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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 hospitals, primary health care centers and some private medical laboratories in Kirkuk. 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.

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الانتشار المصلي للضد ألفا للمقوسات الكوندية وعلاقته بمستوى الحركي الخلوي-4 في الدم بين النساء الحوامل.

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الملخص

داء المقوسات سببه الإصابة بطفيلي المقوسات الكوندية وهي واحدة من أكثر الإصابات الطفيلية الشائعة بين البشر، وهي في الغالب لا توجد أعراض لها ومع ذلك، يمكن أن الإصابة في المرأة الحامل تشكل خطورة شديدة على الجنين. حيث استهدفت الدراسة المقدمة لمعرفة الانتشار المصلي للضد ألفا الخاصة بالمقوسات الكوندية بين النساء الحوامل وتفاعلها مع مختلف مستضدات المقوسات الكوندية وعلاقته بمستوى الحركي الخلوي-4 في مصل الدم. حيث أجريت الدراسة للفترة من حزيران 2017 إلى كانون الثاني 2018 على 300 امرأة حامل راجعن مستشفيات ومراكز الرعاية الصحية الأولية وبعض المختبرات الأهلية في كركوك. تم فحص مصل الدم للنساء الحوامل لإيجاد الضد ألفا للمقوسات الكوندية ومن ثم معرفة قابلية وتفاعل ذلك الضد للمستضدات الخاصة بالمقوسات الكوندية باستخدام اختبار المناعة الخطي الخاص بذلك وكذلك تقييم مستوى الحركي الخلوي-4 في الدم باستخدام تقنية الاليزا. حيث كانت نسبة للضد ألفا في مصل تلك النساء الحوامل 22(7.33٪). وفيما يتعلق بتفاعلية الضد ألفا لمختلف المستضدات الخاصة كانت النتائج 12(54,54٪) و 13(59,09٪) و 11(50,0٪) و 14(63,63٪) و 17(77,27٪) و 13(59,09٪) و 11(50,0٪) و 18(81,81٪) موجبًا للمستضدات ROP1C، MIC3، GRA7، GRA8، p30، MAG1، GRA1 و rSAG1 بالترتيب. فيما يتعلق بمستوى الحركي الخلوي-4 في المصل ، فإن أعلى معدل لمستوى الحركي الخلوي-4 المنخفض في مصل الدم الموجب للضد ألفا وكانت 59,09 ٪. حيث استنتجت الدراسة أن أعلى معدلات انخفاض مستويات الحركي الخلوي-4 في المصل كانت 54,55 ٪ شوهدت في مصل الدم الموجب للضد ألفا للمستضدات GRA7 و GRA1،

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وان معدلات زيادة مستوى الحركي الخلوي–4 تراوحت من 5,88 ٪ إلى 9,09 ٪ للضد ألفا لمستضدات المقوسات الكوندية المختلفة.

الكلمات الدالة: *Toxoplasma* ; IL-4 ; IgA ;MAG1;GRA1 ;rSAG1.

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1. 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 [6] 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 denes secretory organelles specifically micronemes, rhoptries, and denes 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

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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-1as 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].

2. 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 Toxoplasm infection among 300 pregnant women whose age between 18-42 years attended to Azadi General Teaching Hospital, Kirkuk General Hospital and some primary health care centers and private medical laboratories. The pregnant women were examined for Txoxoplasma-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 ≤ 0.05 was considered statistically significant (S) ,and less than 0.01 considered highly significant (HS) and greater than 0.05 considered non-significant.

3. 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.

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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
$X^2 = 5.11$ P = 0.024 P <0.05 Significant				

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 81(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.

T. gondii 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	81.18	4	18.82	22	100
$X^2 = 9.23$ 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

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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
X^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.

T. gondii 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	

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T. gondii antigens	Serum IL-4 Level	Toxo-IgA Seropositive		Total
		No .	%	
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 .

4. 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 *Toxoplasms* 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]. 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 nad 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 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 humeral immune response, also the stimulation of IgA may detect in acute and reactivation states. So the non-significant relation may 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, 29]. 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 anti- bodies 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 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[32-34]. Thus, IL-4 during the course of *T. gondii* infection in an individual host can play both disease-protective and exacerbative roles [32].

Because IL-4 has been demonstrated to significantly modulate immune responses in *T. gondii* infection, the IL-12 acts synergistically with IL-18 to produce IFN-γ by NK cells and IFN-γ 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 [34, 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].

5. 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 .

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