

*Research Article*

# Evaluation Immunological Parameters in Aborted Women with Toxoplasma Gondii

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**Abstract:** Toxoplasma gondii (*T. gondii*), intracellular parasite that cause abortion in infected pregnant women with the parasite. **Aim of the study:** the present investigation aimed to assessment the prevalence of *T. gondii* in aborted women and evaluated level of interferon- gamma, Toll-like receptor 2 (TLR2) and lactoferrin. **Materials and methods:** From September 2024 to May 2025, we examined 90 women, 70 of them spontaneous abortion, while an additional 20 blood samples were obtained from healthy individuals serving as controls, in Tikrit Teaching hospital and many private hospitals in Tikrit city. **Result:** The incidence of parasite infection in aborted women was 56 (80%) out of a total of 70%. The level of IFN, TLR2, and Lactoferrin in the group of aborted women infected with *T. gondii* ( $45.4 \pm 7.1$ ,  $17.04 \pm 2.28$ ,  $59.6 \pm 12.6$ ), as compared with control group ( $19.4 \pm 2.12$ ,  $8.2 \pm 2.1$ ,  $29.2 \pm 9.4$ ). **Conclusion:** The present study concluded increase prevalence of toxoplasma gondii in aborted woman may indicate the main cause of abortion. Furthermore, increase level of immunological parameters that may indicate resolve the parasite infection.

**Keywords:** Aborted Women; Interferon Gamma; Lactoferrin; *T. gondii*; Toll-Like Receptor 2 (TLR2)

## 1. Introduction

Spontaneous abortion also called miscarriage defined as atypical problem for pregnant women. Early miscarriage happens during the first twelve weeks of pregnancy, while late miscarriage happens between twelve and twenty-four weeks. About 10 to 30% of miscarriage are caused by infections. Toxoplasma gondii (*T. gondii*) infections account about 5% for early miscarriage and 66% for late miscarriage. The type of pathogen and the gestational age of infection determine the incidence of pregnancy loss. The acronym TORCH stands for *T. gondii*, other pathogens, Rubella virus, Cytomegalovirus, and Herpes simplex virus, and it describes the main group of pathogens that can infect a pregnant woman and then cross the placenta, causing serious harm to the developing baby. If a woman contracts the obligate intracellular zoonotic parasite *T. gondii* during her pregnancy, it can cause a number of birth defects in her unborn child. Depending on the region, the seroprevalence of *T. gondii* in pregnant women can range from 9% to 48.7% in Europe and from 38% to 77.5% in South America. Peru has exhibit a seroprevalence of 39%. *T. gondii* poses a serious threat due to its able to infect embryonic organs by crossing the placental barrier. Hydrocephalus, Chorioretinitis, low birth weight, and abnormalities of the central nervous system are among the more serious complications linked to infections in the first two and third trimesters of pregnancy. Conversely, infections during the third trimester are linked with late congenital problem and developmental delays. The inflammatory response are essential as mechanism for host defense, but they may have a negative effect on pregnancy and fetal viability when infection with *T. gondii*. Th1 helper T cells are responsible for the induction of proinflammatory cytokines such as interferon gamma (IFN- $\gamma$ ), TNF- $\beta$ , TNF- $\alpha$ , and IL-2. These cells are responsible for activating cytotoxic T cells. Macrophages, on the other hand, are responsible for stimulating the cellular immune response and also play an important role in the rejection

**Received:** September 16, 2025;**Revised:** October 14, 2025;**Accepted:** October 29, 2025;**Published:** November 22, 2025;**Curr. Ver.:** November 22, 2025

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of transplanted tissues. Therefore, the dominance of Th1 helper T cells leads to a cell-mediated immune response or a cytotoxic immune response. Placental immunological function is important for preventing fetal refusal and sustaining pregnancy. Pattern recognition receptor (PRRs), as well as produce cytokines help in the maintenance pregnancy by altering the immunological equilibrium at maternal fetal interface. The immune reaction at the maternal fetal interface and disrupt PRRs- mediate cytokine secretion that induced by *T.gondii* infection which complicating pregnancy maintenance. Toll like receptor is a significant PRRs which control *T. gondii* development. In non-pregnant mice that infected with *T.gondii*, TLR2 triggers Th-1 that is important for eradication the parasite, since it generate INF-  $\gamma$ , IL-2 and nitric oxide. Microglia, neural, and astrocyte need TLR2 in their cytokines and prostaglandin E2production process. This pattern recognition receptor is activated on antigen presenting cell and trophoblasts during early and late gestation, and act to detect glycosylphosphatidylinositol (GPI) from *T.gondii*.

## 2. Materials and Methods

### 2.1. Sample Collection

Between September 2024 and May 2025, we analyzed 90 women, 70 of whom experienced spontaneous abortions, while an additional 20 blood samples were collected from healthy persons serving as controls at Tikrit Teaching Hospital and several private hospitals in Tikrit city. Epidemiological data, encompassing sociodemographic, behavioral, and clinical factors, were collected from all women. Twenty blood samples were taken from healthy women and five milliliters of venous blood was taken from women who had an abortion during the past fifteen days. A sterile, dry medical syringe was used to collect the samples, which were then placed in gel tubes.

The tubes were centrifuged at 3000 rpm for 10 minutes to remove the serum after being left at room temperature for 15-20 minutes to promote coagulation. Before the immunological experiment, the serum was transferred to Eppendorf tubes and kept at -20 °C for subsequent testing. To avoid compromising the quality of the results due to frequent freezing and thawing, all samples were examined at the same time.

### 2.2. Immunological Test

Serodiagnosis of toxoplasmosis was conducted using the Human Anti-*T. gondii* IgM ELISA Kit from Germany (DRG). Various testing was conducted, specifically emphasizing immunological assays including TLR2, IFN- $\gamma$ , and Lactoferrin. Seventy blood samples were collected from women who had undergone abortions, and an additional twenty blood samples were obtained from healthy persons serving as controls. To measure serum concentrations, the ELISA method was employed. The concentrations of TLR2, IFN- $\gamma$ , and lactoferrin were assessed by employing the ELISA method. Antibodies that target human TLR2, IFN- $\gamma$ , and lactoferrin were already applied to the plate. Added to the sample are levels of TLR2, IFN- $\gamma$ , and lactoferrin. As color developed in the substrate solution, the levels of human TLR2, IFN- $\gamma$ , and lactoferrin showed a positive correlation. Applying an acidic stop solution ends the reaction, which is then followed by measuring absorbance at 450 nm.

### 2.3. Analytical Statistics

Statistical analysis was conducted using SPSS 22. The continuous variable format was expressed as means  $\pm$  standard error (SE). The relationship between categorical variables was analyzed. P-values less than or equal to 0.05 were deemed significant.

## 3. Result

The current study, using ELISA, showed that the incidence of parasite infection in women who had aborted a pregnancy was 56 (80%) out of a total of 70%, with 41 (58.6%) samples positive for IgG, while 15 (21%) samples were positive for IgM, as shown in Table .(1)

**Table 1.** Diagnosis of infection with the parasite *T. gondii* using the Toxoplasma IgM/IgG ELISA technique.

| Type of antibody | Percentage |
|------------------|------------|
| IgG              | 41 (58.6%) |
| IgM              | 15(21%)    |
| Total            | 56(80%)    |

The results demonstrated a significant elevation at the level of  $P \leq 0.05$  in the level of IFN, TLR2, and Lactoferrin in the group of aborted women infected with *T. gondii* (**45.4  $\pm$  7.1**,  $17.04 \pm 2.28$ ,  $59.6 \pm 12.6$ ), as compared with control group ( $19.4 \pm 2.12$ ,  $8.2 \pm 2.1$ ,  $29.2 \pm 9.4$ ). As shown in Table (2).

**Table 2.** Level of immunological parameters in aborted women with *T. gondii*

| parameters            | Aborted women with <i>T. gondii</i><br>(n=56) | Control group<br>(n=20) | P-Value |
|-----------------------|---|-------------------------|---------|
| IFN- $\gamma$ (ng/ml) | $45.4 \pm 7.1$                                | $19.4 \pm 2.12$         | 0.001   |
| TLR2(ng/ml)           | $17.04 \pm 2.28$                              | $8.2 \pm 2.1$           | 0.04    |
| Lactoferrin(pg/ml)    | $59.6 \pm 12.6$                               | $29.2 \pm 9.4$          | 0.009   |

#### 4. Discussion

The ELISA test findings indicate that toxoplasma resulted in an 80% abortion rate during the first trimester. This conclusion is consistent with the findings of, which indicate that Toxoplasma is the primary cause of abortion during the first trimester. Maintaining gestation is associated with immune system dynamics during pregnancy. Resorption of the embryo and subsequent abortion can occur as a result of inflammatory reactions triggered by pathogen infection during early pregnancy. Substantial immunomodulation of the Th1 immune response transpires during mid-gestation, leading to a Th2 cytokine milieu at the maternal-fetal contact. As *T. gondii* is unresponsive to Th2 cytokines, infection may result in fetal demise or the progeny may be born with detrimental signs at birth. The current study demonstrated elevated levels of IFN- $\gamma$ , TLR2, and Lactoferrin in women who experienced abortion and tested positive for IgG and IgM serotypes for *T. gondii*, in comparison to the control group. The findings of the present study align with those of, who women who had aborted and tested positive for toxoplasma. Interferon-gamma is pivotal in transitioning from the trophozoite stage present in acute infection to the bradyzoite stage characteristic of chronic infection, while also inhibiting the reverse transition. T helper cells facilitate the immune response to eliminate intracellular parasites by secreting interferon-gamma, the primary agent for parasite destruction, which subsequently activates phagocytic cells. Th2 cells are responsible for eliminating parasites outside of cells by generating immunological complexes between particular antibodies generated by the humoral immune response and parasite antigens, as well as activating the complement system.

Women who had abortion showed increased levels of TLR2 in comparison to the control group in the current study. The observation suggest an intensified innate immune response induced by *T. gondii* infection, which may leading complication in pregnancy problems and fetal loss. TLR2, a pattern-recognition receptor, detects GPIs and other surface molecules of *T. gondii*, triggering intracellular signaling pathway that activate NF- $\kappa$ B and stimulate to synthesis of L-1 $\beta$ , TNF- $\alpha$ , IL-6, and other pro-inflammatory cytokines. These cytokines are essential in regulating the parasite during the early phase of infection; nevertheless, excessive or uncontrolled expression may result in placental inflammation and tissue damage. The increased expression of TLR2 may indicate an overactive immune response that disrupts the essential immunological tolerance necessary for pregnancy. A significant elevation of TLR2 and TLR4 linked with toxoplasmosis-induced miscarriage. The present result agreed with about women infected with the parasite.

Lactoferrin, a natural immunomodulatory substance, can regulate and influence both innate and adaptive immune response. The function is enabled by the presence of lactoferrin receptors on various immune cells and their ability to bind the chemical. Lactoferrin is essential in regulating the innate immune response, serving as a primary defense mechanism against invading pathogens. Furthermore, by activating mediators of the innate response, it

trigger signaling pathways that affect the function of adaptive immune cells. It affect the immune system in multiple ways, such as enhancing the activity of natural killer cells, improving neutrophil function by promoting phagocytosis and macrophage activation, and restricting the dissemination of intracellular infections.

## 5. Conclusion

The present study concluded increase prevalence of *Toxoplasma gondii* in aborted woman may indicate the main cause of abortion. Furthermore, increase level of immunological parameters that may indicate resolve the parasite infection

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