG antibodies**, Clin. Diagn. Lab. Immunol., 355-357(1996).
- [28] GarlTG010en kit. recomLine Toxoplasma IgM/IgA 5974, Instructions for use by Mikrogen GmbH Floriansbogen ,Neuried ,Germany,(2015).
- [29] Harriman W., Volk H., Defranoux N., Wabl M., **Immunoglobulin class switch recombination**, Ann Rev Immunol,11,361–384(1993).
- [30] Mosmann T., Sad S. ,**The expanding universe of T-cell subsets. Th1, Th2 and more**, Immunol Today,17,138–146(1996).
- [31] Szabo S., Jacobson N., Dighe A. , **Developmental commitment to the Th2 lineage by extinction of IL-12 signaling**, Immunity ,2,665–765(1995).
- [32] Gautum S., Tebo M., Hamilton T., **IL-4 suppresses cytokine gene expression induced by IFN-g and/or IL-2 in murine peritoneal macrophages**, J. Immunol,148,1725–1730(1992).
- [33] Roberts C. , "**Different roles for interleukin-4 during the course of Toxoplasma gondii infection**", Infection and immunity 64.3, 897-904(1996).
- [34] Nickdel, M., Lyons R., Roberts F., Brombacher F., Hunter C., Alexander J., Roberts C. , **Intestinal pathology during acute toxoplasmosis is IL-4 dependent and unrelated to parasite burden**, Parasite immunology, 26(2), 75-82(2004).
- [35] Iqbal J., AL-Awadhi M. , **Toxoplasmosis: Role of Cytokines in Disease Modulation & Tissue Pathology**, Ann Clin Pathol, 4,7, 1090(2016).
- [36] Chatterjee P., Chiasson V., Bounds K., & Mitchell B. , **Regulation of the anti-inflammatory cytokines interleukin-4 and interleukin-10 during pregnancy**. Frontiers in immunology, 5, 253(2014).

- [37] Al-doury, S. M., Al-Nasrawi, M. A., & AL-Samarraie, M. Q. (2019). The molecular sequence of Giardia lamblia by using (tpiA) and (tpiB). *International Journal of Drug Delivery Technology*, 9(03), 374-377.
- [38] **4- Shakir, O.M., Abdulla, K.K., Mustafa, A.A., Mustafa, M.A. (2019) Investigation of the presence of parasites that contaminate some fruits and vegetables in the samarra city in Iraq Plant Archives.**