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

Ahlam Ahmed Mahmood1, Zainab Sulaiman Rezaig2
1 Kirkuk Health Directorate, Kirkuk, Iraq.
2 Department of Microbiology College of Medicine, Tikrit University, Tikrit, Iraq.
1dr.ahlamahmaad@gmail.com, 2ah70.tucom@gmail.com

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.

DOI: http://doi.org/10.32894/kujss.2019.14.3.6
علاقة إنترلوكين-8 مع داء المقوسات في النساء الحوامل

أحلام أحمد محمود١، زينب سليمان رزيك٢

١ دائرة صحة كركوك، كركوك، العراق
٢ فرع الأحياء المجهرية، كلية الطب، جامعة تكريت، تكريت، العراق

المرخص

أجريت الدراسة في مدينة كركوك للفترة من 15 حزيران 2018 إلى 15 كانون الأول 2018. شملت الدراسة 100 امرأة حامل و 50 فردًا 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 البممرة المتسلسل PCR للكشف عن الأجسام المضادة ومستوى إنترلوكين-8 في جميع امصال المرضى والإصحاء في الدراسة. أظهرت الدراسة أن أعلى نسبة للأجسام المضادة IgM+ IgG- للمقوسات الكوندية (10%) في النساء الحوامل مقارنة بالحوامل السليمة. في جميع امصال الدراسة، أظهرت الدراسة أن 8% من النساء الحوامل السليمة كان حاملات الأجسام المضادة IgM+IgG+ للمقوسات الكوندية مقارنة ب 6.5% في مجموعة السيطرة. كما أظهرت الدراسة أن 40.91% من النساء الحوامل والمشخصات بفحص الإيلازا أظهرت نتيجة موجبة للإصابة باستخدام مصابات فحص تفاعل البلمرة المتسلسل بين الدراسة 22% من النساء الحوامل في الفئة العمرية 17-26 سنة. كشفت الدراسة أن معدلات إنترلوكين-8 قد سجلت في النساء الحوامل المصابة بالفيروسات الكوندية (79.2±32 نانوغرام/مل) مقارنة مع النساء غير المصابات ومجموعة السيطرة. وهكذا فرق معنوي كبير بين النساء الحوامل والمجموعة السيطرة. وأظهرت الدراسة أن أعلى مستوي إنترلوكين-8 قد سجل في النساء الحوامل اللائي في الثلث الثاني من الحمل (77.61±61 نانوغرام/مل) بالمقارنة مع النساء اللائي في الثلث الثالث من الحمل.
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.
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 |
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 |
|------------------|-----------|-------------------|----------|-------------|-------------|
|                  | 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.**

| Results of RT-PCR | No. | Positive | Negative | P. value | Sensitivity | Specificity |
|-------------------|-----|----------|----------|----------|-------------|-------------|
|                   | 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.
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       |                |        |      |         | 88.46 |

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 |
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   |
| 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].
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 causatieve 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

**References**

[1]  J. Flegr, "*Host Manipulation by Toxoplasma gondii, In: Encyclopedia of Parasitology*, 4th Ed., Springer, Berlin Heidelberg, (2016).

[2]  B. J. Bogitsh, C, E, Carter and T.N, Oeltmann, "*Human parasitology*, 5th Ed., Academic – Elsevier, USA (2018).

[3]  D. Bass, G. D. Stentiford, D.T. Littlewood and H. Hartikainen, "*Diverse applications of environmental DNA methods in parasitology*", Trends in Parasitology, 31(10), 499 (2015).
A. B. Vaidya, "Reflections on an inflection: From virology to parasitology guided by POLARIS", PLoS pathogens, 14(6), e1006941 (2018).

C. L. Pomares and J. G. Montoya, "Laboratory diagnosis of congenital toxoplasmosis", Journal of clinical microbiology, 54(10), 2448 (2016).

J. Matowicka-Karna, V. Dymicka-Piekarska and H. Kemona, "Does Toxoplasma gondii infection affect the levels of IgE and cytokines (IL-5, IL-6, IL-10, IL-12, and TNF-alpha)" , Clinical and Developmental Immunology, 20, 25 (2009).

J. S. Friedland, R. J. Shattock and J. D. Johnson, "Differential cytokine gene expression and secretion after phagocytosis by a human monocyctic cell line of Toxoplasma gondii compared with Mycobacterium tuberculosis", Clinical & Experimental Immunology, 91(2), 282 (1993).

H. S. Al-Rawazq, "Seroprevalence of Immunoglobulin G (IgG) and Immunoglobulin M (IgM) and Risk Factors of Toxoplasmosis for A sample of Pregnant Women in Baghdad", Al-Kindy College Medical Journal, 13(2), 43 (2017).

S. K. Tawfeeq, "Use of specific avidity antigens as a marker for determination of toxoplasmosis in relation to some hormones and cytokines in pregnant women", PhD Thesis, College of medicine, Tikrit University, (2018).

T. M Muslim, A. R. Saeed and W. T. Tawfeeq, "Seroprevalence and associated factors of Toxoplasma infection among sample of pregnant women in Wassit Governorate-Iraq", Medical Journal of Babylon, 9(4), 873 (2012).

B. C. Munoz, L. C. Guardia and M. T. Juncosa, "Toxoplasmosis and pregnancy, Multicenter study of 16,362 pregnant women in Barcelona", Medicina Clinica, 123, 12 (2004).
[12] M. D. Paschale, C. Agrappi and P. Clerici, "Seroprevalence and incidence of Toxoplasma gondii infection in the Legnano area of Italy", Clinical Microbiology and Infection, 14, 186 (2008).

[13] M. Menotti, G. Vilela and S. Romand, "Comparison of PCR-enzyme-linked immunosorbent assay and real-time PCR assay for diagnosis of an unusual case of cerebral toxoplasmosis in a stem cell transplant recipient", Journal of clinical microbiology, 41(11), 5313 (2003).

[14] Q. Liu, Z. D. Wang, S. Y. Huang and X. Q. Zhu, "Diagnosis of toxoplasmosis and typing of Toxoplasma gondii", Parasites & vectors, 8(1), 292 (2015).

[15] S. Belaz, J. P. Gangneux, P. Dupretz, C. Guiguen and F. Robert-Gangneux, "A 10-year retrospective comparison of two target sequences, REP-529 and B1, for Toxoplasma gondii detection by quantitative PCR", Journal of clinical microbiology, 53(4), 1294 (2015).

[16] L. Nimri, H. Pelloux and L. Elkhatib, "Detection of Toxoplasma gondii DNA and specific antibodies in high-risk pregnant women", The American journal of tropical medicine and hygiene, 71(6), 831 (2004).

[17] P. Mousavi, H. Mirhendi and Mohebali M., "Detection of Toxoplasma gondii in acute and chronic phases of infection in immunocompromised patients and pregnant women with real-time PCR assay using TaqMan fluorescent probe", Iranian Journal of Parasitology 13(3), 373 (2018).

[18] M. J. da Silva, M. C. Vinaud and A. M. de Castro, "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, 11, 10(11), e0141700 (2015).
[19] E. F. Al-Hussien, N. F. Nassir and A. Kadhim, "Study of Prevalence and Some Immunological Characteristics of Toxoplasma gondii Infections in Pregnant Women", Journal of University of Babylon, 24(2), 526 (2016).

[20] R. W. Khalil, "Seroprevalence of Toxoplasma gondii Among Aborted Women in Haditha", Al-Anbar Journal of Veterinary Sciences, 10(1), 100 (2017).

[21] H. A. Abdulameer, H. R. Tarish and A. J. Hassan, "Study The Some Aspects of The Immune Response For Pregnant Women Infected with T. gondii and Determine The Genotyping of This Parasite", kufa Journal for Nursing sciences 5(1), 223 (2015).

[22] S. Rougier, J. M. Montoya and F. Peyron, "Lifelong persistence of Toxoplasma cysts: a questionable dogma?", Trends in parasitology, 33(2), 93 (2017).

[23] K. I. Mohamed, M. S. Khadhum and H. Q. Abu-Al-Ess, "The Effect of Toxoplasma gondii on Interleukin-8, Interleukin-10, Leukotriene B4 and Calcium Levels in Aborted Women", International Journal of Medical Research and Health Sciences, 6(11), 76 (2017).

[24] W. R. Ali, "The role of some cytokines and trace elements in pregnant women with acute toxoplasmosis", Ibn Al-Haitham Journal For Pure And Applied Science, 29(2), 23 (2016).

[25] C. F. Denney, L. Eckmann, S. L. Reed, "Chemokine secretion of human cells in response to Toxoplasma gondii infection", Infection and Immunity, 67, 1547 (1999).

[26] C. H. Ju, A. Chockalingam and C. A. Leifer, "Early response of mucosal epithelial cells during Toxoplasma gondii infection", Journal of Immunology, 183,7420 (2009).

[27] M. Borges, T. Magalhães, C. Brito, N. Teixeira and C.W. Roberts, "How does toxoplasmosis affect the maternal-foetal immune interface and pregnancy?", Parasite Immunology, 24, e12606 (2018).