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Determination the Time of Toxoplasmosis among Pregnant Women by using IgG Avidity to Various *Toxoplasma Gondii* Antigens

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Abstract

Toxoplasmosis is caused by *Toxoplasma gondii*, which is an intracellular protozoan parasite. Its main host is the cat. It is one of the most common human parasites. The main objective of this study is to determine the time of *T. gondii* infection among pregnant women by using avidity test of *Toxoplasma* IgG to various *Toxoplasma* antigens. The study period was from August 2017 to February 2018 to study the time of toxoplasmosis among 180 pregnant women and 100 non pregnant married women as control attending to hospitals, primary health care centers and some private medical laboratories. The pregnant women were examined for Toxo-IgG seroprevalence by using ELISA technique, and then examined their avidity of antibodies for specific *Toxoplasma* antigens by using line immune assay. The rates of Toxo-IgG seropositive were64 (35.56 %) among pregnant women. Regarding the reactivity of determined Toxo-IgG against various *Toxoplasma* antigens, the rates were 40(62.50%), 39 (60.93%), 63(98.43%) ,55(85.93%) ,63(98.43%), 62(96.87%) ,41(64.04%) and 42(65.62) positive for *Toxoplasma* ROP1C, MIC3, GRA7, GRA8, p30, MAG1,GRA1,rSAG1 antigens, respectively. Considering the avidity of Toxo-IgG for these antigens, the rates of high avidity were higher than low and intermediate avidity. So, the highest rate of high avidity was 85.0% for ROP1c antigen. It is concluded that the highest rate of predicting *Toxoplasma* infection among pregnant women was for a period of more than 6 months, which makes it less dangerous for maternal and fetal health.

Keywords: *Toxoplasma*; Avidity; ROP1c; MIC3; GRA7.

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تحديد وقت الإصابة بداء المقوسات الكوندية بين النساء الحوامل باستخدام رغبة الأجسام المضادة(جي) لمختلف مستضدات المقوسات الكوندية

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الملخص

داء القطط سببه طفيلي المقوسات الكوندية والتي تعيش داخل الخلية ومضيفها الرئيسي القطط وهي من الإصابات الطفيلية الشائعة في الإنسان. استهدفت الدراسة المقدمة لمعرفة فترة الإصابة بالمقوسات الكوندية بين النساء الحوامل عن طريق استخدام اختبار طمأنة ورغبة الأجسام المضادة نوع (جي) على مختلف مستضدات المقوسات الكوندية. حيث أجريت الدراسة للفترة من آب 2017 إلى شباط 2018 على 180 امرأة حامل و100 امرأة متزوجة وغير حامل راجعن مستشفيات ومراكز الرعاية الصحية الأولية وبعض المختبرات الأهلية. تم فحص مصل الدم للنساء الحوامل لإيجاد الأجسام المضادة نوع (جي) للمقوسات الكوندية باستخدام تقنية الايلايزا، ثم معرفة قابلية وتفاعل تلك الأجسام المضادة للمستضدات الخاصة بالمقوسات باستخدام اختبار المناعة الخطي الخاص بذلك. حيث كانت نسبة الأجسام المضادة نوع (جي) في مصل تلك النساء الحوامل 64(35.56 ٪). وفيما يتعلق بتفاعلية تلك الأجسام المضادة لمختلف المستضدات الخاصة كانت النتائج 40 (٪ 62.50) و 39 (60.93٪) و 63 (98.43٪) و 55 (85.93٪) و 63 (98.43٪) و 62 (96.87٪) و 41 (64.04٪) و 42 (65.62) موجبًا ل ROP1C، MIC3، GRA7، GRA8، p30، MAG1، GRA1 و rSAG1 بالترتيب. أما بالنسبة لقوة الرغبة لتلك الأجسام المضادة للمستضدات حيث كانت معدلات الرغبة العالية أعلى من الرغبة المنخفضة والمتوسطة، وكانت أعلى معدل من الرغبة العالية 85.0 ٪ لمستضد ROP1c. وان أعلى نسبة لتوقع فترة الإصابة بين النساء الحوامل كانت لفترة منذ أكثر من 6 أشهر وهذا ما يجعلها أقل خطورة على صحة الأم والجنين.

الكلمات الدالة:.*Toxoplasma*; Avidity ;ROP1c; MIC3 ; GRA7

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1. Introduction:

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite, infecting a wide range of warm-blooded animals, including humans [1]. Various ways may cause the *T. gondii* infection to humans, including consumption of: uncooked meat of animals having tissue cysts, food or water contaminated with infected cat feces or by infected environmental samples (e.g. soil, cat litter). Adding to that, the infection of a fetus transplacentally from the mother [2,3].

The *T. gondii* infection has a major complication represented by congenital infection [4,5]. Fetus may be infected by *T. gondii* via mother who has this infection for the first time during pregnancy. Because of immature immune response of fetus, it may experience severe sequelae. This includes ocular and/or mental impairment, epilepsy, hydrocephalus or intrauterine death and abortion [6]. As the pregnancy advances to the end, the transplacental transmission risk becomes higher; however, the first trimester of pregnancy is exposed to the highest risk of severe sequelae [7].

The *T. gondii* possesses specialized electron dense secretory organelles, namely micronemes, rhoptries and dense granules containing characteristic proteins, like microneme proteins (MICs), rhoptry proteins (ROPs) and dense granule proteins (GRAs), respectively. These proteins are considered to play an essential role in intracellular parasitism invasion of vertebrate cells by the protozoan *T.gondii*. Binding to the host cell, triggered apical release of the micronemal protein MIC at the tight attachment zone was formed between the parasite and the host cell. In the second step, invagination of the host cell plasma membrane was initiated by discharging the rhoptry protein ROP to form a nascent parasitophorous vacuole (PV). ROP is fully discharged into the vacuole upon completion of the invasion time. In contrast to these very rapid early events, release of the dense granule markers GRA [8-10].

The net antigen binding force of antibodies population is defined by terms of “avidity” or “functional affinity”. These terms are more preferred than the “affinity” term. The IgG avidity can be measured by determining the antigen binding force of specific IgG antibodies [11] by using reagents to remove IgG from the immobilized antigen. As a result, IgG antibodies of low avidity are almost completely dissociated, in conditions where high avidity antibodies mostly remain antigen-bound [12,13]. The presence of high avidity IgG antibodies can be used to exclude recently acquired infection [11,13]. The IgG avidity test was developed to

help discriminate between past and recently acquired infection [11]. The *T. gondii* infection is most commonly diagnosed by detecting anti-*Toxoplasma* immunoglobulin including IgG antibodies in the blood [12,14,15].

The IgG avidity index (AI) results are calculated depending on the strength between the IgG and the microbial antigens epitope following treatment with denaturing agents and expressed as percentage. The interpretation of IgG avidity index (AI) results has been determined as follows: AI < 50% = low avidity antibodies indicating acute primary infection, AI 50-60% = intermediate avidity and AI > 60% = high avidity antibodies indicating past infection [16-18].

2. Materials and Methods:

A cross sectional study was carried out in Kirkuk governorate from August 2017 to February 2018 for studying the time of toxoplasmosis among 180 pregnant women whose age rangesbetween18-42 years attending Azadi General Teaching Hospital, Kirkuk General Hospital and some primary health care centers and private medical laboratories. The pregnant women were examined for Toxo-IgG seroprevalence by using ELISA technique, and then examined for the avidity of determined Toxo-IgG for various specific *Toxoplasma* antigens (ROP1C, MIC3, GRA7, GRA8, p30 MAG1, GRA1, rSAG1) separately by using line immune (*RecomLine; Mikrogen, GmbH, Germany*) assay. Computerized statistical 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 to be statistically significant (S), less than 0.01 is considered as highly significant (HS), then greater than 0.05 is considered as non-significant.

3. Results:

A total 180 pregnant women whose age ranged between (18-42 years old) were examined for seroprevalence of specific Toxo-IgG by using ELISA technique. The seroprevalence of Toxo-IgG was 64(35.56%) among pregnant women. While the seroprevalence among 100 control (non pregnant) married women was 5 (5.0 %), as shown in Table 1.

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Table 1: The seroprevalence of specific Toxo-IgG among pregnant women and control by using ELISA technique.

Results	Toxo- IgG Antibody			
	Pregnant women		Control	
	No .	%	No .	%
Positive	64	35.56	5	5
Negative	116	64.44	95	95
Total	180	100	100	100
$X^2 = 32.32$ P = 0.00001 P <0.01 Highly Significant				

Regarding the specificity of the determined specific Toxo-IgG against the various *Toxoplasma* antigens (ROP1C, MIC3, GRA7, GRA8, p30 MAG1, GRA1, rSAG1) was identified separately by using line immunoassay. The rates of Toxo-IgG against these antigens among the total 64 Toxo-IgG seropositive pregnant women were 40(62.50%), 39(60.93%), 63(98.43%), 55(85.93%), 63(98.43%), 62(96.87%), 41(64.04%) and 42(65.62) seropositive for *Toxoplasma* ROP1C, MIC3, GRA7, GRA8, p30, MAG1, GRA1, rSAG1 antigens, respectively as shown in Table 2.

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

T. gondii antigens	Toxo-IgG seropositive					
	Positive		Negative		Total	
	No .	%	No .	%	No .	%
ROP1c	40	62.50	24	37.50	64	100
MIC3	39	60.93	25	39.07	64	100
GRA7	63	98.43	1	1.57	64	100
GRA8	55	85.93	9	14.07	64	100
p30	63	98.43	1	1.57	64	100
MAG1	62	96.87	2	3.13	64	100
GRA1	41	64.06	23	35.94	64	100
rSAG1	42	65.62	22	34.38	64	100
$X^2 = 83.80$ P =0.000001 P <0.01 Highly Significant						

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Regarding the avidity of the determined Toxo-IgG to various used *Toxoplasma* antigens, the rates of high avidity was higher than low and intermediate avidity. So, the highest rate of high avidity was 85.00% for ROP1c antigen; while the highest rates of low avidity was 24.39% for GRA1 antigen and for intermediate avidity was 4.76% for p30 antigen, as shown in Table 3.

Table 3: The avidity of Toxo-IgG seropositive to various *Toxoplasma* antigens.

T. gondii antigens	Toxo-IgG seropositive							
	IgG avidity							
	High		Low		Intermediate		Total	
	No .	%	No .	%	No .	%	No .	%
ROP1c	34	85.00	5	12.50	1	2.50	40	100
MIC3	30	76.92	8	20.51	1	2.57	39	100
GRA7	51	80.95	10	15.85	2	3.20	63	100
GRA8	45	81.81	8	14.54	2	3.65	55	100
p30	48	76.19	12	19.05	3	4.76	63	100
MAG1	50	80.64	10	16.12	2	3.24	62	100
GRA1	30	73.17	10	24.39	1	2.44	41	100
rSAG1	32	76.19	9	21.42	1	2.39	42	100
X^2 = 4.09 P =0.995 P > 0.05 Non significant								

Considering the time of toxoplasmosis among Toxo-IgG seropositive pregnant women depending on Toxo-IgG avidity to various Toxo-antigens, as described in Table 4. From the total 64 pregnant women whose suspected toxoplasmosis was for more than 6 months were 50.00% .So, 35.95% of them are suspected to probably infection since 3-6 months; while the remain 14.06% of them are suspected to have toxoplasmosis within less than 3 months ago.

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Table 4: Determination of the time of toxoplasmosis using avidity testing by evaluation of the avidity of bands.

Suspicion of toxoplasmosis	Toxo-IgG avidity for specific Toxo-antigens				Total seropositive	
	p30	MAG1	GRA1	rSAG1	No.	%
<3months	No high avidity for p30, MAG1and GRA1, rSAG1				9	14.06
3-6months	If high avidity for p30 or MAG1and/or GRA1				23	35.94
> 6 months	High avidity for rSAG1				32	50.00
Total					64	100

4. Discussion:

In this study, the rate of Toxo-IgG seropositive was 35.56% among pregnant women in comparison to the lower rate which was 5.0% among control group by using ELISA technique with highly significant relation P< 0.01, as shown in Table 1. The different results recorded among these two groups may be due to the immunological change in hormonal imbalance during pregnancy which leads to increasing the rate of toxoplasmosis among pregnant women. In agreement with other studies in Iraq and other countries that used ELISA technique for Toxo-IgG seroprevalence among pregnant women, this study showed occasionally the same or close results as in studies done in Najaf 30.76%, in Erbil 29.05%, in Turkey 37% and in Sudan 34.1% [19-23]. This low variation of ELISA result may be attributed to the differences in hygienic, socioeconomic, and cultural factors. Also, most of these countries are regarded as developed countries. This variation in prevalence can be explained by several factors including the number and presence of the cats, climate and cultural and ethnic practices. Direct contact with cats is not required for transmission due to the longevity of oocysts in the environment [24,25]. Studies from different countries showed that the prevalence of antibody to *T. gondii* among women of childbearing age in developing countries and in populations with low socioeconomic status is generally higher than that in developed countries [26].

Concerning the reactivity of determined Toxo-IgG, the current study showed the different rates of Toxo-IgG reactivity with various *Toxoplasma* antigens. The highest rate of reaction was 98.43% for GRA7 and p30 antigens; while the lowest rate was 60.93% for MIC3 antigen with highly significant relation p <0.01, as shown in Table 2. The different rates of Toxo-IgG

with the various *Toxoplasma* antigens may be due to the stimulation of immune response to the *T. gondii* depending on the process of *T. gondii* exposure and expression of 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 of the humeral immune response. "The IgG response is particularly characterized by GRA7, GRA8, p30, MAG1, GRA1 and rSAG1. While IgG antibodies against GRA7 are normally already detected at the beginning, followed by p30, IgG antibodies against MAG1 and GRA1 only occur somewhat later" [16].

The current study revealed that the rates of high avidity of Toxo-IgG to various *Toxoplasma* antigens is higher than low and intermediate avidity. The range of high avidity was between 73.17% and 85.00%; while the highest rate of low avidity was 24.39% for GRA1 and the highest rate of intermediate avidity was 4.76% for p30 antigen with non-significant relation, as shown in Table 3. The different rates of the Toxo-IgG to various *Toxoplasma* antigens may be due to the specificity of theses antigen and its epitopes as a target for humoral immune responses following the period infection. So, the realization of non-significant relation may be due to that the rate of high avidity is equal or similar for most *Toxoplasma* antigens.

To estimate the time of infection, various serologic markers were used. In general, positive immunoglobulin G (IgG) confirms *Toxoplasma* infection, but gives no indication as to when this event took place [12,14,15]. An IgG avidity test is now widely used to differentiate between acute and chronic *Toxoplasma* infections [27-29].

Evaluation of avidity: p30, MAG1, GRA1 and rSAG1 was done by using the *Toxoplasma* antigens. In most cases, determination of the avidity for individual antigens enables determining the status of infection more accurately. Particularly, this is important in the distinction between a subsiding and an acute *Toxoplasma* infection. Typically, the IgG antibodies reactions against specific antigens begin in different phases of infection. These antibodies, which are successively mature from low-avidity to high-avidity, usually enables differentiating the status of infection by analyzing the IgG bands and avidity patterns [16].

The current study showed that the rate of suspecting toxoplasmosis among examined pregnant women depending on the typical Toxo-IgG reaction pattern was 50.0% for more

than 6 months of infection. This may be due to that most pregnant women are exposed to *Toxoplasma* infection before pregnancy. So, this reflects the lowering risk of maternal *Toxoplasma* infection to their fetus as seen in our community, which was few or rare case of fetal *Toxoplasma* infection and its complication from infected mother. While the rate of infection since less than 3-6months was 35.95% and the lowest was the rate of suspicion to toxoplasmosis since less than 3 months ago, which was 14.09%,as showed in Table 4.

5. Conclusions:

The highest rate of pregnant women was suspected to be infected by *Toxoplasma* infection since more than 6months and this makes it less dangerous for maternal and fetal health.

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