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Interleukin-8 Level in Pregnant Women with Toxoplasmosis in Kirkuk City

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Abstract

A cross sectional study was carried out in Kirkuk city from 15th of June 2018 to 15th of December 2018. The study included 100 pregnant women and 50 healthy individuals. Their ages ranged between 17-45 years old who were admitted to Kirkuk general hospital.. Molecular tests for real-time PCR and serological testing for detection specific *Toxoplasma gondii* IgM and IgG and Interleukin-8 level by using ELISA technique was done for patients and control. The study showed that the highest rate of anti *T. gondii* IgM+ IgG- antibodies (10%) was recorded among pregnant women compared with 8% in the control group, while 22% of pregnant women were IgM+IgG+ compared 6.5% of the healthy control group. The study revealed that 40.91% of pregnant women with positive ELISA was positive by PCR compared with 0% of patients with negative ELISA results. The study showed that the highest rate of *T. gondii* infection (diagnosed by PCR) were recorded among pregnant women at age group 27-36 year (22.55%) and the lowest rate was within the age group 17-26 year. The highest mean level of IL-8 recorded PCR +ve groups, in pregnant women (79.2 ±53.2 ng/ml) compared with PCR –ve groups. There was a highly significant differences of IL-8 between pregnant women and the control group. The study showed that the highest mean level of IL-8 (77.61±60.4 ng/ml), in pregnant at 2nd trimester of pregnancy, followed by 3rd trimester. This study was concluded that a highly elevation of IL-8 level was correlated Toxoplasma infection in pregnant women and real time PCR is golden method in diagnosis of toxoplasmosis.

Keywords: *Toxoplasma gondii*, Interleukin-8, Pregnant women, PCR.

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علاقة انترلوكين-8 مع داء المقوسات في النساء الحوامل

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الملخص

أجريت الدراسة في مدينة كركوك للفترة من 15 حزيران 2018 الى 15 كانون الأول 2018. شملت الدراسة 100 امرأة حامل و 50 فرداً سليماً كمجموعة سيطرة واللاتي كانت اعمارهن 17-45 سنة وكن يراجعن مستشفى كركوك العام. أجريت فحوصات الكشف عن الحمض النووي للمقوسات الكوندية باستخدام فحص تفاعل البلمرة المتسلسل PCR وفحص الايلايزا ELISA للكشف عن الاجسام المضادة ومستوى انترلوكين-8 في جميع امصال المرضى والاصحاء في الدراسة. أظهرت الدراسة ان اعلى نسبة للأجسام المضادة IgM+ IgG- للمقوسات الكوندية (10%) في النساء الحوامل مقارنة 8% في مجموعة السيطرة بينما كان 22% من النساء الحوامل حاملات للأجسام المضادة نوع IgM+IgG+ للمقوسات الكوندية مقارنة بـ 6،5% في مجموعة السيطرة. كما أظهرت الدراسة أن 40،91% من النساء الحوامل والمشخصات بفحص الايلايزا أظهرن نتيجة موجبة للإصابة باستخدام مصابات فحص تفاعل البلمرة المتسلسل بينت الدراسة ان 22% من النساء الحوامل المصابات بالطفيلي كن ضمن الفئة العمرية 27-36 سنة وان اقل نسبة من الإصابة كانت ضمن الفئة العمرية 17-26 سنة. كشفت الدراسة ان اعلى معدلات انترلوكين-8 قد سجلت في النساء الحوامل المصابات بالمقوسات الكوندية (97،2 ±53،2 نانوغرام/مل) مقارنة مع النساء غير المصابات ومجموعة السيطرة وان هنالك فرق معنوي كبير بين النساء الحوامل ومجموعة السيطرة بما يخص مستوى انترلوكين-8. أظهرت الدراسة أن اعلى مستوى لانترلوكين-8 قد سجل في النساء الحوامل اللاتي كن في الثلث الثاني هن الحمل (77،61 ±60،61 نانوغرام/مل) يليه النساء اللاتي في الثلث الثالث من الحمل.

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يستنتج من الدراسة ان هنالك علاقة قوية بين إصابة الحوامل بداء المقوسات وارتفاع مستوى انترلوكين-8 وان فحص تفاعل البلمرة المتسلسل PCR هو افضل طريقة لتشخيص الطفيلي.

الكلمات الدالة: المقوسات الكوندية، انترلوكين-8، النساء الحوامل، تفاعلات البلمرة المتسلسة.

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1. Introduction:

Toxoplasmosis is a very common infection caused by the obligate intracellular protozoan parasite [1]. This parasite is called *Toxoplasma gondii* widely distributed around the world. *Toxoplasma gondii* can be vertically transmitted to the fetus during pregnancy and may cause wide range of clinical manifestations in the offspring depending on the gestational age at which the primary maternal infection was acquired, the virulence of the parasite and the immunologic development of the fetus [2]. The women may have spontaneous abortions, stillbirths, or premature delivery in addition to various fetal anomalies [3]. The frequency of severe congenital infections can be limited by early screening for specific antibodies to *Toxoplasma gondii* in the serum of pregnant women [1]. Toxoplasmosis during pregnancy can cause congenital infection and manifest as mental retardation and blindness in the infant, the severity of fetal disease varies inversely with gestational age at which maternal infection occurs [4]. Interleukin-8 (IL-8) is produced by macrophages and other cell types such as epithelial cells and endothelial cells. Primary function of IL-8 is the induction of chemotaxis in its target cells like neutrophil and granulocytes [5]. IL8 has an important role in the innate immune response. Interleukin-8 is often associated with inflammation. It has been cited as a pro-inflammatory mediator in Toxoplasmosis [6]. It is well recognized that T cell-mediated immunity plays a central role in the host response to intracellular pathogens [7]. T cell- mediated immunity and activated macrophages have been shown to play important roles in resistance to *T. gondii* infection [6]. The aim of the study was to evaluate the role of IL-8 level in pregnant women in the presence of *T. gondii* DNA and compared with healthy control.

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2. Material and Methods:

A cross sectional study was carried out in Kirkuk city from 15th of June 2018 to 15th of December 2018. The number of pregnant women understudy were 100. The ages of the patients were between 17-45 years. Those patients admitted to Kirkuk general hospital. The control group were matched to the patients, included 50 healthy individuals (patient's relatives). Five ml of blood was collected by vein puncture using 5 ml syringe from each patient and control group enrolled in this study. Blood samples were placed in two tubes, one of them containing anticoagulant EDTA and used for molecular tests of *Toxoplasma gondii* (using kit from Sacace biotechnology-Iraly, Toxo-DNA Real-TM Qualit). The second part of the sample (2) ml was placed in plane tubes, left for 30 minutes at 37 °C for clotting and centrifuged at 3000 rpm for 15 minutes, the obtained sera was aspirated using automatic micropipette and transferred to Eppendorf tubes and stored in deep freeze at -20°C for serological testing for detection of specific *Toxoplasma gondii* IgM and IgG and IL-8 level by using ELISA technique (Komabiotech, India).

3. Results:

The study showed that the highest rate of anti *T. gondii* IgM+ IgG- antibodies (10%) was recorded among pregnant women compared with 8% in the control group, while 22% of pregnant women were IgM+IgG+ compared with 6% of the healthy control group as shown in Table 1.

Table 1: Results of anti *T. gondii* IgM and IgG antibodies among the study groups.

Study groups	No. of examined	Results of ELISA	No. of infected	%
Pregnant women	100	IgM(+) IgG+ve	22	22
		IgM(+) IgG-ve	10	10
		IgM(-) IgG+ve	12	12
		IgM(-) IgG-ve	56	56
Control group	50	IgM(+) IgG+ve	3	6
		IgM(+) IgG-ve	4	8
		IgM(-) IgG+ve	3	6
		IgM(-) IgG-ve	40	80

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The study revealed that 40.91% of pregnant women with positive ELISA was positive by PCR compared with 0% of patients with negative ELISA results with sensitivity and specificity of 40.91% and 100% respectively with highly significant relation (P: ≤0.01) as shown in Table 2.

Table 2: Comparison between *Toxoplasma* total antibodies by ELISA and real-time PCR in pregnant women.

Results of ELISA	No. (100)	Results of RT-PCR				P. value	Sensitivity	Specificity
		Positive		Negative				
		No.	%	No.	%			
Total positive	44	18	40.91	25	59.09	0.0004 HS	40.91%	100%
Negative	56	0	0	17	100			

The Table 3 shows that the highest rate of those who had IgM+ and IgG - *T.* in pregnant women was positive by PCR (70%) and 50% of patients with IgM+ IgG+ antibodies with non-significant relation (P: >0.05).

Table 3: Comparison of the result for *Toxoplasma* IgM and IgG antibodies by ELISA and real-time PCR in pregnant women.

	No.	Results of RT-PCR				P. value
		Positive		Negative		
		No.	%	No.	%	
IgM(+) IgG+ve	22	11	50	11	50	0.11 NS
IgM(+) IgG-ve	10	7	70	3	30	
IgM(-) IgG+ve	12	0	0	12	100	
IgM(-) IgG-ve	56	0	0	56	100	
Total	100	18	18	82	70	

The present study showed that the maximum rate (30%) of pregnant women in third trimester of pregnancy were positive by PCR followed by 16.67 of second trimester pregnant women and 7.41 of first trimester pregnant women, Fig. 1.

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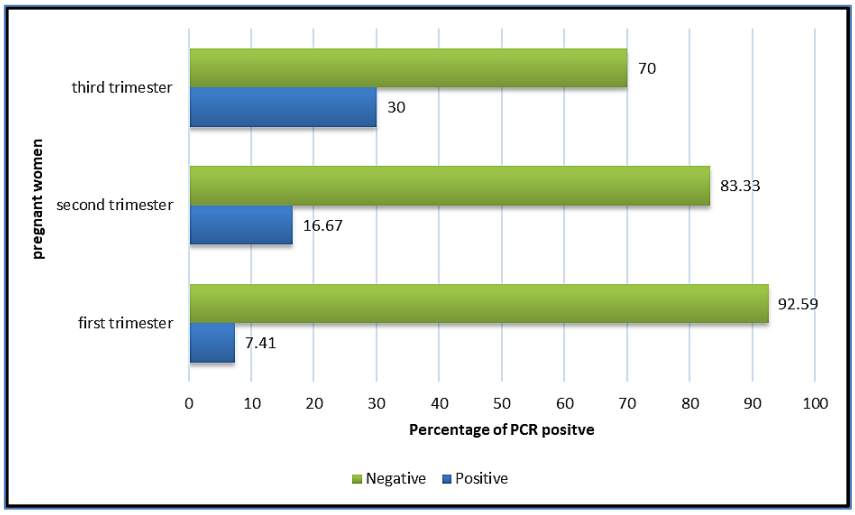

Fig. 1: Distribution of real-time PCR of *T. gondii* DNA result according to trimester of pregnancy, (P. value: 0.081 (NS)).

The study showed that the highest rate of *T. gondii* infection (diagnosed by PCR) were recorded among pregnant women within the age group 27-36 year (22.55) and the lowest rate was within the age group 17-26 year with non-significant relation (P: >0.05) as shown in Table 4.

Table 4: Distribution of *T. gondii* according to age groups by using real-time PCR.

Age groups (years)	Total No.	Pregnant women			
		PCR +ve	%	PCR -ve	%
17-26	26	3	11.54	22	88.46
27-36	40	9	22.5	31	77.5
37-46	34	6	17.65	28	82.35
Total	100	P. value: 0.88 (NS)			

The study showed that the highest mean level of IL-8 was recorded in PCR +ve groups in pregnant women (79.2 ±53.2 ng/ml) compared with PCR –ve groups, Table 5.

Table 5: Level of IL-8 among pregnant women in relation to *T. gondii* infection

Study groups	IL-8 (ng/ml)	PCR +ve	PCR -ve	P. value
Pregnant women	No.	18	82	0.0005
	Mean± SD.	79.2 ±53.2	53.3 ±38.17	

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The study demonstrated that there was a highly significant differences in the level of IL-8 between pregnant women and the control group (P: 0.0001), Table 6.

Table 6: Interleukin-8 level in pregnant women infected by (PCR result) *Toxoplasma* and the control group.

IL-8 level (ng/ml)	Pregnant women (PCR +ve)	Control group (PCR-ve)	P. value
No.	18	44	0.0001
Mean±SD.	79.2 ±53.2	50.29±20.3	

The study showed that the highest mean level of IL-8 (77.61±60.4 ng/ml), was recorded in pregnant women in the 2nd trimester of pregnancy, followed by 3rd trimester. The result was significant, Table 7.

Table 7 Relation of IL-8 level with gestational time of pregnancy

Pregnant women	No.	IL-8 level (ng/ml)	
		Mean ±SD.	P. value
1st trimester	28	47.21±22.9	0.0433 (S)
2nd trimester	42	77.61±60.4	
3rd trimester	30	73.5±52.5	

4. Discussion:

Regarding the seroprevalence of *T. gondii* in pregnant women, Al-Rawazq [8] found that the seroprevalence of anti-IgG and IgM antibodies in pregnant women were 40 (36.4 %) and 16 (13.6 %) respectively which is near of the current findings. The current result of pregnant women was in agreement with Tawfeeq [9] who demonstrated that the rates for anti Toxo-IgM (-) / IgG (+) was 25.0 % ,Toxo-IgM (+)/IgG (-) was 7.90% and Toxo-IgM (+) / IgG (+) was 7.37%. A slightly higher prevalence was reported by Muslim *et al* [10], Munoza *et al* [11] and Paschale *et al* [12] there was significant differences between anti-IgG, IgM antibodies. The variation in the rates of *T. gondii* antibodies may be attributed to the fact that different techniques and different companies , each differs in sensitivity and specificity, while the wide variation in the rates within same and different countries may be due to differences in hygienic, socioeconomic, and cultural factors [9].

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The real-time PCR offered the possibility of assessing disease progression and treatment efficacy [1,2]. Menotti *et al* [13] found that *T. gondii* DNA was detected more accurately by PCR optimum sensitivity when compared with ELISA. Although serological testing has been one of the major diagnostic techniques for toxoplasmosis, it has many disadvantages, for example, it may fail to detect specific anti-*Toxoplasma* immunoglobulin G (IgG) or IgM during the active phase of *T. gondii* infection, because these antibodies may not be produced until after several weeks of parasitaemia [14]. Therefore the high risk of congenital toxoplasmosis of a fetus may be undetected because the pregnant mother might test negative during the active phase of *T. gondii* infection [15]. Nimri *et al* [13] showed that 47.7% and 36.15% of high risk pregnant women had positive results for ELISA anti-*T. gondii* IgG and IgM respectively and 56.1% of them had positive PCR results with sensitivity and Specificity of ELISA IgG were 71. % & 63% respectively. Although the diagnosis of patients with toxoplasmosis has been faced by a number of problems, the most frequent challenge encountered by physicians all over the world [17]. While infection in early pregnancy poses a small risk of fetal transmission (less than 6%), rates of transmission range between 60% and 81% in the third trimester [2]. Conversely, although the transmission of *T gondii* during embryogenesis is rare, it results is far more serious effects on the fetus [7]. In contrast, maternal infection in the third trimester often results in asymptomatic newborns. However, if not treated appropriately, these newborns might develop retinochoroiditis and neurologic deficits in childhood or early adulthood [8,9] da Silva *et al* [18] found that 92.3% of pregnant women with toxoplasmosis were during the first trimester of gestational age. Tawfeeq [9] revealed that 49.48 % of seropositive Toxo- IgG was seen within 3rd trimester of pregnancy with highly significant relation $P<0.01$. Al-Hussien *et al* [19] found that the highest infection rates were found at 26- 30 age group, while the lowest infection rate were found at age groups 36-40. Al-Rawazq [8] found that the seropositivity was observed higher in the age group between 20 to 30 years (37.1%). Khalil [20] found that *Toxoplasma* antibodies increase with age especially in the age group 25-30 years. This association does not mean that older age is a risky factor to predisposed to infection but might be explained by the older the person, the longer time being exposed to the causatiev agent and may retain a steady level of anti-*Toxoplasma* IgM in serum [21].

The chemokine response is dependent on invasion by live tachyzoites and subsequent host cell lysis. IL-8 is responsible for activation and recirculation of neutrophils and neutrophils

can phagocyte and kill or inhibit tachyzoites of *Toxoplasma* and showed that human intestinal epithelial cells infected with *T. gondii* elicit rapid secretion of IL-8 [22]. Mohamed *et al* [23] indicated an increase of IL-8 in patients compared with healthy control and the highest mean level was recorded within the age group 29-39 year. Ali *et al* [24] indicated that the mean serum concentration of IL-8 in chronic and acute phase of *T. gondii* infection in pregnant women were more elevated than in healthy control. The chemokine response is dependent on invasion by live tachyzoites and subsequent host cell lysis. Furthermore, supernatants or lysates from *T.gondii* infected fibroblasts could elicit significant IL-8 secretion [25].

Increased level of IL-8 correlates with early acute inflammation or with a reactive form of toxoplasmosis. IL-8 is responsible for activation and recirculation of neutrophils and neutrophils can phagocytose and kill or inhibit tachyzoites of *Toxoplasma* and showed that human intestinal epithelial cells infected with *T. gondii* elicit rapid secretion of IL-8, so it has an important role in innate immunity in response to *Toxoplasma* [26]. In agreement with the present results, Borges *et al* [27] found that IL-8 was significantly increased in acute with early acute inflammation or with a reactive from toxoplasmosis in pregnant women.

5. Conclusions:

It was concluded that *T. gondii* infection was a highly related to elevation of IL-8 level in pregnant women and real time PCR is golden method in diagnosis of toxoplasmosis

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