

Immune response in *Toxoplasma gondii* seropositive Iraqi aborted pregnant women in comparison with seronegative women

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Abstract

Background:

Toxoplasmosis is a disease caused by *Toxoplasma gondii* which affects one-third of the human population worldwide.

Materials and Methods:

The aim of this study was to determine the levels of Interferon (IFN- γ), Tumor Necrosis Factor (TNF- α), and Interleukin 10 (IL-10) in the blood of aborted pregnant women who were seropositive for *T. gondii* in comparison with the women in the first (pregnant and non-aborted) and second (non-pregnant and non-aborted) seronegative control groups living in Diyala and Erbil Provinces. IFN- γ , TNF- α , and IL-10 activities were determined by ELISA.

Results:

Aborted pregnant women living in Erbil Province who were seropositive for *T. gondii* had significantly higher ($P < 0.05-0.001$) levels of IFN- γ , TNF- α , and IL-10 when were compared with the women in the control groups. In contrast, no significant differences have been observed in the concentrations of IL-10 and TNF- α in the sera of the aborted pregnant women living in Diyala Province who were seropositive for *T. gondii* in comparison with the women in the control groups. Concerning the levels of IFN- γ , the results showed that there was a significant increment in the sera of the aborted pregnant women who were seropositive for *T. gondii* in comparison with the women in the first control group ($P = 0.027$). The results showed that the concentration of IFN- γ in the sera of the aborted pregnant women who were seropositive for *T. gondii* living in Erbil Province was significantly higher ($P = 0.0193$) than that in their counterparts living in Diyala Province, while no significant differences have been observed in the levels of both IL-10 and TNF- α .

Conclusions:

The immune response of the aborted pregnant women living in Erbil Province who were seropositive for *T. gondii* responded differently when compared to their age-matched counterparts living in Diyala Province.

Keywords: Toxoplasmosis; pregnant and aborted women, IL-10, IFN- γ and TNF- α .



INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by an obligate protozoan parasite called *Toxoplasma gondii* which affects one-third of the human population worldwide. The life cycle of this parasite includes mainly two stages, tachyzoite which is responsible for the acute infection and the bradyzoite which is responsible for the chronic infection. The bradyzoite is found in tissue cyst, which remains largely dormant for the life of host, but can reactivate and cause disease. It is important to mention that although the bradyzoite is not affected by any of the current drug treatments, it has been reported that the immune system can eliminate *T. gondii* cysts from the brains of infected hosts when immune T- cells are transferred into infected immunodeficient animals that have already developed large numbers of the cysts (1). Although the infection with *T. gondii* is asymptomatic in the majority of cases, it can have serious consequences in immunocompromised individuals. In addition, the congenital infection usually has serious clinical symptoms such as ocular infection, and posterior retinochoroiditis (2-4). The prevalence of toxoplasmosis and the severity of infection vary greatly from one country to another and some researchers have attributed that to a number of factors such as the status of the host immune system, the genotype of infective parasite strains, and the host genetic background (4-7). In Iraq, toxoplasmosis is prevalent all over the country and the prevalence rate ranges between 20% and over 60% (8-10).

It is important to mention that the immune system of the immunocompetent individuals can control the multiplication of the parasites and stops their dissemination, and stimulates the transformation of tachyzoites into bradyzoites, and the formation tissue cysts in many organs including the brain (11). Cytokines include a wide range of relatively low molecular weight, pharmacologically active proteins that are secreted by one cell for the purpose of altering either its own functions (autocrine effect) or those of adjacent cells (paracrine effect) (12). Cytokines are primarily synthesized by T- cells, monocytes, macrophages and endothelial cells and possess various important functions such as facilitation the communication among immune system cells, regulation of transcription factors, and control of inflammation, cell differentiation, proliferation and antibody secretion (13). Many studies have shown that cytokines play an important role in the pathogenesis of toxoplasmosis (7, 14, 15).

It has been reported that the infection with *T. gondii* enhances both humoral and cell-mediated immunity (16). The innate response to *T. gondii* includes the involvement of a wide range of cytokines such as interleukins, Interferon gamma (IFN- γ), Tumour Necrosis Factor (TNF), Nitrogen monoxide (NO), a Reactive Oxygen species (ROS) and many other factors. In addition, IFN- γ and α are important for controlling tachyzoite replication during both acute and chronic phases of infection, while IL-10 and IL-12 are important at the initial phase of infection but less important during the chronic stage of infection. Moreover, it has been reported that IL-12 is important in initiating an effective cell -mediated immunity against *T. gondii* tachyzoites, while IL-10 seems to modulate the production of both IL-12 and IFN - γ *in vivo*, in order to avoid an excessive immune response that could cause extensive inflammation and host tissue damage. In fact IL-10 and IL-12 have different roles in regulating IFN- γ synthesis during the initial stage of infection (17).

Although it has been known that some alterations in levels of antibodies and cytokines during the reactivation of *T. gondii* infection happen (11, 18), the role and function of cytokines, in cellular mediation, in the humoral response, as well as their impaired action during pregnancy and abortion processes need to be investigated. The present study was aimed to determine the levels of Interferon gamma (IFN- γ), Tumor Necrosis Factor alpha (TNF- α), and Interleukin 10 (IL-10) in the blood of aborted pregnant women who were seropositive for *T. gondii* in comparison with the healthy women in the first (pregnant and non-aborted) and second (non-pregnant and non-aborted) control groups of the same age group who live in Diyala and Erbil Provinces, Iraq.

MATERIALS AND METHODS

Subjects and Methods

One hundred and eighty (90 samples from women living in Diyala Province and 90 samples from the women who live in Erbil Province) blood samples were used in this study. The concentrations of cytokines were measured in the sera of aborted pregnant women (Group 1) who were seropositive for *T. gondii* as compared to the seronegative first control group (pregnant and non-aborted) (Group 2) and to the second control group (non-pregnant and non-aborted) (Group 3) living in the two Provinces. The blood samples were obtained during the period from March 2018 to February 2019. The ages of the participant women ranged between 20 and 40 years. A questionnaire has been given to each participant in order to get the required information regarding the age, the period of pregnancy, the number of miscarriages, the presence of chronic diseases or other inflammatory diseases, and the presence of animals or cats at home. Written informed consent was obtained from each participant in the study. The Human Ethics Committee at the College of Sciences (University of Diyala) has approved this study (Protocol 5/2019).

ELISA technology for determining the concentrations of some cytokines

The concentrations of three selected cytokines [Interferon gamma (IFN-γ), Tumor Necrosis Factor Alpha (TNF-α) and interleukin 10 (IL-10)] were measured in the sera of the selected women using the Enzyme Linked Immunosorbent Assay (ELISA) according to the instructions of the manufacturer.

Statistical Analysis

The concentration of each cytokine, per group of participants was determined by means (in pg/mL). The statistical analyses were made by the one way ANOVA for comparison between different groups using statistical software (SPSS version 21). P-value ≤ 0.05 was considered statistically significant.

RESULTS

It can be seen from Table 1 that the concentrations of the three cytokines (IL-10, TNF-α and IFN-γ) increased significantly in the sera of the aborted pregnant women who were seropositive for *Toxoplasma gondii* in comparison with the seronegative women in the first (P < 0.05-0.005) and second (P < 0.008-0.001) control groups of the same age group and live in Erbil Province.

It can be seen from Table 2 that the statistical analysis did not show significant differences in the concentrations of IL-10 and TNF-α in the sera of the aborted pregnant women who were seropositive for *T. gondii* in comparison with the women in the first (P > 0.05) and second (P > 0.08) control groups of the same age group and live in Diyala Province. Concerning the concentrations of IFN-γ, the results showed that there was a significant increment (P = 0.027) in the sera of the aborted pregnant women and infected with *T. gondii* in comparison with the women in the first control group, while the comparison with the second control group showed a marginal significant difference (P = 0.053).

Tables 3, 4, and 5 showed the comparison between the aborted pregnant women who were seropositive for *T. gondii* and the first control group (Non-aborted pregnant women) living in Erbil and Diyala Provinces regarding the sera levels of IL-10, TNF-α and IFN-γ, respectively. The statistical analysis showed no significant differences in the concentrations of both IL-10 (Table 3) and TNF-α (Table 4) between the aborted pregnant women living in Erbil and Diyala Provinces. Similarly, no significant differences in the concentrations of both IL-10 (Table 3) and TNF-α (Table 4) were found between the women in both control groups living in Erbil Province and their counterparts living in Diyala Province.

In contrast, the results showed that the concentration of IFN-γ in the sera of the aborted pregnant women who were seropositive for *T. gondii* living in Erbil Province was significantly higher (P = 0.0193) than that in their counterparts living in Diyala Province (Table 5). In addition, no significant differences were observed in the levels of IFN-γ in the sera of both control groups living in Erbil Province and their counterparts living in Diyala Province (Table 5).

Table 1. Concentrations of cytokines in aborted pregnant women (Group 1) who were seropositive for *Toxoplasma gondii* as compared to the seronegative first control group (pregnant and non-aborted) (Group 2) and to the second control group (non-pregnant and non-aborted) (Group 3) living in Erbil Province.

Concentration (Pg/mL)* / Mean ± SE					
Cytokines	Group 1	Group 2	Group 3	P-value (Group 1 vs:	
				Group 2	Group 3
IL-10	0.396 ± 0.094	0.169 ± 0.04	0.123 ± 0.02	0.018	0.004
TNF-α	0.512 ± 0.111	0.296 ± 0.075	0.093 ± 0.01	0.059	0.001
IFN-γ	0.573 ± 0.139	0.188 ± 0.004	0.217 ± 0.02	0.005	0.008

*(Pg : Picogram). ; (SE: Standard error)

Table 2. Concentrations of cytokines in aborted pregnant women (Group 1) who were seropositive for *Toxoplasma gondii* as compared to the first control group (pregnant and non-aborted) (Group 2) and to the second control group (non-pregnant and non-aborted) (Group 3) living in Diyala Province.

Concentration (Pg/ml) / Mean ± SE					
Cytokines	Group 1	Group 2	Group 3	P-value (Group 1 vs:	
				Group 2	Group 3
IL-10	0.398 ± 0.13	0.415 ± 0.23	0.181 ± 0.08	0.476	0.0812
TNF-α	0.394 ± 0.11	0.461 ± 0.23	0.269 ± 0.18	0.399	0.283
IFN-γ	0.234 ± 0.02	0.191 ± 0.01	0.197 ± 0.01	0.027	0.053

Table 3. Comparison the concentration of Interleukin 10 (IL-10) cytokine between aborted pregnant women (Group 1) who were seropositive for *Toxoplasma gondii* as compared to the seronegative first control group (pregnant and non-aborted) (Group 2) and to the seronegative second control group (non-pregnant and non-aborted) (Group 3) living in Erbil and Diyala Provinces.

Groups	Concentration of IL-10 (Pg/ml) / Mean ± SE		P-value
	Erbil	Diyala	
Group 1	0.396 ± 0.09	0.398 ± 0.13	0.991
Group 2	0.169 ± 0.04	0.415 ± 0.23	0.312
Group 3	0.123 ± 0.02	0.181 ± 0.08	0.468

Table 4. Comparison the concentration of Tumor Necrosis Factor-α (TNF-α) between aborted pregnant women (Group 1) who were seropositive for *Toxoplasma gondii* as compared to the seronegative first control group (pregnant and non-aborted) (Group 2) and to the seronegative second control group (non-pregnant and non-aborted) (Group 3) living in Erbil and Diyala Provinces.

Groups	Concentration of TNF-α (Pg/ml) / Mean ± SE		P-value
	Erbil	Diyala	
Group 1	0.512 ± 0.11	0.296 ± 0.08	0.444
Group 2	0.394 ± 0.11	0.461 ± 0.23	0.515
Group 3	0.093 ± 0.01	0.269 ± 0.18	0.350

Table 5. Comparison the concentration of Interferon-γ (IFN-γ) between aborted pregnant women (Group 1) who were seropositive for *Toxoplasma gondii* as compared to the seronegative first control group (pregnant and non-aborted) (Group 2) and to the seronegative second control group (non-pregnant and non-aborted) (Group 3) living in Erbil and Diyala Provinces.

Groups	Concentration of IFN-γ (Pg/ml) / Mean ± SE		P-value
	Erbil	Diyala	
Group 1	0.188 ± 0.01	0.573 ± 0.14	0.019
Group 2	0.191 ± 0.01	0.234 ± 0.02	0.543
Group 3	0.217 ± 0.02	0.197 ± 0.01	0.306

DISCUSSION

The principal findings of the present study were the significant increments in the concentrations of the cytokine (IFN- γ) in the sera of pregnant women who were seropositive for *T. gondii* in comparison with healthy control women of the same age groups living in both Erbil and Diyala Provinces, while the concentrations of the cytokines (IL-10 and TNF- α) increased significantly in the sera of pregnant women who were seropositive for *T. gondii* in comparison with healthy control women living in Erbil Province but not in those living in Diyala Province.

The result of the present study was consistent with that conducted by Al-Obaidi and Habib (19) which showed an increase in the concentration of interferon gamma in Iraqi women who have recurrent miscarriages and were seropositive for *T. gondii*, as well as an increase in the concentration of IL-10 in order to inhibit the excessive immune response and protect the body cells from damage. The authors concluded that the high concentration of IL-10 can protect fetuses from recurrent miscarriages by inhibiting and regulating the production of Th1 and T- cells. The results of the current study also in agreement with the that of Mohamed et al. (20) which was conducted on a group of Iraqi women who were infected with toxoplasmosis and have recurrent miscarriages. The results showed high level of IL-10 in the sera of these women when compared with healthy seronegative women (control group). The authors suggested that the increase in the level of IL-10 in the sera of the women with toxoplasmosis may be due to its importance in inhibiting excessive cellular immune response by inhibiting the effectiveness of MHC class II antigens in dendritic cells and macrophage cells (21). In addition, IL-10 plays an important role in inhibiting the production of a number of cytokines that are produced by the T-cells such as IL-12, TNF- α , and IFN- γ . It also plays an important role in inhibiting the production of cellular kinematics which are produced by the natural killer cells (22).

In the present study, the significant increase in the levels of IL-10 in the sera of the pregnant and aborted women who were seropositive for *T. gondii* in comparison with the healthy women may be related to the capacity of this parasite to enhance Th2-cytokines including IL-10. Meira et al. (11) analyzed the synthesis of Interferon gamma (IFN- γ), Tumor Necrosis Factor alpha (TNF- α), and Interleukin 10 (IL-10) in chronically infected patients who developed the symptomatic disease as cerebral or ocular toxoplasmosis and reported that the deviation to Th2 immune response, including the production of anti-inflammatory cytokines such as IL-10, may promote the parasite's survival causing the tissue immune destruction.

Interferon gamma works in conjunction with phagocytic and T- cells of all kinds to resist the *T. gondii* parasite. Likewise, the dendritic cells (DC) and the killer cells (NK) all serve as the final result of the immune response that eliminates the parasite or inhibits its activity. The current study showed that the average concentration of interferon gamma in the serum of women with toxoplasmosis is higher than the rate of its concentration in the serum of uninfected (healthy) women, and this confirms the role of interferon gamma in the immune response as it works to resist the parasite and inhibit the growth via many mechanisms or convert it from a rapidly reproducing phase (Tachzoite) to a slow-multiplying phase (Bradyzoite) by a group of effects including nitric oxide (23). It also destroys the amino acid tryptophan, which is important for parasite growth by increasing the effectiveness of the Indoleamine 2,3 dioxygenase enzyme (24). The results of the current study also coincided with the results of the study of Kadum and Abbas (25), which has been conducted in Al-Qadisiyah Province (Iraq) and showed a high concentration of interferon gamma in the sera of the aborted women who were seropositive for *T. gondii* when compared with the control group of healthy and non-infected women. In addition, the results of this study coincided with other studies conducted in Iraq which demonstrated a high rate of in interferon gamma in women infected with *T. gondii* in comparison with the control group (uninfected women) (26, 27).

Although the interferon gamma has a protective role in the immune response, excessive production which may lead to uncontrolled immune response may damage the host cells, where the host needs a balancing process in the immune response, reducing damage to the host and killing pathogens (28). Therefore, cytokine IL-10 is important in inhibiting the excessive immune response of cytokine interferon gamma and working to balance the immune response to reduce immune diseases (29).

The results of the current study showed that there were significant differences between the concentrations of tumor necrosis factor alpha type (TNF- α) in sera of pregnant and aborted pregnant women who were living in Erbil and Diyala Provinces and who were seropositive for *T. gondii* as well as in sera of the first control group (non-aborted pregnant women) and the second control group (pregnant and non-pregnant women).

The results of this study were consistent with the results of the study conducted by El-Hashimi et al. (30) in Iraq regarding the levels of TNF- α and IL-6 in the sera of three groups of women (aborted pregnant women who were seropositive for toxoplasmosis, aborted pregnant women who were seronegative for *T. gondii*, and healthy uninfected women) and the authors reported a significantly higher level of TNF- α in the sera of the first group in comparison with the other groups.

The results of the current study agreed with that of Al-Kady (31) which has been conducted on Egyptian women infected with *T. gondii*, where he found an increase in the level of TNF- α in these patients compared with the healthy group. In addition, the results of the present study were consistent with that of Alfonzo et al. (32) on French women infected with toxoplasmosis where they found an increase in the level of TNF- α when compared with the healthy women who were not infected with this parasite. In addition, Matowicka-Karna et al. (33) reported a significant increase in the levels of both IL-10 and TNF- α in the sera of pregnant women who were infected with toxoplasmosis when compared with the women in the control group who were seronegative for *T. gondii*. In contrast, Lang et al. (34) observed a decrease in the

concentration of TNF- α in the sera of women who were seropositive for *T. gondii* in comparison with the control group (non-infected women).

Recently, El-Sherbini et al. (35) studied the gene expression profile of some pro- and anti-inflammatory cytokines in *Toxoplasma* seropositive women with variant pregnancy conditions and reported that the sera of women who suffered from repeated abortion showed higher levels of both IFN- γ and TNF- α than the sera of the women who did not suffer from any abortion and the authors suggested that this indicates a predominant inflammatory state in the aborted women. The authors concluded that these cytokines have a potential role in the upcoming prognostic or/and therapeutic concerns of toxoplasmosis among pregnant women.

CONCLUSION

The present study showed for the first time that the immune response of the aborted pregnant women living in Erbil Province who were seropositive for *T. gondii* responded differently when compared to their age-matched aborted pregnant women living in Diyala Province.

DECLARATIONS

Authors' contributions: Administration (NY); investigation (NY and ALM); design of the research methods (SH); conducted the study (ZA); analyzed the data (ZA); interpreted the results (ALM); wrote the first draft of the manuscript (ZA); reviewed and edited the manuscript (ALM and NY); all authors read and approved the final manuscript before submitting for consideration to publish.

Competing interests: The authors declare no conflict of interest.

REFERENCES

1. Suzuki, Y., Wang, X., Jortner, B.S., Payne L, Ni, Y. et al. (2010). Removal of *Toxoplasma gondii* cysts from the brain by perforin-mediated activity of CD8+ T cells. *American Journal of Pathology*, 176: 1607–1613.
2. Delair, E., Latkany, P., Noble, A.G., Rabiah, P., McLeod, R., and Brezin, A. (2011). Clinical manifestations of ocular toxoplasmosis. *Ocular Immunology and Inflammation*, 19: 91–102.
3. Olariu, T.R., Remington, J.S., McLeod, R., Alam, A., and Montoya, J.G. (2011). Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. *Pediatric and Infectious Diseases Journal*, 30: 1056–1061.
4. Dubey, J.P., Lago, E.G., Gennari, S.M., Su, C. and Jones, J.L. (2012). Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. *Parasitology*, 139: 1375–1424.
5. Garweg, J.G., and Candolfi, E. (2009). Immunopathology in ocular toxoplasmosis: facts and clues. *Memorials do Instituto Oswaldo Cruz*, 104: 211–220.
6. Pereira-Chiocola, V.L., Vidal, J.E. and Su, C. (2009). *Toxoplasma gondii* infection and cerebral toxoplasmosis in HIV-infected patients. *Future Microbiology*, 4: 1363–1379.
7. Sullivan, W.J. Jr., and Jeffers, V. (2012). Mechanisms of *Toxoplasma gondii* persistence and latency. *FEMS Microbiology Review*, 36: 717–733.
8. Molan, A.L. and Ismail, M.H. (2017). Study the possible association between toxoplasmosis and Diabetes mellitus in Iraq. *World Journal of Pharmacy and Pharmaceutical Sciences*, 6: 85–96.
9. Hussein, S. and Molan, A.L. (2019). Prevalence of *Toxoplasma gondii* Infection in Hemodialysis Patients with Chronic Renal Failure and Risk Factors in Diyala Province, Iraq. *Malysian Journal of Medicine and Health Sciences*, 15(1): 31–36.
10. Mahmoud, S.F., Molan, A.L. and Rathi, M.H. (2020). Seroprevalence of *Toxoplasma gondii* infection in children with visual and/or hearing disability in comparison with healthy children in Iraq. *Malaysian Journal of Medicine and Health Sciences*, 16(3): 140–147.
11. Meira, C.S., Pereira-Chiocola, V.L., Vidal, J.E., Mattos, C.C., Motoie, G., Costa-Silva, T.A. et al. (2014). Cerebral and ocular toxoplasmosis related with IFN- γ , TNF- α , and IL-10 levels. *Frontiers in Microbiology*, 5: 1–7.
12. Patrizi, R. M. (2008). The influence of acute resistive exercise on inflammatory markers in the blood of obese, postmenopausal women. MS.c. Thesis, Texas Christian University.
13. Salazar-Onfray, F., Lopez, M.N. and Mendoza-Naranjo, A. (2007). Paradoxical effects of cytokines in tumor immune surveillance and tumor immune escape. *Cytokine and Growth Factor Reviews*, 18: 171–182.
14. Costa-Silva, T.A., Borges, M.M., Galhardo, C.S., and Pereira-Chiocola, V.L. (2012). Immunization with excreted/secreted proteins in AS/n mice activating cellular and humoral response against *Toxoplasma gondii*

- infection. *Acta Tropica*, 124: 203–209.
15. Ghasemi, H., Ghazanfari, T., Yaraee, R., Owlia, R., Hassan, Z.M., and Faghihzadeh, S. (2012). Roles of IL-10 in ocular inflammations: a review. *Ocular Immunology and Inflammation*, 20: 406–418.
 16. Phuangphet, W. (2008). Toxoplasmosis: Pathogenesis and immune response. *Thammasat Medical Journal*, (8): 487.
 17. Butcher, B.A., Kim, L., Panopoulos, A.D., Watowich, S.S., Murray, P.J. and Denkers E.Y. (2005). IL-10-independent STAT3 activation by *Toxoplasma gondii* mediates suppression of IL-12 and TNF- α in host macrophages. *Microbiology and Immunology*, 174(6): 3148–52.
 18. Hoti, S. L., and Tandon, V. (2011). Ocular parasitoses and their immunology. *Ocular Immunology and Inflammation*, 19: 385–396.
 19. Al-Obaidi, A. B. and Habib, M. A. (2009). IFN- γ versus IL-10 in situ expression in recurrent spontaneous abortion. *Iraqi Journal of Medical Sciences*, 7: 21–29.
 20. Mohamed, K.I.A., Khadhum, M.S., Abu-Al-Ess, H.Q.M., Ali, S.H.M. et al. (2017). 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: 76–82.
 21. Wilson, D. C., Matthews, S. and Yap, G.S. (2008). IL-12 signaling drives CD8+ T cell IFN- γ production and differentiation of KLRG1+ effector subpopulations during *Toxoplasma gondii* Infection. *Journal of Immunology*, 180: 5935–5945.
 22. Aldebert, D., Durand, F., Mercier, C., Delauw, M. and Pellowx, H. (2007). *Toxoplasma gondii* triggers secretion of interleukin-12 but low level of interleukin-10 from the THP-1 human monocyte cell line. *Cytokine*, 37: 206–211.
 23. Fox, B. A, Gigley, J.P. and Bzik, D.J. (2004). *Toxoplasma gondii* lacks the enzymes required for de novo arginine biosynthesis and arginine starvation triggers cyst formation. *International Journal for Parasitology*, 34(3): 323–331.
 24. Nascimento, F.R.F., Calich, V.L.G., Rodríguez, D. and Russo, F.M. (2002). Dual role for nitric oxide in paracoccidioidomycosis: Essential for resistance, but overproduction associated with susceptibility. *Infection and Immunity*, 79(6): 2470–2480.
 25. Kadum, S.A. and Abbas, A.F. (2013). A study of some immunological features in aborted women infected with *Toxoplasma gondii* and Cytomegalovirus in Hilla city. *AL-Qadisiya Journal of Sciences*, 18: 62–70.
 26. Al-Sorchee, S.M.A. (2005). Immunological study on women infected with Toxoplasmosis with a History of Abortion/Baghdad. M.Sc. Thesis. College of Education (Ibn Al- Haitham), University of Baghdad.
 27. Abdullah, D. A., Mahmood, M. A. and Mohammed, D.B. (2011). The levels of cytokines IL-4, IL-10, IL12, P40, IFN- γ during acute Toxoplasmosis. *Journal of the Faculty of Medicine, Baghdad*, 53: 1–5.
 28. Shaw, M.H., Freeman, G. (2006). Tyk2 negatively regulates adaptive Th1 immunity by mediating IL-10 signaling and promoting IFN- γ dependent IL-10 reactivation. *Journal of Immunology*, 176: 7263–7271.
 29. Zhao, M., Zhang, R., Xu, X., Liu, Y. Zhang, H. Zhai, X. and Hu, X. (2013). IL-10 reduces levels of apoptosis in *Toxoplasma gondii* infected trophoblasts. *PLoS ONE* 8(2): e56455.
 30. El-Hashimi, W.K., Khalil, H.I. Al-Mayah, Q.S. and Al-Bashier, N.M. (2014). The role of cytokines, TNF- α , IL-6 and pregnancy associated hormones in *Toxoplasma gondii* induced abortion. *European Journal of Experimental Biology*, 4: 98–104.
 31. El-Kady, I. M. (2011). T-cell immunity in human chronic Toxoplasmosis. *Journal of the Egyptian Society of Parasitology*, 41: 17 – 28.
 32. Alfonzo, M., E. Badell, E., Pourcel, C. and Scott-Algara, D. (2005). Cell mediated and not humoral immune response is responsible for partial protection against toxoplasmosis in SCID mice reconstituted with human PBMC. *Immunology*, 24: 273–282.
 33. Matowicka-Karna, J., Dymicka-Piekarska, V. and Kemon, H. (2009). Does *Toxoplasma gondii* infection affect the levels of IgE and cytokines (IL-5, IL-6, IL-10, IL-12, and TNF- α)?. *Clinical and Developmental Immunology*, 8: 1–4.
 34. Lang, C., Gross, U. and Luder, G. (2007). Subversion of innate and adaptive immune responses by *Toxoplasma gondii*. *Parasitology Research*, 100: 191–203.
 35. El-Sherbini, M. S., AbdEl-Aal, A.A., El-Sherbiny, W.S., Attia, S.S. et al. (2019). Toxoplasmosis and abortion: pro- and anti-inflammatory cytokines gene expression of the host immune cells. *Egyptian Journal of Medical Human Genetics*, 20: 1–10.