

Assessment of Serum Interleukin-17A, Tumor Necrosis Factor Alpha, and Lactoferrin levels Among Women Infected with Toxoplasmosis

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Abstract

Background: *Toxoplasma gondii* is an intracellular parasite. It is considered one of the major causes of miscarriage and inhibits fetal development, in early pregnancy. **Objectives:** This study aimed to estimate interleukin-17A (IL-17A), tumor necrosis factor alpha (TNF- α), and LTF levels in Toxoplasmosis-infected pregnant women who miscarried. **Materials and Methods:** The research was conducted from July 2022 to February 2023. A total of 91 serum samples were collected from pregnant women who had experienced abortion and who attended medical attention in public hospitals and private medical clinics in the governorates of Salah al-Din and Kirkuk. The enzyme-linked immunosorbent assay (ELISA) technique was used to evaluate IL-17A, TNF- α , and LTF levels in Toxoplasmosis-infected pregnant women. **Results:** The IgG ELISA test revealed 77 positive samples (84.61%) and 14 negative samples (15.3%), whereas IgM ELISA test showed 84 positive samples (92.3%) and 7 negative samples (7.6%). IL-17A levels were significantly higher in patients, with an average concentration of 330.8 ± 17.4 pg/mL, compared to the control group, whose average concentration was 249 ± 73.3 pg/mL. Similarly, TNF- α levels in patients were significantly higher compared to the control group, with a mean of 166.4 ± 13.1 pg/mL, 100.5 ± 13.2 pg/mL, respectively. LTF levels were significantly higher in patients, with an average concentration of 50.8 ± 3.2 pg/mL, compared to the control group, whose average concentration was 29.6 ± 12.4 pg/mL. **Conclusions:** The study shows elevated levels of IL-17A, TNF- α , and Lactoferrin in patients.

Keywords: ELISA IgG, IgM, IL-17A, Lactoferrin, TNF- α , *Toxoplasma gondii*

INTRODUCTION

Toxoplasmosis, one of the most common parasitic zoonoses worldwide, is caused by the obligate intracellular protozoa *Toxoplasma gondii*.^[1] This disease has a global distribution, infecting approximately 30%–50% of the world's population.^[2] *Toxoplasma gondii* infects a wide range of mammal and bird species as intermediate hosts, whereas felids, the definitive hosts, harbor the sexual form of the parasite in their intestinal cells and are responsible for the dissemination of oocysts into the environment through feces. The immune response to *T. gondii* infection is strongly associated with cellular response.^[3]

Pro-inflammatory cytokines play a vital role in the pathogenesis of Toxoplasmosis.^[4] The type of cytokine produced depends on the type of cellular kinetics, with

TM1 cells producing tumor necrosis factor-alpha (TNF- α) or beta (TNF- β), whereas TH17 cells produce interleukin-17A (IL-17A).^[5]

TNF- α is responsible for the production of acute inflammatory responses, activating the adaptive immune response and the immediate immune response against the *Toxoplasma* parasite. It also stimulates natural killer cells to produce interferon-gamma (IFN- γ) during acute *Toxoplasma* infection. TNF- α also plays a role in activating the immediate immune response by stimulating

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the cellular killing mechanism of macrophage cells during acute toxoplasmosis infection.^[6] IL-17A is involved in host defense against pathogens, and it appears to play a crucial role in protective immunity against *T. gondii* by stimulating neutrophil cells at sites of inflammation. In addition, IL-17A promotes neuroinflammation during chronic toxoplasmosis infection.^[7]

Lactoferrin (LF) is a glycoprotein found in various bodily fluids, such as milk, external secretions, saliva, tears, vaginal fluids, semen, nasal secretions, bronchi, bile salts, intestinal fluids, urine, and neutrophil cell granules. The molecular mechanism of LF's activity against parasite infestation is complex, but it typically interferes with the iron uptake required by the parasite for development. LF has also been found to reduce and inhibit the activity of *T. gondii*.^[4,8]

AIM OF THE STUDY

This study aimed to determine the Serodiagnosis of toxoplasmosis and levels of IL-17A, TNF- α , and Lactoferrin in pregnant women miscarriage (20–40 years old)

MATERIALS AND METHODS

Time and location

The research was conducted from July 2022 to February 2023. A total of 91 serum samples were collected from pregnant women who had experienced abortion and who attended medical attention in public hospitals and private medical clinics in the governorates of Salah al-Din and Kirkuk. Each participating woman in the study had a unique questionnaire form filled out during interviews.

Collection of blood samples

Five milliliters of venous blood were taken from each subject. The blood samples were placed in gel tubes and then centrifuged at 3000rpm for 10min to extract the serum. The tubes were left at room temperature for 15–20min to allow the blood to clot. To preserve sample quality, the acquired serum was transferred to Eppendorf tubes (200 μ L) for various assays and stored at -20°C until immunological testing was conducted ^[3]. All samples were examined simultaneously after the completion of sampling to avoid repeated freezing and thawing, which could impact the quality of the findings.

Diagnosis of *Toxoplasma gondii* by ELISA test

The diagnosis of chronic or acute stages of infection was established by showing specific IgG and IgM antibodies using the enzyme-linked immunosorbent assay (ELISA) technique (*T. gondii* IgM/IgG DRG) ^[14].

Human IL-17A, TNF- α , Lactoferrin ELISA test

The serum concentrations of IL-17A, TNF- α , and lactoferrin were assessed using the enzyme-linked immunosorbent assay (ELISA) (Germany DRG, China Sunlong). The ELISA plates were precoated with human IL-17A, TNF- α , and lactoferrin antibodies. The samples were added to the plates, and the color development in the substrate solution correlated with the level of IL-17A, TNF- α , and lactoferrin. The process was stopped by adding a stop solution, and absorbance was measured at 450nm ^[15].

Ethical approval

The research was carried out in accordance with the ethical guidelines. The objective of this study was verbally conveyed to the participants. Before collecting any samples, ethical approval was obtained and patients were selected for inclusion in the study. The goal and process of the survey were effectively communicated by the researcher to the patients, accompanied by standardized instructions and guidance for the completion of the questionnaire. The study design, patient information, and permission form underwent a thorough evaluation and received approval from the local Ethics Committee 12947 on September 6, 2022.

Statistical analysis

To perform statistical analysis, IBM SPSS software program, version 22.0 was used. Frequencies and percentages were used to present categorical information. The format for continuous variables was mean \pm SE. Values of $P \leq 0.05$ were regarded as significant by using the *t* test.

RESULTS

Diagnosis of *Toxoplasma gondii* by ELISA test

The total number of this study was 91; 77 samples' (84.61%) test result was positive for IgG antibodies, with an average concentration of 178.1 ± 4.9 IU/mL. For IgM antibodies, 84 samples (92.3%) tested positive, with an average concentration of 65.4 ± 3.8 IU/mL. In the control group, 14 samples (15.3%) tested negative for IgG antibodies, with an average concentration of 11.7 ± 2.8 IU/mL. For IgM antibodies, seven samples (7.6%) tested negative, with an average concentration of 11.2 ± 1.7 IU/mL [Table 1].

Serum IL-17A level in patients and control groups

The study observed a significant increase in the average concentration of IL-17A in infected women (330.8 ± 17.4 pg/mL) compared to the control group (249.4 ± 73.3 pg/mL) [Table 2].

Serum TNF- α level in patients and control groups

The study showed a significant increase ($P \leq 0.05$) in the average concentration of TNF- α in women with

Table 1: Diagnosis of *Toxoplasma gondii* by ELISA test

Method	The number of samples examined	The number of positive samples	Percentage %	Mean \pm SE	The number of negative samples	Percentage %	Mean \pm SE
IgG	91	77	84.61	178.1 \pm 4.9IU/ mL	13	14.2	11.7 \pm 2.8IU/ mL
IgM	91	84	92.3	65.4 \pm 3.8 IU/ mL	7	7.6	11.2 \pm 1.7IU/ mL

Table 2: Serum IL-17A level in patients with *Toxoplasma gondii* and control groups

IL-17A Pg/mL	Patients <i>N</i> = 70	Control <i>N</i> = 20	<i>P</i> Value
Mean \pm SE	330.8 \pm 17.4	249.4 \pm 73.3	0.0021*

P* Value is significant at *P* \leq 0.05Table 3: Serum TNF- α level in patients with *Toxoplasma gondii* and control groups**

TNF- α Pg/ mL	Patients <i>N</i> = 70	Control <i>N</i> = 20	<i>P</i> Value
Mean \pm SE	166.4 \pm 13.1	100.5 \pm 13.2	0.012*

**P* Value is significant at *P* \leq 0.05

toxoplasmosis (166.4 \pm 13.1 pg/mL) compared to uninfected women (100.5 \pm 13.2 pg/mL) [Table 3].

Serum Lactoferrin level in patients and control groups

According to the results of the study, a significant increase was found in the concentration of lactoferrin in infected women (50.8 \pm 3.2 pg/mL) compared to the control group (29.6 \pm 12.4 pg/mL) [Table 4]. The difference was statistically significant at a level of *P* \leq 0.05.

DISCUSSION

The IgG and IgM antibodies play a crucial role in defending against the antigens of the fast-reproducing tachyzoite and slow-reproducing Bradyzoite phases of *T. gondii*, helping to control the infection by reducing the number of formed phases.^[9] The findings of this study are consistent with the research by^[10] in terms of higher IgM antibody levels in aborted women infected with toxoplasmosis compared to IgG antibody levels. However, the results differ from,^[11] who reported higher IgG antibody levels in toxoplasmosis patients than IgM antibody levels.

This study highlights the elevated levels of IL-17A in pregnant women during the acute and chronic stages of infection compared to uninfected women. This increase in IL-17A may be attributed to the immune response against the parasite, which involves the recruitment and attraction of neutrophil cells to sites of inflammation.^[12] Previous studies conducted on laboratory mice with RH parasitic basic and pollinated by ROPI3 DNA for the parasite support these findings.^[13] The results agree with

Table 4: Serum lactoferrin level in patients with *Toxoplasma gondii* and control groups

Lactoferrin Pg/mL	Patients <i>N</i> = 70	Control <i>N</i> = 20	<i>P</i> Value
Mean \pm SE	50.8 \pm 3.2	29.6 \pm 12.4	0.001*

**P* Value is significant at *P* \leq 0.05

the research by^[3] and,^[14] but contradict the findings of^[15] and.^[16]

TNF- α is a powerful cytokine that has diverse effects on various cells. It has been shown to affect hormone production, placental formation, fetal development, and steroid formation. It also plays a role in placental differentiation and the birthing process while also being implicated in pregnancy termination.^[17] Recent studies indicate that infection with the parasite suppresses TNF- α production as a strategy for successful infection.^[18] TNF- α acts as a pyrogenic factor, stimulating acute inflammatory responses and exerting antimicrobial activity by activating macrophage cells to produce TNF- γ .^[19] It plays a critical role in protective immunity against toxoplasmosis, functioning synergistically with IFN- γ to develop resistance against *T. gondii* infection.^[6]

In toxoplasmosis, TNF- α is essential for macrophage activation and inhibition of parasite replication, but this action requires synergy with IFN- γ . TNF- α is believed to have a protective effect in both the acute and chronic phases of the disease, stimulating IFN- γ production by NK cells, which plays a crucial role in the early non-specific response to toxoplasmosis. However, the role of TNF- α in toxoplasmosis is still debated, as some studies have associated it with fatal infection in mice and harmful actions in cerebral and hepatic tissues. TNF- α may also contribute to the intracerebral dissemination of *T. gondii* and be increased in toxoplasmic chorioretinitis during primary infection in humans.^[20-23] These findings are in agreement with the studies conducted by,^[2,18] and.^[17]

Lactoferrin, as a natural immunomodulatory molecule, has the ability to modulate and impact the response of both the innate and adaptive immune systems. This activity is facilitated by the presence of lactoferrin receptors on various immune cells and their ability to bind to the molecule. Lactoferrin plays a significant role in regulating the innate immune response, serving as a first-line host defense mechanism against invasive pathogens. In addition, lactoferrin induces mediators of the innate response and triggers signaling pathways that subsequently impact the function of adaptive immune cells. Lactoferrin affects the innate immune system in multiple ways, including increasing natural killer (NK) cell activity, enhancing neutrophil function by promoting phagocytosis, activating macrophages, and limiting intracellular pathogen proliferation.^[8] The findings of this study align with the results of^[21] in Samarra, with lactoferrin levels reaching 18.894 ± 3.1 pg/mL in women infected with the parasite, compared to 10.51 ± 1.2 pg/mL in the control group.

CONCLUSIONS

This study shows that elevated interleukin in pregnant women with Toxoplasmosis. Serum levels of IL-17A, TNF- α , and Lactoferrin increased in patients with Toxoplasmosis who experienced pregnancy.

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Conflicts of interest

There are no conflicts of interest.

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