Impact of Toxoplasma gondii Seropositivity on Thyroid Function and Anti-TPO Antibody Status

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Abstract

Background: Thyroid dysfunction is one of the most common endocrine illnesses worldwide, with autoimmune mechanisms significantly contributing to its aetiology. Toxoplasma gondii, a prevalent intracellular parasite, has been proposed as a potential catalyst for autoimmune thyroid disorders (AITDs), however its function remains inadequately comprehended. **Objective:** This study sought to examine the correlation between Toxoplasma gondii infection and thyroid dysfunction, specifically with the positivity of anti-thyroid peroxidase (anti-TPO) antibodies. **Methods:** A cross-sectional investigation was executed in Kirkuk, Iraq, from October 1, 2024, to April 30, 2025, encompassing 100 patients with thyroid dysfunction (13 with hyperthyroidism and 87 with hypothyroidism) and 50 healthy controls. Serum samples were examined for thyroid hormones (TSH, T3, T4), anti-TPO antibodies (CLIA), and T. gondii-specific IgM and IgG antibodies (ELISA). **Results:** Marked disparities in age distribution were seen between patients and controls ($p = 0.0003$), with hyperthyroidism prevalent in individuals over 55 years, and hypothyroidism more common in middle-aged cohorts. Despite a higher prevalence among females, the sex differences were not statistically significant ($p = 0.083$). Anti-TPO positivity was markedly elevated in patients (23%) vs to controls (0%) ($p = 0.0025$). The infection of T. gondii shown a substantial correlation with thyroid dysfunction, especially in Anti-TPO positive patients, where the co-positivity of IgM/IgG was 34.78% compared to 5.19% in Anti-TPO negative individuals ($p = 0.0001$). Co-positivity for CMV IgM/IgG was significantly greater in Anti-TPO positive patients (34.78%) than in negative patients (2.60%) ($p = 0.0001$). In seronegative individuals for T. gondii, those who tested positive for Anti-TPO exhibited a substantially greater mean age (55.71 ± 12.23 years) compared to those who tested negative for Anti-TPO (39.59 ± 14.78 years; $p = 0.016$). Moreover, infection markers exhibited a robust correlation with Anti-TPO positivity by gender, with Anti-TPO positive males demonstrating the highest co-positivity rates for both T. gondii and CMV (66.67%). **Conclusion:** The results suggest a possible immunopathogenic involvement of Toxoplasma gondii infection in autoimmune thyroid dysfunction, specifically hypothyroidism linked to anti-TPO positive.

Keywords: Toxoplasma Gondii, Thyroid Dysfunction, Anti-TPO Antibodies, IgM/IgG.

Introduction

Thyroid dysfunction is one of the most prevalent endocrine disorders. The thyroid gland synthesises the hormones thyroxine (T4) and triiodothyronine (T3) in response to thyrotrophin (TSH) released by the pituitary gland. Overt hyperthyroidism is characterised by diminished serum TSH levels and elevated free serum triiodothyronine (T3) or free thyroxine (T4) levels. Subclinical hyperthyroidism is defined by reduced serum TSH levels while maintaining normal free triiodothyronine (FT3) and free thyroxine (FT4) levels. Overt hypothyroidism is defined by reduced free thyroxine (T4) levels and elevated TSH, while subclinical hypothyroidism is characterised by modestly increased TSH levels with normal T3 and T4 levels.[1]

Access This Article Online

Quick Response Code:

Website: www.jnsbm.org

DOI: https://doi.org/10.5281/zenodo.17129198

Thyroid dysfunction is prevalent throughout diverse ages, genders, races/ethnicities, and geographic areas, due to differences in dietary iodine intake. Women, those aged over 60, those with a personal or significant familial history of thyroid diseases, and postpartum women are at an increased risk of thyroid dysfunction. Toxoplasma gondii (T. gondii) is an obligatory intracellular protozoan parasite. T. gondii is responsible for global morbidity and death.[2-4] This parasite causes a disorder termed toxoplasmosis, which often remains undetected and is

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Submitted: 05th July, 2025 **Received:** 28th August, 2025
Accepted: 30th August, 2025 **Published:** 03rd September, 2025

How to Cite This Article: Sabir I F, Obaid H M. Impact of Toxoplasma gondii Seropositivity on Thyroid Function and Anti-TPO Antibody Status. J Nat Sc Biol Med 2025;16(3):7-12

inadequately managed. The transmission of T. gondii predominantly occurs through the oral route, with raw or undercooked meat acting as a major vector for its dissemination.[5] Humans may get the infection by consuming sporulated oocysts found in contaminated food or water. T. gondii may be transmitted by organ transplantation and blood transfusion.[6] T. gondii can cross the placenta of an infected pregnant mother and potentially infect the foetus congenitally. Toxoplasmosis has a wide spectrum of clinical symptoms, ranging from asymptomatic instances to severe, life-threatening illnesses.[7,8] There is scant information concerning T. gondii infection in the thyroid gland. In a study of nine autopsy cases of disseminated toxoplasmosis, researchers noted the involvement of the thyroid gland. Antibodies targeting T. gondii have been associated with autoimmune thyroid conditions.[9] A prior infection with T. gondii was associated with an elevation in autoantibodies against thyroid peroxidase. Latent toxoplasmosis was associated with a little increase in thyroid hormone production during gestation. Moreover, impaired thyroid function was recorded in murine toxoplasmosis.[10-11] This study aimed to investigate the relationship between Toxoplasma gondii infection and thyroid dysfunction, particularly with the presence of anti-thyroid peroxidase (anti-TPO) antibodies.

Materials and methods

This observational study was carried out in Kirkuk city, Iraq, from October 1, 2024, until the conclusion of April 2025. The main aim was to investigate the correlation between Toxoplasma gondii infection and thyroid dysfunction, particularly focussing on the connection with anti-thyroid peroxidase (anti-TPO) antibody positivity. A total of 150 people were enrolled, comprising 100 patients diagnosed with thyroid dysfunction and 50 ostensibly healthy persons serving as the control group. Among the individuals with thyroid dysfunction, 13 were diagnosed with hyperthyroidism and 87 with hypothyroidism. Control patients were matched by age and sex and had no known history or clinical symptoms of thyroid illness. Participants were sourced from outpatient endocrine clinics and internal medicine departments in Kirkuk. Participants were required to be at least 16 years old and have thyroid insufficiency verified through clinical and biochemical assessment. Control subjects were selected based on normal thyroid hormone levels and the absence of thyroid-related complaints. The exclusion criteria were pregnancy, lactation, existing systemic autoimmune or infectious disorders, recent immunosuppressive treatment, or organ transplantation. Aseptic venous blood samples (5 mL) were obtained from each participant and processed without delay. The serum was isolated via centrifugation, aliquoted, and preserved at –20°C for subsequent analysis. Thyroid hormone levels, including TSH, T3, and T4, along with anti-TPO antibodies, were quantified using chemiluminescent immunoassay (CLIA) on the Cobas e411 analyser (Roche Diagnostics). The reagent kits utilised were Cobas TSH, Cobas T3, Cobas T4, and Cobas anti-TPO kits.

Detection of Toxoplasma gondii infection was conducted with commercial qualitative ELISA kits for the assessment of both IgM and IgG antibodies. The data were analysed in accordance with the manufacturer's guidelines, categorising individuals as IgM/IgG positive.

Statistical analysis was performed utilising version 26.0 of the Statistical Package for the Social Sciences (SPSS) software (IBM Corp., Armonk, NY, USA). Categorical variables were presented as frequencies and percentages, whereas continuous variables, such as age, were reported as mean ± standard deviation. The Chi-square test (χ^2) or Fisher's exact test was utilised to assess the relationships among thyroid dysfunction, anti-TPO antibody status, and viral serological indicators. An independent samples t-test was employed to compare mean age based on Anti-TPO status within infection subgroups. A p-value below 0.05 was deemed statistically significant for the entirety of the analysis.

Prior to engaging in the project, each patient was provided with comprehensive information regarding the study methodology and signed an informed consent document. The study adhered to ethical standards and received clearance from the Kirkuk Health Directorate, with official reference number 216 dated 1/10/2024.

Results

The demographic and hormonal parameters of individuals with thyroid dysfunction exhibited substantial changes compared to the control group. The age distribution exhibited significant differences ($p = 0.0003$), with a greater percentage of hyperthyroid patients (50%) over the age of 55, whereas hypothyroidism was predominantly observed in the middle-aged demographic, specifically those aged 26–45 years. Individuals aged 16 to 25 were more prevalent in the control group (26%) compared to those with thyroid problems. While females constituted the majority of both hyperthyroid (83.33%) and hypothyroid (76.14%) patients, the sex distribution lacked statistical significance ($p = 0.083$). In terms of thyroid hormone levels, hyperthyroid patients presented dramatically diminished TSH and elevated T3 and T4 levels, whereas hypothyroid individuals demonstrated substantially increased TSH and decreased T3 and T4 levels in comparison to controls, with all differences being statistically significant ($p = 0.001$). In this investigation, Toxoplasma IgG positive was identified in 8 (61.54%) hyperthyroid individuals, but concurrent IgM and IgG positivity was found in 1 (7.69%) patient. Among hypothyroid patients, 30 (34.48%) tested positive for Toxoplasma IgG, whereas 11 (12.64%) demonstrated positivity for both IgM and IgG. The percentage of patients exhibiting negative Toxoplasma serology (IgM/IgG negative) was diminished in hyperthyroid instances, with 4 (30.77%) compared to 45 (51.72%) in hypothyroid individuals.

Table 1: Demographic and Hormonal Characteristics of Patients with Thyroid Dysfunction.

Parameters	Patients with Thyroid Dysfunction			Control Group n(%)	P-value
Age Groups (years)	No.	Hyperthyroidism n(%)	Hypothyroidism n(%)		
16–25	6	2 (16.67)	4 (5.59)	13 (26)	0.0003
26–35	27	2 (16.67)	25 (28.41)	21 (42)	
36–45	30	2 (16.67)	28 (31.82)	9 (18)	
46–55	11	1 (8.33)	10 (11.36)	5 (10)	
>55	26	6 (50)	20 (22.73)	2 (4)	
Total	100	13(100)	87(100%)	50(100)	
Sex					
Female	77	10 (83.33)	67 (76.14)	30 (60)	0.083
Male	23	2 (16.67)	21 (23.86)	20 (40)	
Total	100	12(100%)	88(100)	50(100)	
Thyroid Hormone					
TSH		0.08 ± 0.08	14.68 ± 15.03	2.73 ± 0.75	0.001
T3		3.32 ± 4.13	1.71 ± 0.79	1.76 ± 0.53	0.001
T4		116.19 ± 44.97	106.56 ± 34.10	92.52 ± 13.28	0.001

Table 2: T. Gondii Serological Markers among Patients with Thyroid Dysfunction and the Control Group.

Toxoplasma ELISA Result	Patients with Thyroid Dysfunction			Control Group n(%)	P-value
	No	Hyperthyroidism n(%)	Hypothyroidism n(%)		
IgG Positive	38	8 (61.54)	30 (34.48)	0 (0)	0.0066
IgM Positive	1	0 (0)	1 (1.15)	0 (0)	
IgM/IgG Positive	12	1 (7.69)	11 (12.64)	0 (0)	
IgM/IgG Negative	49	4 (30.77)	45 (51.72)	50 (100)	
Total	100	13 (100)	87 (100)	50 (100)	

The assessment of Anti-TPO antibody levels in patients with thyroid dysfunction revealed a statistically significant correlation (p = 0.0025). In the hyperthyroid cohort, 5 (38.46%) patients tested positive for Anti-TPO, whereas 8 (61.54%) tested negative. In hypothyroid patients, Anti-TPO positivity was detected in 18 cases (20.69%), while 69 cases (79.31%) were Anti-TPO negative. In contrast, no control subject tested positive for Anti-TPO.

Table 3: Anti-TPO Antibody Status among Patients with Thyroid Dysfunction and the Control Group.

Anti-TPO	Patients with Thyroid Dysfunction			Control Group n(%)
	No	Hyperthyroidism n(%)	Hypothyroidism n(%)	
Positive	23	5 (38.46)	18 (20.69)	0 (0)
Negative	77	8 (61.54)	69 (79.31)	50 (100)
Total	100	13 (100)	87 (100)	50 (100)

P-value: 0.0025

Table 4 examines the correlation between anti-thyroid peroxidase (Anti-TPO) antibody positivity and Toxoplasma gondii serological status in individuals with thyroid dysfunction. The connection is statistically significant (P = 0.0001), suggesting a possible correlation between T. gondii infection and thyroid autoimmunity. Significantly, mixed IgM/IgG seropositivity was observed in 34.78% of Anti-TPO positive patients, in contrast to merely 5.19% of Anti-TPO negative individuals, indicating that chronic or reactivated T. gondii infection may contribute to the initiation or aggravation of autoimmune thyroid disease. Conversely, IgM-only positive was more common in Anti-TPO negative patients (40.26%) compared to those with Anti-TPO positivity (30.43%). Seronegativity (IgM/IgG negative) was substantially more prevalent in Anti-TPO negative patients (54.55%) compared to Anti-TPO positive patients (30.43%), further substantiating the potential immunological role of T. gondii. Isolated IgG positivity was infrequent and exclusively identified in a patient positive for Anti-TPO.

Table 4: Association between anti-TPO Antibody Positivity and T. Gondii Infection among Thyroid Dysfunction Patients.

T. gondii IgM/IgG Status	Patients with Thyroid Dysfunction			P-value
	No.	Anti-TPO Negative n(%)	Anti-TPO Positive n(%)	
IgM Positive	38	31 (40.26%)	7 (30.43%)	0.0001
IgG positive	1	0 (0%)	1 (4.35%)	
IgM/IgG Positive	12	4 (5.19%)	8 (34.78%)	
IgM/IgG Negative	49	42 (54.55%)	7 (30.43%)	
Total	100	77 (100)	23 (100)	

The analysis of average ages among thyroid dysfunction patients with positive and negative Anti-TPO antibody status indicated a significant difference in those who tested negative for both Toxoplasma IgM and IgG. Patients who tested positive for Anti-TPO and negative for Toxoplasma IgM/IgG had a higher mean age of 55.71 ± 12.23 years, in contrast to 39.59 ± 14.78 years in Anti-TPO negative patients (p = 0.016).

Table 5: The Comparison of Mean Ages among Thyroid Dysfunction Patients with Positive and Negative Anti-TPO Antibody.

Toxoplasma IgM/IgG Status	Anti-TPO Positive	Anti-TPO Negative	P-value
IgM Positive	45.00 ±	0	
IgG Positive	36.38 ± 13.77	41.49 ± 14.81	0.435
IgM/IgG Positive	40.75 ± 20.13	45.50 ± 24.99	0.645
IgM/IgG Negative	55.71 ± 12.23	39.59 ± 14.78	0.016
Total	43.83 ± 16.91	40.40 ± 15.05	

Table 6 demonstrates a statistically significant correlation (p = 0.001) among gender, anti-TPO antibody status, and infection markers for both CMV and Toxoplasma gondii in patients with thyroid dysfunction. CMV IgM/IgG co-positivity was markedly widespread in anti-TPO positive males (66.67%) and females (30%), in contrast to considerably lower rates in anti-TPO negative males (5%) and females (1.75%). T. gondii IgM/IgG co-positivity was found in 66.67% of anti-TPO positive males and 30% of females, in contrast to 10% and 3.51% in their anti-TPO negative counterparts, respectively. Moreover, the negatives for CMV and T. gondii IgM/IgG was predominantly observed in anti-TPO negative females (77.19% and 57.89%), suggesting reduced exposure or the absence of active or chronic infection within this cohort.

Table 6: The Comparison of Gender among Thyroid Dysfunction Patients with Positive and Negative Anti-TPO Antibody.

Toxoplasma IgM/IgG Status	Anti-TPO				P-value
	Positive		Negative		
	Males, n(%)	Females n(%)	Males, n(%)	Females n(%)	
IgM Positive	0 (0)	1 (5)	0 (0)	0 (0)	0.001
IgG positive	1 (33.33)	6 (30)	9 (45)	22 (38.60)	
IgM/IgG Positive	2 (66.67)	6 (30)	2 (10)	2 (3.51)	
IgM/IgG Negative	0 (0)	7 (35)	9 (45)	33 (57.89)	
Total	3 (100)	20 (100)	20 (100)	57 (100)	

Discussion

The classical profiles of thyroid dysfunction were observed in the present study, wherein hyperthyroid patients showed significant TSH suppression and increased T3 and T4, whereas hypothyroid patients demonstrated elevated TSH and decreased levels of thyroid hormones (p = 0.001). These patterns are consistent with the biochemical profiles described by Kawther *et al*.[5] and in which value is given to anti-TPO antibodies for confirmation of thyroid dysfunction. The presence or absence of thyroid dysfunction was also significantly dependent on anti-thyroid peroxidase (anti-TPO) antibody positivity (p = 0.0025). Here, 38.46 percent of hyperthyroid persons tested positive for anti-TPO, while in hypothyroids, 20.69 percent tested positive. These findings corroborate that of Hamim and Almankhee[6], who established that anti-TPO levels are most important for the diagnosis of thyroiditis and may speak to the autoimmune nature of the disease. Aziz *et al*.[7] found elevated anti-TPO titers in patients with Hashimoto's thyroiditis, thereby reinforcing its importance in clinical diagnosis. In our study, the association between Toxoplasma gondii and thyroid dysfunction was also investigated by. Toxoplasma IgG seropositivity was higher among hyperthyroid patients (61.54%) than among hypothyroid patients (34.48%), which suggests a significant role of latent infections in thyroid autoimmunity. Bansal *et al*.[8] reported the high prevalence of autoimmune thyroid disorders in northern India and speculated that environmental or infectious triggers might be responsible for initiating the disease. The association of anti-TPO positivity and T. gondii infection was strongest among hypothyroids with a significant correlation (p = 0.0001). Vasilj-Mihaljević *et al*.[9] showed similar observations of a strong association between anti-TPO levels and thyroid hormone abnormalities. These observations suggest that latent infections could have cause or aggravating effects on autoimmune thyroid disease. Another noteworthy association observed was that between advanced age and Toxoplasma seronegativity in anti-TPO-positive patients (p = 0.016), possibly a reflection of immune senescence or decreased immune responsiveness in the elderly. Shrestha and Shrestha[10] noted a higher incidence of thyroid disorders in postmenopausal women, attributed to hormonal changes and immune alterations due to aging. Analysis of genders indicated a higher incidence of hyperthyroidism and hypothyroidism in women by 83.33% and 76.14%, respectively. This can be explained by the trend reported by Khan *et al*.[11], which showed that anti-TPO positivity is higher among women due to the possibility of hormonal modulation of the immune system.

Moazzam *et al.*[12] also reported similar gender disparities in thyroid hormone profiles and autoantibody levels. Our study reconfirmed the fact that anti-TPO antibody counts were higher in autoimmune thyroiditis than in other thyroid disorders. Both Shrestha *et al.*[13] and Mahmod and Jaffat[14] reported anti-TPO and anti-TG antibodies as the main markers in autoimmune thyroid diseases, especially in premenopausal and reproductive-age women, indicating the value of antibody testing in the at-risk population. Interestingly, this was in accordance with the results of Jeena *et al.*[15] and Tipu *et al.*[16], who stressed the clinical importance of anti-TPO testing on subjects with abnormal thyroid profiles to facilitate appropriate treatment. Zohora *et al.*[17] also demonstrated high positivity for anti-TPO among infertile females, suggesting systemic effects of thyroid autoimmunity beyond metabolic. Frayyeh *et al.*[18] also found a strong association between thyroid autoimmunity and infertility in Iraqi women, similar to the autoimmune phenotype observed in our female subjects. Gayathri *et al.*[19] further added that subclinical hypothyroidism, usually underdiagnosed, may also show elevated anti-TPO antibodies, emphasizing the importance of the early screening. Moreover, Jose *et al.*[20] conducted a hospital-based study confirming hypothyroidism as one of the highest prevalent forms of thyroid disorders in rural populations and commonly associated with anti-TPO positivity. Ghimire *et al.*[21] endorsed this by revealing that thyroid-related awareness and testing remain scarcely available in the developing world, hence the late diagnosis To summarize, the data showed that the well-known hormonal trends in thyroid dysfunction occur; anti-TPO antibodies are useful in diagnosis; and latent infections, especially Toxoplasma gondii, are strongly implicated in thyroid autoimmunity. These observations are in line with clinical expectations and diagnostics considered valid by thyroid authorities such as the ATA. Further investigations into environmental and infectious agents may aid in uncovering means to prevent autoimmune thyroid disorders.

Conclusion

The results suggest a possible immunopathogenic involvement of Toxoplasma gondii infection in autoimmune thyroid dysfunction, specifically hypothyroidism linked to Anti-TPO positive.

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