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Original Article

Interleukin Activity in Miscarried Women with Toxoplasmosis and Anti Thyroid Peroxidase (Anti-TPO)

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Abstract

Background: We aimed to investigate the presence of thyroid peroxidase antibodies (anti-TPO) in women with spontaneous abortion, both with and without toxoplasmosis. This was achieved by evaluating and comparing the serum levels of interleukins IL-4, IL-6, IL-10, IL-17, and TNF-α in women who experienced abortion due to toxoplasmosis, categorized by positive and negative anti-TPO status, with those who were toxoplasmosis-negative and also negative for anti-TPO.

Methods: We evaluated the serological presence of IgG and IgM antibodies to *Toxoplasma* using an ELISA method, in Samarra City, Salah al-Din Governorate, Iraq in 2021-2022. A sample of 153 women with spontaneous abortion were enrolled. We also measured the serum levels of interleukins IL-4, IL-6, IL-10, IL-17, TNF-α, and anti-TPO using the same technique.

Results: Overall, 103 were *Toxoplasma*-positive. Of these, 14 had positive anti-TPO results (13.5%), compared to only 3 positive cases among the 50 matched controls who were *Toxoplasma*-negative (6.0%). The difference between *Toxoplasma*-positive and *Toxoplasma*-negative women regarding anti-TPO status was statistically significant for interleukins IL-4 (P=0.010), IL-6 (P=0.017), and IL-10 (P=0.003), but not for IL-17 or TNF-α. Additionally, the statistical analysis revealed a highly significant difference in the average concentrations of interleukins IL-4 (P=0.013) and IL-10 (P<0.001) between the *Toxoplasma*-positive/anti-TPO-positive group and the *Toxoplasma*-negative/anti-TPO-negative group of aborted women.

Conclusion: Elevated concentrations of IL-4, IL-10, and IL-6 have been associated with women undergoing recurrent miscarriages and negative anti-TPO results. The complex interaction between pro-inflammatory and anti-inflammatory cytokines is essential for immunological balance and pregnancy outcomes in a condition of toxoplasmosis. An increase in IL-4 and IL-10 levels in anti-TPO-positive individuals may lead to an imbalance in immune response, facilitating the development of autoimmune thyroid disease.

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Introduction

Toxoplasma gondii is a protozoan parasite that infects humans and causes significant health issues worldwide. It is responsible for toxoplasmosis, a condition that can result in neurological abnormalities, stillbirth, and miscarriage. This parasite can lead to various health complications and affects multiple human organs and glands, including the thyroid gland (1). Unlike many other parasites, the first trimester of pregnancy is the period during which miscarriages are most common, and that the prevalence of TORCH infection, including toxoplasmosis (2). The immune system transforms *Toxoplasma* tachyzoites into bradyzoites, leading to the formation of tissue cysts and the disease entering a latent state, known as latent toxoplasmosis (LT).

Balance of cytokine secretion during toxoplasmosis infection in pregnancy is crucial for maintaining fetal tolerance and preventing excessive inflammation (3). Mast cells and basophils are sources of Interleukin 4 (IL-4) Th2 T cells the role in *T. gondii* infection regulates immunological response and stimulates Th2 response participated in chronic infection. The levels of IgG, IL-4, and IL-8 were elevated in women who experienced recurrent miscarriages and infected with *T. gondii* (4). Th2 cells, B cells, and monocytes produce interleukin 10 (IL-10), which serves a crucial anti-inflammatory function, hence aiding in the regulation of the immune response. Influence on Pregnancy Suppressive immune response can lead recurrence of latent infections and contribute to abortion (5). Th1-secreted interleukin 17 (IL-17) plays a role in *T. gondii* infection, with elevated levels linked to inflammation and tissue damage, potentially impacting pregnancy outcomes (6). Macrophages release Interleukin 6(IL-6) in response to certain microbial compounds known as pathogen-associated molecular patterns. IL-6 is a major immune response regulator, with a well-defined role in modulating inflammation progression and T cell development. The female reproductive tract and gestational tissues produce IL6 in a controlled manner (7).

In a study, 33% of patients diagnosed with autoimmune thyroid disorders (AITD) were infected with *T. gondii*. This study also demonstrated a connection between the gender of patients suffering from AITD and their infection with *T. gondii* (8). Valizadeh et al (9) conducted research to evaluate the prevalence and association between AITD and toxoplasmosis in pregnant women. The findings suggest that *Toxoplasma* IgG+ can increase the risk of developing AITD by 10.39 times in individuals with LT, leading to higher anti-TPO antibody levels. *T. gondii* may contribute to the development of thyroiditis in pregnant women, likely due to the antigenic similarities between *Toxoplasma* and thyroid peroxidase, which could trigger immune responses contributing to AITD. Future research could focus on exploring the molecular mimicry between *Toxoplasma* antigens and thyroid peroxidase. The enzyme thyroid peroxidase anti-TPO, which attaches to membranes, reduces iodide to add iodine to tyrosyl residues in thyroglobulin (10). Anti-TPO antibodies may cause damage to thyrocytes. The high level of Anti-TPO is an elevated risk of pregnancy complications, such as preterm delivery, placenta rupture, and abortion (11).

Antibodies against thyroid peroxidase anti-TPO, also known as anti-microsomal antibodies, characterize Hashimoto's thyroiditis and Graves' disease (12). Imbalances in T helper 17 (Th17) cells and regulatory T cells (Tregs) significantly impact idiopathic abortions (13). In Hashimoto's thyroiditis, high Anti-TPO levels are associated with an increased frequency of T cells producing Th/Tc1 cytokines, which may contribute to thyroid cell damage and death (14). Based on the findings from previous study, we hypothesize that thy-

roid autoimmunity may intensify inflammatory states and influence pregnancy outcomes (15).

Our study examined the prevalence of anti-TPO in women who experienced miscarriage due to latent toxoplasmosis. We also measured the activity of interleukins (IL-4, IL-6, IL-10, IL-17, and TNFα) in two groups: women infected with toxoplasmosis who were Anti-TPO-negative and those who were Anti-TPO-positive, and compared them with a control group of women without toxoplasmosis and negative for Anti-TPO. This was done to investigate the potential role of toxoplasmosis in autoimmune thyroid diseases and the involvement of interleukins in autoimmune thyroiditis and late spontaneous abortions in women who miscarried due to toxoplasmosis.

Materials and Methods

Sample collection and design of the study groups

We evaluated the serological presence of IgG and IgM antibodies to *Toxoplasma* using an ELISA method, in Samarra City, Salah al-Din Governorate, Iraq in 2021-2022. A sample of 153 women with spontaneous abortion were enrolled. Blood samples were taken, after informed consent. We distributed a questionnaire to each patient to collect clinical characteristics and risk factors. This covers age, number of abortions, month of miscarriage, place of living (rural or urban), and medical history of hypertension, diabetes, and autoimmune thyroid disease. We drew 5 ml of blood from each patient, centrifuged it at 3000 RPM for 5 min, and stored it at -20 °C until use.

Following the collection of samples, an antibody test was performed to identify toxoplasmosis and anti-TPO, using the ELISA technique. The study samples categorized into three groups 1): The first group of women who experienced abortion and were infected with toxoplasmosis, negative for anti-TPO (n=89); 2) the second group women who had abortions, were infected with toxoplasmosis, and positive for anti-TPO (n=14); and 3) the third group, serving as the control group, comprises women who underwent abortion, were not infected with toxoplasmosis, and had negative for anti-TPO (n=47). Subsequently, we evaluated several cytokines (IL-4, IL-6, IL-10, IL-17 and TNF-α) that might affect immune regulation and their role in the stimulation of autoantibodies.

Research methods

All isolated sera were tested by ELISA for detection of specific antibodies *T. gondii* (IgG and IgM) using the bioactive kit (Germany), Anti thyroid peroxidase Ab (anti-TPO) ELISA kit AESKULIS(Germany) Interleukin (IL-4, IL-6, IL-10, IL-17 and TNF-α) ELISA kit(ylbiont). All sera from positive women (103) and *T. gondii* negative (50) were kept in-20 °C for later use in detecting anti-TPO and cytokines activities. The normal values of TPO are 0–60 U/ml elevated values (positive anti-TPO): above 60 U/ml.

Statistical analysis

The means of cytokine plasma levels were compared between groups using independent sample t-test with equal variances or unequal variances (after checking equality of variance with Levine's test). Normality of distribution of the cytokine plasma levels was checked using Shapiro-Wilks' test and a log-transform was performed in case of non-Normality.

Ethical approval

The research was approved by the Medical Ethics Committee at Samarra General Hospital, Salah al-Din Health Department in Iraq on 25/3/2021 (reference number 3047).

Results

The rate of infection with toxoplasmosis was (92.2%) positive for IgG antibodies, while only (7.8%) were positive for IgM antibodies.

In 103 women who miscarried due to toxoplasmosis, 14 of them tested positive for thyroid peroxidase antibodies (anti-TPO) at a rate of 13.51%, compared to 3 positive results in 50 negative control groups for toxoplasmosis (6.0%) (Fig. 1). The rate of positive results for autoimmune antibodies of the thyroid gland in women with recurrent miscarriages is higher compared to women with recurrent miscarriages not infected with toxoplasmosis, although the statistical analysis does not show a significant difference between them *P*-value (0.07). This concentration of anti-TPO indicates that autoimmune thyroiditis may be one of the causes of thyroid dysfunction, as seen in Hashimoto's disease.

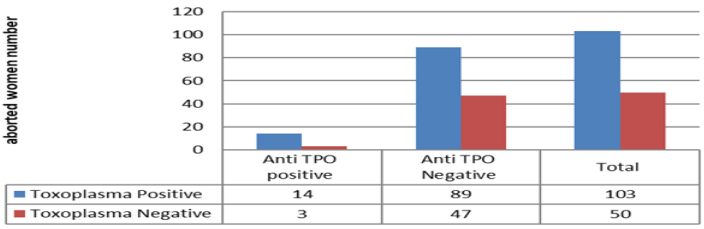

Fig. 1: Distribution of anti-TPO status in *toxoplasma* positive and *toxoplasma* Negative aborted women

The results in Table 1 and Fig. 2 showed that the mean concentration of IL-4, IL-6, IL-10 in group I women is significantly higher compared to group III (control) women, while no significant difference was observed for IL-17 and TNF-α.

Table 1: Statistical comparison of cytokine levels in group I(*Toxoplasma* Positive/anti-TPO Negative) compared to group III (*Toxoplasma* Negative /anti-TPO Negative) control women

Cytokine	Group I (n=89)	Group III (n=47)	*P*-value
IL-4	98.13±29.33	32.21±29.33	.010
IL-6	47.78±33.79	39.72±13.46	.017
IL-10	39.34±22.65	27.89±7.11	.017
IL-17	61.06±28.20	59.64±21.11	.176
TNF-α	74.07±26.58	73.70±27.50	.900

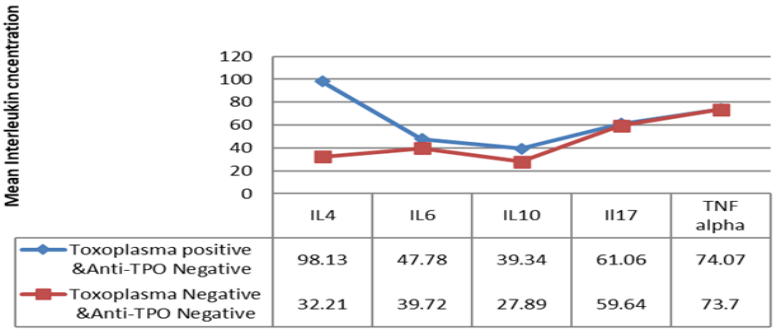

Fig. 2: Mean interleukin level in group I (*Toxoplasma* Positive/anti-TPO Negative) and group III (*Toxoplasma* Negative /anti-TPO Negative) control women

While the results in Table 2 and Fig. 3 showed that the mean concentration of IL-4 and IL-10 in group II women is significantly higher compared to group III (control) women, while no significant difference was observed for IL-17, IL6 and TNF-α.

Table 2: statistics analysis of Interleukin activity group II (*Toxoplasma* Positive/anti-TPO Positive) women and group III (*Toxoplasma* Negative /anti-TPO Negative)

Cytokine	Group II (n=14)	Group III (n=47)	*P*-value
IL-4	79.36±56.83	32.21±29.33	.013
IL-6	38.21±13.18	39.72±13.46	.953
IL-10	35.57±16.70	27.89±7.11	.000
IL-17	62.86±27.29	59.64±21.11	.316
TNF-α	68.14±12.47	73.70±27.50	.099

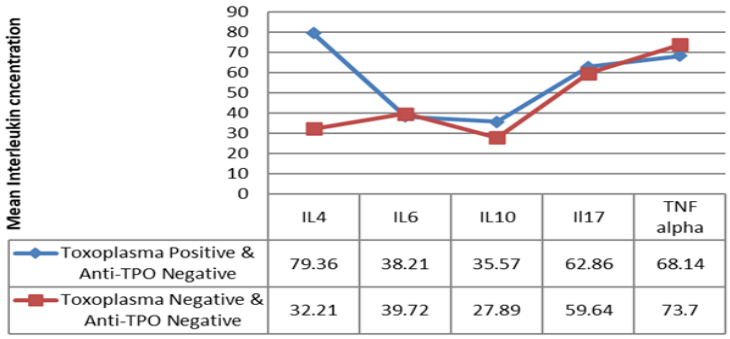

Fig. 3: Mean interleukin level in (*Toxoplasma* Positive/anti-TPO Positive) and (*Toxoplasma* Negative/anti-TPO Negative) aborted women

Discussion

Overall, 14 out of 103 samples gave positive results for thyroid antibodies (13.6%), while the positive results in the group of non-toxoplasmosis were 3 out of 50 samples (6%), which means that latent toxoplasmosis infection may lead to the stimulation of autoimmune antibodies including anti-TPO antibodies compared to toxoplasmosis-negative women, leading to the emergence of autoimmune thyroid disorders after toxoplasmosis infection.

28.3% of women with seropositive anti-*Toxoplasma* IgG antibodies had auto immune thyroid disorders, compared to 13.9% of those with seronegative antibodies. A significant association is present between toxoplasmosis and AITD ($P<0.05$). Latent toxoplasmosis could stimulate the immune system and cause AITD in pregnant women (16). Another study found that women with latent toxoplasmosis had significantly higher anti-TPO levels ($P=0.004$). Toxoplasmosis-related AITD (autoimmune thyroid disorder) measures may not be clinically relevant, but they may reveal how autoimmune thyroid problems begin (17). Latent toxoplasmosis is common during pregnancy and may elevate the risk of AITD (18). The overall prevalence of latent toxoplasmosis was found to be 22.6%, particularly among women with high anti-TPO levels. In addition, *T. gondii* infection directly impacts thyroid gland function and hormone synthesis (19). Hypothyroidism individuals with parasitoid toxoid plasma infection had lower calcitonin levels. Early thyroid antibody detection during pregnancy predicts thyroid disease complications and morbidity in the mother. Thus, screening techniques

should include anti-TPO titers and TSH levels since antibodies increase the risk of postpartum thyroiditis and pregnancy complications (20). Another study found a significant association between toxoplasmosis and anti-TPO levels. A virtually simultaneous study confirms that previous *T. gondii* infection increases anti-TPO (21).

In our study, levels of IL-4, IL-10, and IL-6 were significantly elevated in women with toxoplasmosis (anti-TPO -ve) compared to those without toxoplasmosis (anti-TPO -ve). In chronic toxoplasmosis infections, higher levels of the pro-inflammatory cytokine IL-6 and the anti-inflammatory cytokines IL-10 and IL-4 were seen, hence modulating the immune system in infected women.

Women with congenital toxoplasmosis who suffer spontaneous miscarriage or stillbirth have a skewed immune response to Th2 cells. Th2 cells secrete cytokines such as IL-4 and IL-10, believed to regulate the inflammatory response and facilitate tissue healing (22). Factors such as infection timing, dose, gestational age, and the host's genetic background can influence the multifaceted involvement of IL-4 in the specific immune response to *T. gondii* during pregnancy. Research has highlighted the potential role of IL-4 in the development of congenital toxoplasmosis and abortion (23). In one of the studies compared to the control group, the percentage INF-γ / IL-4 in the peripheral blood of patients with recurrent spontaneous abortion is abnormally high with 33.77%. The flow cytometry technique has high clinical value in detecting the INF-γ / IL-4 ratio in peripheral blood (24). The mean IL-4 levels in the patients aborted due to toxoplasmosis increased compared to the sample of control women. The results of the current study agreed with the study that found several cytokines, such as IL-4, IL-5, and IL-10 are increased in patients chronically infected with toxoplasmosis compared to uninfected patients (25). The pathology of toxoplasmosis-associated abortion has linked the IL10 pathway to immune regulation during *T. gondii* infection (26). Interleukin-10 suppresses the immunological response to *T. gondii* reducing inflammatory and tissue damage. Nonetheless, suppressing the immune response may enhance vulnerability to *T. gondii i*nfection and boost parasite survival. *T. gondii* infection in pregnant women can cause abortion, and IL-10 may contribute to this by suppressing the body's immune response to the parasite and enhancing its persistence in the placenta (27). One study also showed a significant increase in the levels of IL-6, IL-17, and dopamine in the sera of patients infected with toxoplasmosis compared to healthy controls (28). A significant rise was found in IL-6 and IL-12 blood levels in *T. gondii* women who had miscarriages compared to pregnant women (29). *T. gondii* infection causes the production of anti-inflammatory and pro-inflammatory cytokines, such as IL-6 and IL-10, via a modified host immune system that manipulates host gene transcription and signaling pathways (30).

Our study identified a highly significant relationship between IL-4 and IL-10 concentrations in women who had abortion and were infected with Toxoplasma (+ve) (anti-TPO +ve) compared to those uninfected with Toxoplasma (–ve) (anti-TPO -ve). This indicates that women positive for toxoplasmosis produce anti-inflammatory cytokines IL-4 and IL-10 to prevent excessive immune responses and thyroid autoimmune disorders.

Research in Tikrit City, Iraq (31) revealed that pregnant women infected with *T. gondii* showed increased levels of IL-6 in comparison to healthy controls and the general infected population. The concentration of IL-10 demonstrated a significant variation. Denis et al. reported four different cytokine profiles representing the temporal dynamics of Toxoplasma infection phases in pregnant French women (32). During the acute stage, inflammatory mediators such as IL-1β, IL-17A, IL-18, and TNF-α showed little change. Chronically infected people have much reduced inflammatory mediators. A recent meta-analysis by Dos Santos et al (33) encompassing eight

studies and a total sample of 868 pregnant women, revealed that toxoplasma-positive women exhibited elevated levels of IL-4, IL-6, and IL-17 compared to toxoplasma-negative women. IL-6 and IL-17 levels were two folds higher in the blood of toxoplasmosis-positive mothers who had an abortion compared to toxoplasmosis-negative controls (P<0.001). Elevated serum levels of IL-4, IL-10, and hs-CRP correlate with persistent or recurrent disease in patients with PTC and PTC combined with HT. Our findings indicate that these biomarkers could enhance patient categorization based on recurrence risk, particularly in individuals with PTC + HT, where thyroglobulin levels are unreliable due to the presence of thyroglobulin Abs (34). Ninety-eight euthyroid women diagnosed with Hashimoto's thyroiditis participated in the study. The diagnosis was corroborated by higher serum levels of anti-TPO. Women with Hashimoto's thyroiditis had markedly increased serum levels of IL6 in comparison to controls (35). People with Hashimoto's thyroiditis had higher levels of IL1B, IL-6, IL-8, IL-10, IL-12, and TNF-α in their blood than healthy controls. Serum TSH, anti-TG, and anti-TPO levels were generally lower in controls and significantly higher in individuals with Hashimoto's thyroiditis (36). One study of Japanese women found a negative association between thyroid autoimmunity and normal pregnancy rates (37). The results of the current study may facilitate future research and enhance the diagnosis and management of autoimmune thyroid disease (38). In addition, new research has established a correlation between thyroid autoimmune diseases and an increased rate of spontaneous abortion (39).

Our study showed that toxoplasmosis can stimulate the emergence of some autoimmune diseases, including thyroid gland autoimmune diseases, and that women infected with toxoplasmosis who were negative for Anti-TPO antibodies had a high level of inflammatory IL6 and anti-inflammatory IL-10 and IL-4, which produced an immune balance, especially in chronic infections, which protected the body. For women with toxoplasmosis who tested positive for Anti-TPO, IL-4 and IL-10 levels were high, which may cause immunological problems as a result to an imbalance between inflammatory and anti-inflammatory interleukins.

Conclusion

The immunological response to *Toxoplasma* infection depends on pro- and anti-inflammatory cytokines. In women infected with *T. gondii* who test negative for anti-TPO, elevated IL-6, IL-4, and IL-10 may indicate an immunological imbalance between inflammatory and anti-inflammatory interleukins. Women positive for both anti-TPO and *T. gondii* had high IL-4 and IL-10 levels only, indicating an immune system imbalance that may cause immunological diseases. Our research suggests that inflammatory and anti-inflammatory interleukins may help diagnose and treat autoimmune thyroid disorders.

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Conflicts of Interest

All the authors declare that they have no conflict of interest.

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