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Hormonal and Immunological Biomarkers (Testosterone, Oxytocin, and IL-5) Associated with Toxoplasma gondii Infection in Relation to Autism Spectrum Disorder

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ABSTRACT:

Background: Toxoplasma gondii is an intracellular protozoan parasite capable of crossing the placental barrier and influencing fetal neurodevelopment. Increasing evidence suggests that maternal toxoplasmosis may contribute to the development of Autism Spectrum Disorder (ASD) through hormonal and immunological alterations.

Objective: This study aimed to investigate the association between maternal Toxoplasma gondii infection and serum levels of testosterone, oxytocin (OXT), and interleukin-5 (IL-5), and to evaluate their possible relationship with Autism Spectrum Disorder.

Methods; A case–control study was conducted between January and October 2025. A total of 180 peripheral blood samples were collected from 60 families, including mothers of children with ASD, affected children, and their siblings. Maternal T. gondii infection was determined by detecting anti-T. gondii IgG and IgM antibodies using a TORCH rapid cassette test. Serum testosterone, oxytocin, and IL-5 levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. Results: Maternal Toxoplasma gondii infection was associated with a markedly higher frequency of Autism Spectrum Disorder (ASD) among offspring (76.2%, 96/126) compared with non-infected mothers (13.4%, 54/404). Infected mothers exhibited significantly higher serum testosterone levels than non-infected mothers (0.69 ± 0.00 vs. 0.65 ± 0.00 ng/mL, P = 0.001) and significantly elevated serum IL-5 concentrations (1.991 ± 0.010 vs. 1.480 ± 0.011 pg/mL, P = 0.001). In contrast, serum oxytocin levels were significantly lower in infected mothers compared with non-infected mothers (0.90 ± 0.00 vs. 2.35 ± 0.02 pg/mL, P = 0.001).

Conclusion: Maternal Toxoplasma gondii infection was associated with significant changes in serum testosterone, oxytocin, and IL-5 levels. These alterations may contribute to the biological mechanisms linking maternal toxoplasmosis with Autism Spectrum Disorder and could serve as potential biomarkers for evaluating the impact of maternal infection on neurodevelopment.

INTRODUCTION

Toxoplasma gondii is one of the most widespread parasites globally, with estimates suggesting that between 30 and 60% of the world's population is infected. It has been found that about one-third of the world's population has antibodies against the

parasite. In recent years, this parasite has been carefully studied by many doctors, researchers, and economists due to the serious effects it causes on humans and farm animals, which constitute the main source of food (1).

The parasite is most often transmitted to humans through the consumption of undercooked meat or meat contaminated with the parasite's tissue cysts, unpasteurized milk, organ transplantation, blood transfusion, via the placenta, direct contact with the feces of infected cats, or even by ingesting sporozoites in contaminated water, food and soil, or by inhalation (2) Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder arising from the interaction of genetic, environmental, and immunological factors that affect brain development and communication functions. In recent decades, there has been increased interest in studying environmental influences, particularly parasitic infections during pregnancy, as a major trigger for what is known as Maternal Immune Activation (MIA). MIA is a systemic inflammatory condition that can alter the fetal neurological environment and increase the risk of neurodevelopmental disorders, most notably autism (3) .

Studies (4, 5). show that primary maternal infection during pregnancy can lead to transmission of the parasite to the fetus, causing congenital toxoplasmosis, which results in neurological disorders and defects in cortical and visual development. Recent studies have also indicated that even chronic, asymptomatic infection can induce persistent, mild immune responses and alterations at the neuronal and mitochondrial gene levels, creating a risk environment for the development of autism. Emerging evidence suggests that maternal *T. gondii* infection may influence neurodevelopment not only through direct parasitic invasion but also by altering endocrine and immune pathways(7 ,6) . Hormonal biomarkers such as testosterone and oxytocin play essential roles in brain development, neuronal differentiation, synaptic plasticity, and social behavior, whereas interleukin-5 (IL-5) is an important immunoregulatory cytokine involved in inflammatory and immune responses. Dysregulation of these hormonal and immunological mediators during pregnancy may contribute to abnormal fetal brain development and increase susceptibility to ASD(8,9) . Therefore, evaluating these biomarkers in mothers infected with *T. gondii* may provide valuable insights into the biological mechanisms linking maternal toxoplasmosis with ASD.

Therefore, the present study aimed to assess maternal *T. gondii* infection with testosterone, oxytocin, and IL-5 serum levels and their potential relationship with ASD.

MATERIALS AND METHODS

Study design: This case–control study was conducted between January and October 2025 to investigate the association between *Toxoplasma gondii* infection and hormonal and immunological biomarkers in relation to Autism Spectrum Disorder (ASD). The study included 60 families, comprising mothers of children with ASD, their affected children, and their siblings. A total of 180 peripheral blood samples were collected from the study participants. Samples were obtained from Dar Al-Ataa Autism Center (Tikrit–Al-Qadisiyah), the National Autism Center at Baghdad Medical City, outpatient clinics in Salah Al-Din Governorate, and through personal contacts, as well as through personal contacts. The study population was classified into three groups: Group A, mothers of children diagnosed with ASD; Group B, siblings of children with ASD; and Group C, children diagnosed with ASD.

Blood Sample Collection: Approximately 5 mL of venous blood was collected aseptically from each participant. Blood samples were transferred into gel separator tubes for serum separation to perform hormonal and immunological assays.

Serological Diagnosis of *Toxoplasma gondii:* Maternal serum samples were screened for *Toxoplasma gondii* infection using the TORCH Rapid Test Cassette, according to the manufacturer's instructions. The test was used for the qualitative detection of anti-*T. gondii* IgG and IgM antibodies. Mothers who tested positive for either IgG or IgM antibodies were considered seropositive and were subsequently included in the infected group, whereas mothers negative for both antibodies were classified as the non-infected group.

ELISA Kit: Serum testosterone, oxytocin (OXT), and interleukin-5 (IL-5) levels were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. Testosterone and oxytocin were measured using competitive ELISA kits, whereas IL-5 was determined using a Elabscience ELISA kit. Absorbance was measured at 450 nm using a microplate reader, and concentrations were calculated according to the respective standard curves.

Statistical Analysis: The obtained data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) software. Results were expressed as mean ± standard deviation (Mean ± SD). Differences among groups were assessed using F-value. Statistical significance was considered at $P \leq 0.05$.

RESULTS

Maternal *T. gondii* Infection and Autism Spectrum Disorder

Table 1 shows the distribution of Autism Spectrum Disorder (ASD) among children according to maternal *T. gondii* infection status. A substantially higher proportion of ASD was observed among children born to *T. gondii*-infected mothers (96/126, 76.2%) compared with children born to non-infected mothers (54/404, 13.4%). In contrast, non-ASD children were more frequently observed among mothers without *T. gondii* infection (350/404, 86.6%) than among infected mothers (30/126, 23.8%). These findings indicate a markedly higher frequency of ASD among offspring of mothers with ***T. gondii*** infection.

Table 1. Distribution of Autism Spectrum Disorder among children according to maternal *Toxoplasma gondii* infection status.

Maternal *T. gondii* infection	Total (n)	ASD children n (%)	Non-ASD children n (%)
Positive	126	96 (76.2)	30 (23.8)
Negative	404	54 (13.4)	350 (86.6)

Immunological markers

Serum testosterone

Table 2 compares serum testosterone levels between *Toxoplasma gondii*-infected and non-infected mothers. The mean serum testosterone level was significantly higher in infected mothers (0.69 ± 0.00 ng/mL) than in non-infected mothers (0.65 ± 0.00 ng/mL). Statistical analysis revealed a significant difference between the two groups (t = 5.22, mean difference = 0.04, P = 0.001).

Table 2. Comparison of serum testosterone levels between *T. gondii*-infected and non-infected mothers.

Study Groups	N	Mean (ng/mL)	Std. Error	t-test	Mean Difference	P-value
T. gondii-infected mothers	60	0.69	0.00	5.22	0.04	0.001
Non-infected mothers	60	0.65	0.00	—	—	—

Oxytocin Hormone

Table 3 compares serum oxytocin Hormone levels between *T. gondii*-infected and non-infected mothers. The results showed a significant decrease in oxytocin levels in infected mothers compared to healthy mothers. Infected mothers showed a significant decrease (mean 0.90 ± 0.00) compared to healthy mothers (2.35 ± 0.02), with a significance level of (P = 0.001).

Table 3 compares serum oxytocin Hormone levels between T. gondii-infected and non-infected mothers.

Study Groups	N	Mean (pg/mL)	Std. Error	t-test	Mean Difference	P-value
T. gondii-infected mothers	60	0.90	0.00	49.87	1.45	0.001
Non-infected mothers	60	2.35	0.02			

IL-5 Levels

Table 4 compares serum IL-5 levels between *T. gondii*-infected and non-infected mothers. The mean serum IL-5 concentration was significantly higher in infected mothers (1.991 ± 0.010 pg/mL) than in non-infected mothers (1.480 ± 0.011 pg/mL). Statistical analysis demonstrated a highly significant difference between the two groups (t = 33.62, mean difference = 0.511, P = 0.001).

Table 4 compares serum IL-5 levels between *T. gondii*-infected and non-infected mothers.

Study Groups	N	Mean (pg/mL)	Std. Error	t-test	Mean Difference	P-value
T. gondii-infected mothers	60	1.991	0.010	33.62	0.511	0.001
Non-infected mothers	60	1.480	0.011			

Reference range (IL-5): > 1.5 pg/mL

DISCUSSION

The results showed a significant increase in the rate of autism among children of mothers infected with the *T. gondii* parasite (76.1%) compared to children of uninfected mothers (13.3%). This demonstrates a statistically significant variation in the incidence of ASD in children depending on the mother's health status and her *T. gondii* infection Al-Taei and Jasim (2021) These high pathophysiological results indicate the high ability of *T.gondii* to cross the placenta, reach the fetal central nervous system, and establish itself in glial and neuronal brain cells. This tissue establishment leads to a state of chronic neuroinflammation accompanied by excessive secretion of inflammatory cytokines in utero, which directly interferes with the processes of neurogenesis and synapse formation in the fetus, thus increasing the likelihood of the future emergence of autistic behaviors and traits. This general trend in our study's findings is consistent with the study conducted by Nayeri (2020), which concluded that toxoplasmosis infection is a statistically significant risk factor for autism. Al Malki's study (2021) observed in a small Saudi sample a correlation between maternal toxoplasmosis infection and the occurrence of mutations in mitochondrial DNA in her autistic children, which supports the hypothesis of a possible biological mechanism linking infection to autism through oxidative stress associated with mitochondrial dysfunction.

The results of the current study showed a significant increase in testosterone levels in infected mothers compared to healthy mothers. This increase can be explained by the fact that chronic infection with the parasite *T. gondi* may affect hormonal regulation through its impact on the hypothalamic-pituitary-adrenal, as well as the effect of the chronic inflammatory response on steroid hormone secretion. This finding is particularly important because several studies have indicated that increased fetal exposure to androgens may be associated with an increased likelihood of autism spectrum disorder. Simon Baron-Cohen *et al.* demonstrated that elevated fetal steroid activity is associated with an increased risk of autism, supporting the hypothesis of an overactive male brain.

This result is consistent with the study by Schwarz *et al.* (2020), which demonstrated that mothers with high androgen levels during pregnancy have an increased risk of having children with autism, thus supporting your finding regarding elevated hormone levels in infected mothers. A study by Croen *et al.*, 2016, in which researchers measured steroid hormone levels in stored serum samples from mothers during pregnancy, found that mothers who gave birth to children with autism had significantly higher testosterone levels, with a probability value (P = 0.01) and an increased risk of (1.62) for every one standard deviation increase in the hormone.

The results showed a significant and clear decrease in the level of oxytocin in infected mothers compared to healthy ones. Oxytocin plays a crucial role in social behavior, emotional communication, and the regulation of social relationships. Current data indicate that disruption of this hormone is the most relevant mechanism associated with autism spectrum disorder. The decrease recorded in the current study can be attributed to the chronic inflammation resulting from infection with the Toxoplasma parasite, which may cause dysfunction in the oxytocin-secreting neurons in the hypothalamus, which may lead to a decrease in its level. Furthermore, the inflammatory cellular mechanisms have the ability to modify the functions of the glandular nerve axons responsible for secreting this hormone.

IL-5 is a Th2-type immune response cytokine responsible for activating antibody-dependent humoral immunity and eosinophils. The marked increase in IL-5 levels in immunocompromised mothers can be explained by the fact that the body's response to *T. gondii* primarily relies on a Th1 cellular immune response through the secretion of interferon-gamma (IFN-γ) and interleukin-12 (IL-12). This, in turn, stimulates certain Th2-related cytokines, including IL-5, in mothers to maintain control against the parasite (Ashwood, 2013).

IL-5 is a Th2-type immune response cytokine responsible for activating humoral immunity, which relies on antibodies and eosinophils. The results of our current study on the significant increase of IL-5 are consistent with the findings of the study by (Goines *et al*., 2011), which indicated that there is a significant increase in the levels of cytokines associated with the Th2 pathway, including interleukin IL-5, in maternal plasma during pregnancy. Similarly, the study by (Croen *et al*., 2017), which showed that maternal blood samples during pregnancy indicated that an increase, rather than a decrease, of some cytokines is associated with autism. The reason here lies in the immunological specificity of chronic toxoplasmosis infection, which induces the body to produce a Th1-directed cellular response as a primary line of defense, which leads to an immune stimulation of Th2 cytokines, including IL-5, in mothers in order to reduce the severity of cellular inflammation and protect maternal and placental tissues to ensure the stability of the pregnancy successfully.

CONCLUSION

This study shows that maternal infection with *T. gondii* is associated with significant changes to levels of testosterone, oxytocin and IL-5 in the serum, as well as a higher incidence of ASD in offspring. Our results indicate the possibility that maternal toxoplasmosis may result in biological mechanisms related to ASD via endocrine and immunological dysregulation. We recommend further prospective studies with larger cohorts to elucidate the causal link and explore other relevant biomarkers that may assist in early prediction and prevention of maternal *T. gondii* induced ASD.

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