ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2025;11(1):51-61 10.52547/iem.11.1.51

Seroprevalence of Toxoplasmosis and Interferon Gamma Levels in Autoimmune Thyroiditis Patients

ARTICLE INFO

Article Type
Original Article

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How to cite this article
Haddad A.H., Khalaf A.K. Seroepidemiological Prevalence of Toxoplasmosis and Interferon Gamma Levels in Autoimmune Thyroiditis Patients . Infection Epidemiology and Microbiology. 2025;11(1): 51-61.

Article History
Received: July 29, 2024
Accepted: September 16, 2024
Published: February 22, 2025

ABSTRACT

Background: In thyroiditis, the cellular immune response plays a crucial role in triggering the production of autoantibodies. *Toxoplasma gondii* elicits a strong innate and adaptive immune response within the host organism. This study aims to assess the seroepidemiological prevalence of toxoplasmosis and the levels of interferon-gamma (IFN-γ) in patients with autoimmune thyroiditis (AITD) in Iraq.

Materials & Methods: This case-control survey was conducted on 100 patients diagnosed with AITD and 70 healthy individuals (non-AITD) who attended general hospitals in Thi-Qar Province, Iraq, between July and November 2023. The prevalence of anti-*Toxoplasma gondii* antibodies was evaluated using enzyme-linked immunosorbent assay (ELISA) kits. Serum levels of IFN-γ were measured using ELISA kits, while the expression levels of IFN-γ were assessed using real-time polymerase chain reaction (PCR).

Findings: Among the participants, 33 patients (33.00%) in the AITD group and 9 patients (12.85%) in the non-AITD group tested positive for *T. gondii* IgG antibodies ($p < 0.001$). Additionally, 2.00% of AITD patients and 1.40% of non-AITD patients were positive for anti-*Toxoplasma* IgM antibodies. PCR analysis revealed the presence of *T. gondii* parasites in 2.00% of AITD patients and 1.4% of non-AITD patients. In AITD patients with *T. gondii* antibodies, both serum levels and gene expression of IFN-γ were significantly elevated compared to AITD patients without *T. gondii* antibodies ($p < 0.05$).

Conclusion: Current findings suggest that individuals infected with *T. gondii* may experience direct effects on the thyroid gland due to elevated levels of IFN-γ. However, further analyses are necessary to validate these results.

Keywords: *Toxoplasma gondii*, Gravis disease, Hashimoto's disease, ELISA, Real-time PCR.

CITATION LINKS

[1] Dardé, M. L.; Ajzenberg, D.; Smith, J. Population structure... [2] Havelaar AH, Kemmeren JM, Kortbeek LM . Disease burden of... [3] Cook TB, Postolache TT ""Latent" infection with Toxoplasma gondii... [4] Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii... [5] Cappa M, Bizzarri C, Crea F. Autoimmune thyroid diseases... [6] Taylor PN, Global epidemiology of hyperthyroidism ... [7] Al-Khamesi MB. Effect of Toxoplasmosis on lipid... [8] Alvarado-Esquivel C, Association between Toxoplasma gondii ... [9] Clark JT, IL-33 promotes innate lymphoid cell-dependent... [10] Tozzoli R, Infections and autoimmune thyroid diseases... [11] Shapira Y, Prevalence of anti-Toxoplasma antibodies... [12] Wasserman EE, Infection and thyroid autoimmunity: a seroepidemiologic... [13] Kaňková Š, Effects of latent toxoplasmosis on autoimmune... [14] STAHL W., KANEDA Y. Impaired thyroid function in... [15] Dubey JP, Newell TK, Verma SK, Calero-Bernal R, Stevens EL. Toxoplasma gondii... [16] Basso W, Assessment of diagnostic accuracy of a... [17] Moshfe A, Arefkhah N, Sarkari B, Kazemi S, Mardani A. Toxoplasma gondii... [18] Bergallo M, Evaluation of IFN-γ polymorphism... [19] Anandkumar S., Chacko J., and Usha, M. Thyroid Disorder... [20] Jenkins D, HLA class II gene polymorphism... [21] Tozzoli R, et al. (2008) Infections and autoimmune ... [22] Sadoon, M. M. & Khalaf, A. K . Estimation the Level of... [23] Abdullah D. A. The level of cytokines IL-4, IL-12p40... [24] Rufash H. N., & Yousif J. J. effective of genotypes of... [25] Abdul-Lateef H. I., AL-Najar S. A., & Majeed N. G. A. The levels of IFN-, IL-12... [26] Ali M. I., Abd El Wahab W. M., Hamdy D. A., & Hassan A. Toxoplasma gondii... [27] Arranz-Solís D., Mukhopadhyay D., & Saeij J. J. Toxoplasma effectors... [28] Abed. A. A & Khalaf, A. K . Serological detection of some... [29] Yan X., Sun Y., Zhang G. and et al. Study on the... [30] Ibtisam Al Aboosi, et al. Toxoplasmosis and Relation with Some Immunological Markers for... [31] Azab Hameed, F., & Khalaf, A. K. The effect of infection...

Introduction

Toxoplasma gondii is a parasitic apicomplexan protozoan responsible for the global spread of toxoplasmosis [1]. This parasite can only undergo sexual reproduction in felids, specifically in cats, which are the definitive hosts [2]. In humans, *T. gondii* infections are often asymptomatic, especially in newborns and individuals with weakened immune systems, but they have the potential to result in severe cases of toxoplasmosis [2]. Following exposure, individuals may experience mild flu-like symptoms within the first few weeks, although in healthy adult humans, the infection typically remains asymptomatic [3]. The transmission of *T. gondii* occurs via the ingestion of undercooked meat that harbors tissue cysts or through the consumption of oocysts that are excreted in soil, water, vegetables, or other materials contaminated by the feces of an infected animal [4]. Transmission can also occur through organ transplant or blood transfusion [4]. Various organs and glands, including the thyroid gland, are vulnerable to *T. gondii* infection, impacting bone, heart, and brain functions, as well as metabolism regulation.

Autoimmune thyroid diseases (AITD), characterized by thyroid gland inflammation, can be caused by factors such as radiation, drugs, trauma, viruses, or parasites [5]. Hypothyroidism and hyperthyroidism are two distinct types of thyroid gland abnormalities with specific symptoms [6]. Hyperthyroidism results from an overproduction of thyroid hormones, while hypothyroidism occurs when insufficient thyroid hormones are released [6]. In thyroiditis, the cellular immune reaction plays a key role in triggering the production of autoantibodies and reactive T cells affecting thyroglobulin (Tg) and some autoantigens such as thyroid peroxidase [5]. AITD is characterized by the infiltration of immune cells, accompanied by the enlargement of thyroid, fibrosis, and the progressive destruction of thyrocytes, which ultimately leads to the development of hypothyroidism [6]. Upon the commencement of the immune response directed against thyroglobulin (Tg), specialized T lymphocytes that are specific to the thyroid gland transfer to the tissue [6]. These lymphocytes produce interferon (IFN)-γ, which in turn prompts thyrocytes to express major histocompatibility complex (MHC) class II molecules. This mechanism facilitates the subsequent proliferation of self-reactive T cells and elicits an inflammatory reaction, culminating in the aggregation of activated immune cells within the thyroid gland [6].

T. gondii targets various organs and glands within the body, including the thyroid gland responsible for the secretion of vital thyroid hormones namely triiodothyronine (T3), thyroxine (T4), and calcitonin [7]. Anti-T. gondii antibodies have been linked to AITD. Latent toxoplasmosis has been connected to an increase in autoantibodies targeting thyroid peroxidase. It has also been linked to a modest increase in thyroid hormone synthesis during gestation. Additionally, compromised thyroid function has been documented in cases of murine toxoplasmosis [8].

T. gondii replicates within any host cell that contains a nucleus, triggering the production of various inflammatory markers. This results in the onset of acute inflammation and the initiation of a targeted immune reaction against the *T. gondii* antigen [7]. Among the cytokines involved, IFN-γ stands out as a crucial mediator that belongs to the type 2 interferon class, playing a fundamental function in both the innate and adaptive immune responses against the protozoan parasite [9]. IFN-γ is responsible for activating several antimicrobial mechanisms, such as stimulating macrophages to generate inducible nitric oxide synthase (iNOS), which is essential for restricting parasite replication. Through a

variety of mechanisms involving different substances, IFN-γ significantly contributes to the immune defense against *T. gondii* by impeding parasite growth and inducing a shift from rapid to slow multiplication.

The current body of literature regarding the infection of the thyroid gland by *Toxoplasma gondii* is relatively sparse. An investigation into autopsy cases of disseminated toxoplasmosis identified the thyroid gland as being affected [10]. Additionally, the detection of anti-T. gondii antibodies has been associated with AITD [11, 12]. Prior studies have suggested that a history of *T. gondii* infection is correlated with an elevation in autoantibodies directed against thyroid peroxidase [13]. Furthermore, latent toxoplasmosis has been linked to a modest increase in thyroid hormone production during gestation [14]. In addition, compromised thyroid function has been observed in murine models of toxoplasmosis [15].

Objectives: Accordingly, the objective of the present study was to assess the seroepidemiological prevalence of toxoplasmosis and IFN-γ levels in AITD patients from Iraq.

Materials and methods

Study design and participants: This case-control study was performed on 100 patients with AITD (Gravis disease (GD) and Hashimoto's disease (HD)) and 70 healthy individuals (Non-AITD) who were referred to general hospitals of Thi-Qar Province, Iraq, between July to November 2023. The diagnosis was established through clinical evaluations corroborated by laboratory analyses and diagnostic imaging techniques, such as ultrasound and scintigraphy, conducted by qualified specialist physicians. As for exclusion criteria, participants who had taken systemic antibiotics within the past three months (e.g., cotrimoxazole, clindamycin, sulfadiazine, atovaquone, and azithromycin) and immunocompromised individuals, including patients undergoing chemotherapy for cancer, individuals with transplants, and those with acquired immunodeficiency syndrome (AIDS) were excluded from the study.

Questionnaire: Before the collection of blood samples, participants were required to fill out a printed questionnaire that gathered demographic information, including variables such as gender, age, and residential location.

Sampling: Blood samples were procured by drawing 5 mL of blood from each participant via sterile syringes. Following the coagulation process, the blood was subjected to centrifugation at 5000 rpm for a duration of 5 min. The resultant serum was subsequently divided into two aliquots and transferred for the purpose of immunological assays (T. gondii IgG, IgM, and IFN-γ) and was kept at -20°C.

Enzyme-linked immunosorbent assay (ELISA) for anti-Toxoplasma antibodies: A variety of serological techniques have been employed to identify *T. gondii*-specific antibodies in both human and animal populations. These methods include the indirect hemagglutination assay (IHA). Among these, ELISA is particularly prevalent for diagnosing exposure to this protozoan with a sensitivity and specificity of higher than 92%, as it is considered the most reliable, cost-effective, and practical option available [16]. The prevalence of anti-*T. gondii* antibodies was assessed using ELISA kits (Nova Tec Immunodiagnostica GmbH, Germany) to test all the collected sera according to the manufacturer's instructions.

Molecular detection of *T. gondii*: The utilization of polymerase chain reaction (PCR) for molecular diagnostics in various clinical specimens has proven to be an exceptionally effective technique for the identification of *T. gondii* DNA, demonstrating a specificity rate of 100% [17]. DNA samples were acquired from non-coagulated blood

using the DNA extraction kit manufactured by Qiagen, Germany, following the provided guidelines. The PCR assay employed specific primers with the following sequences: Toxo-F (5'-CAGGGAGGAAGACGAAAGTTG-3') and Toxo-R (5'-CAGACACAGTGCATCTGGATT-3'), as well as the master mix (Fermentas, Germany). The thermal cycling protocol included an initial denaturation step conducted at 95°C for a duration of 5 min, followed by cycles of 95°C for 30 s, 54°C for 30 s, and 73°C for 30 s utilizing a thermal cycler from Bio-Rad, USA. The final extension phase was carried out at 73°C for 10 min. Subsequently, the amplified products were observed on a 1% agarose gel and examined using the PCR Gel Documentation System. Positive controls consisted of *T. gondii* DNA (RH strain), while negative controls were devoid of any DNA [17].

Determining the serum level of IFN-γ : The serum levels of IFN-γ were measured in the participants using a Human IFN-γ ELISA Kit (China).

Evaluating the IFN-γ gene expression : Total RNA was extracted from peripheral blood utilizing a protocol provided by Qiagen, Germany. The quality of the extracted RNA was assessed using a Nanodrop spectrophotometer from Biotek Epoch. Subsequently, the RNA samples were converted to complementary DNA (cDNA) employing the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Sankt Leon-Rot, Germany) in accordance with the manufacturer's guidelines. Quantitative real-time PCR was conducted using the SYBR Green master mix 2X (Thermo Fisher Scientific, Heiligen, Germany) with primers specifically designed for the IFN-γ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, reference gene) gene as described by Bergallo et al. [18]. The PCR protocol included an initial denaturation step at 96°C for 10 min, followed by 42 amplification cycles, concluding with a final extension at 73°C for 5 min. Relative gene expression levels were determined using the 2-ΔΔCt method, calculated using Bio-Rad iQ5 Optical System Software (USA).

Statistical analysis: The statistical analysis of the results was performed using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA). Chi-square tests were used to examine the difference in the distribution of the participants in case and control groups, as well as the associations between the infection and the studied factors. Variables significantly related to Toxoplasma prevalence were analyzed as possible risk factors by means of univariate logistic regression, with a significance level set at $p < 0.05$.

Findings

Participants: The mean age of the participants in the AITD and non-AITD groups was 36.6 and 39.4 years old, respectively. The predominant demographic in both the AITD group (75 participants, representing 75.0%) and the non-AITD group (48 participants, accounting for 68.55%) was female. In terms of residential distribution, 58 participants (58.00%) in the AITD group and 39 participants (55.70%) in the non-AITD group resided in urban areas, while the rest of the participants were located in rural regions.

Prevalence of anti-*T. gondii* antibodies: Out of 100 AITD patients, 29 individuals (29.00%) were diagnosed with Graves' disease (GD), while 71 individuals (71.00%) were found to have Hashimoto's disease (HD). Among the AITD patients, 33 individuals (33.00%) tested positive for anti-Toxoplasma gondii IgG antibodies. In contrast, only nine individuals (12.85%) from the healthy control group exhibited the presence of anti-*T. gondii* IgG antibodies. The observed difference in the prevalence of anti-*T. gondii* IgG antibodies between AITD patients and non-AITD individuals was statistically significant ($p < 0.001$). Conversely, statistical analysis did not reveal a signifi-

cant difference (p = 0.91) in the prevalence of anti-*T. gondii* IgM antibodies between the two groups, with two AITD patients (2.00%) and one non-AITD individual (1.42%) testing positive (Table 1).

In the analysis of age-related subcategories, no statistically significant correlation was identified between the prevalence of *T. gondii* antibodies and the age of individuals in both the AITD group (p = 0.19) and the non-AITD group (p = 0.26). However, a significant correlation was observed between gender and the presence of *T. gondii* antibodies in both the AITD (p < 0.001) and non-AITD (p < 0.001) groups. Furthermore, a significant association was noted between the participants' residential location and the prevalence of *T. gondii* antibodies in the AITD group (p < 0.001), whereas no such association was found in the non-AITD

Table 1) The prevalence of anti-*Toxoplasma gondii* antibodies among the studied groups.

Group	No. of participants	IgG antibody		IgM antibody	
		Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)
Healthy (control)	70	9 (12.85)	61 (87.15)	1 (1.42)	69 (98.58)
Gravis disease	29	9 (31.90) *	20 (68.10)	1 (3.44)	28 (96.55)
Hashimoto's disease	71	24 (33.80) *	47 (66.20)	1 (1.40)	70 (98.60)

* A statistically significant difference was observed in comparison to the control group, with a p-value of less than 0.05.

Table 2) Frequency of *T. gondii* antibodies in in patients suffering from autoimmune thyroid diseases (AITD) and healthy individuals (Non-AITD) based on the demographic characterizations.

Variable		*T. gondii* antibodies		*P* value Chi-Square	Crude OR	95%CI	*P* value
		Positive No. (%)	Negative No. (%)				
AITD	Age						
	>34 yrs	11 (33.30)	22 (66.70)	0.21	1.64	0.84-2.96	0.192
	34- 55 yrs	19 (32.64)	39 (67.36)	-	1	1	-
	55< yrs	3 (33.30)	6 (66.70)	-	1	1	-
	Gender						
	Male	4 (16.00)	21 (84.00)	-	1	1	-
	Female	29 (38.60)	41 (61.40)	0.01	2.21	1.20-3.62	0.00*
	Residence						
	Rural	7 (16.66)	35 (83.64)	-	1	1	-
	Urban	26 (44.80)	32 (55.20)	0.00	2.41	1.43-4.26	0.00*
Non-AITD	Age						
	>34 yrs	2 (13.33)	13 (86.6)	0.32	1.72	0.64-2.92	0.26
	34- 55 yrs	6 (13.33)	39 (86.64)	-	1	1	-
	55< yrs	1 (10.00)	9 (90.00)	-	1	1	-
	Gender						
	Male	1 (4.55)	21 (95.45)	-	1	1	-
	Female	8 (16.60)	40 (83.30)	0.04	1	1.43-3.96	0.00*
	Residence						
	Rural	4 (12.18)	27 (87.82)	0.65	1.31	0.56-2.49	0.52
	Urban	5 (12.80)	34 (87.20)	-	-	1	-

* p<0.05 significant difference

group (p = 0.52) (Table 2).

PCR analysis revealed the presence of *T. gondii* parasites in two individuals (2.00%) with AITD and in one individual (1.40%) without AITD (Fig. 1). Among patients diagnosed with GD, 9 individuals (31.00%) tested positive for anti-*T. gondii* IgG antibodies, and 24 individuals (33.80%) with HD also tested positive for these antibodies. Statistical analysis indicated no significant difference in the prevalence of anti-*T. gondii* antibodies between patients with GD and HD (p = 0.84).

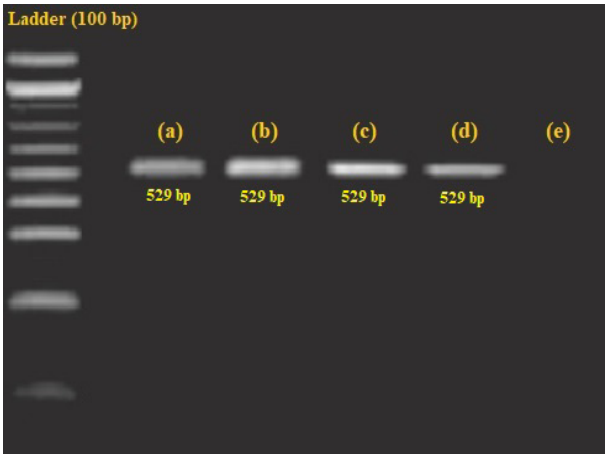

Figure 1) Gel electrophoresis of PCR products obtained from all participants. Lane (a): positive control (529 bp); lanes b, c, and d: positive samples (529 bp); lane (e): negative sample.

Evaluating the serum level of IFN-γ: Mean serum levels of IFN-γ among GD patients with or without seropositivity to *T. gondii* antibodies were 614.17 and 507.12 pg/mL, respectively. In addition, mean serum levels of IFN-γ among HD patients with or without seropositivity to *T. gondii* antibodies were 581.15 and 435.82 pg/mL, respectively. Statistical analysis showed that significant differences were observed among AITD patients seropositive for *T. gondii* compared with AITD patients who were seronegative for *T. gondii* antibodies (p < 0.05) (Fig. 2).

Evaluating the IFN-γ gene expression : The gene expression level of IFN-γ among GD patients with or without seropositivity to T. gondii antibodies was 3.82 and 2.21-fold, respectively (Fig. 3). In addition, the gene expression level of IFN-γ among HD patients with or without seropositivity to

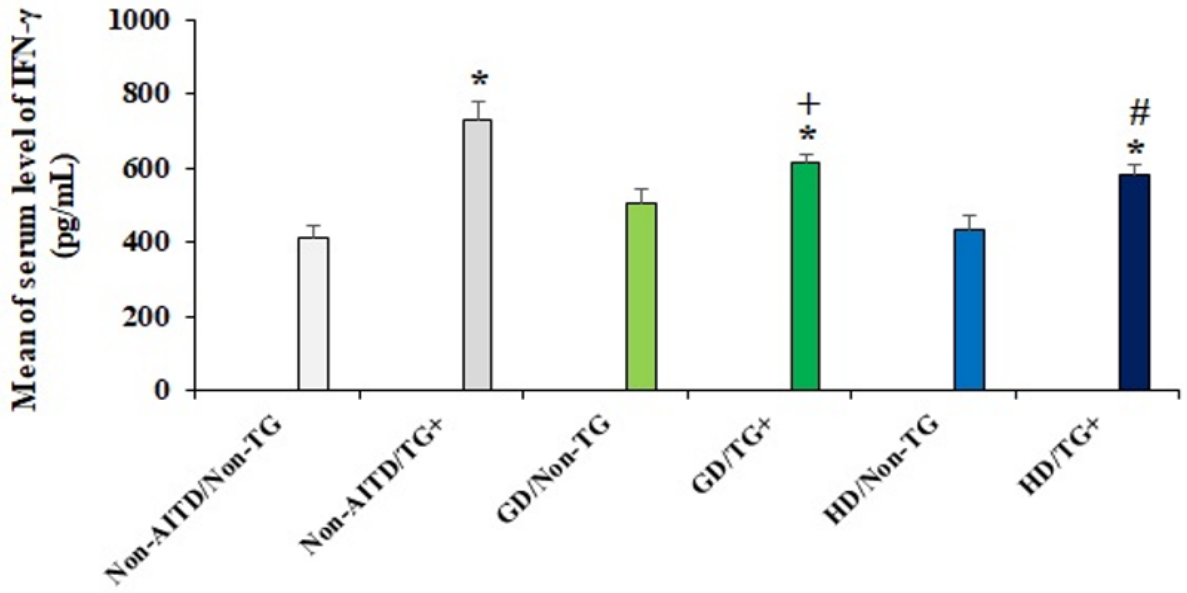

Figure 2) The mean of the serum level of IFN-γ among AITD (Gravis disease (GD) and Hashimoto's disease (HD)) and non-AITD people with or without seropositivity to *T. gondii* antibodies (TG) by ELISA method. Data are indicated as Mean±SD. * p< 0.05 A statistically significant difference was observed in comparison to the control group; + p< 0.05 A statistically significant difference was observed in comparison to the GD/Non-TG; # p< 0.05 A statistically significant difference was observed in comparison to the HD/Non-TG.

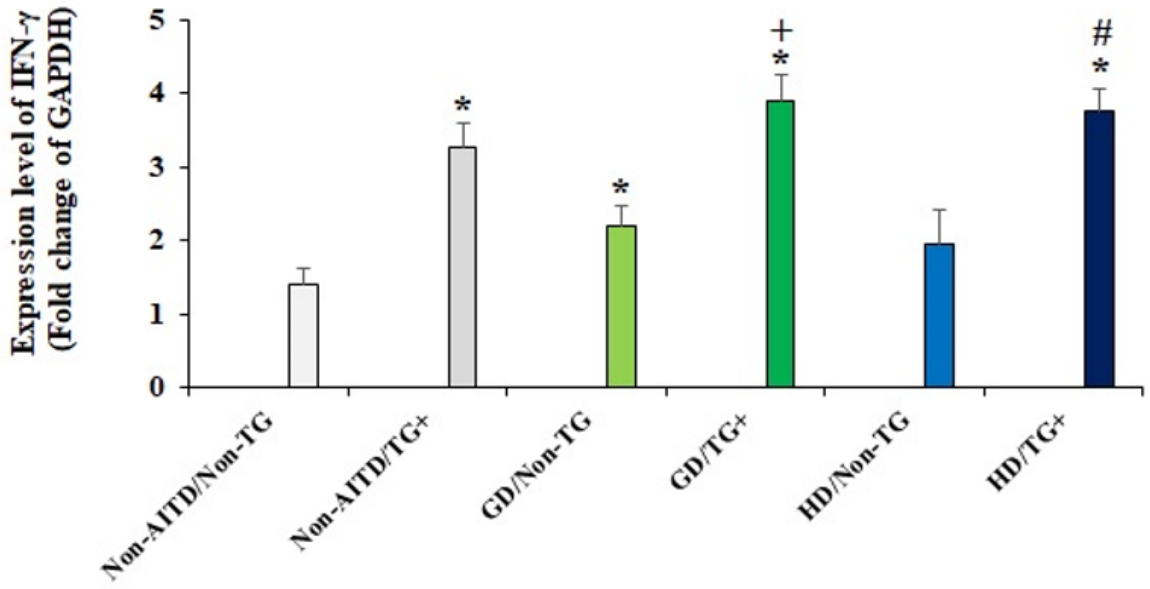

Figure 3) The gene expression level of IFN-γ among AITD (Gravis disease (GD) and Hashimoto's disease (HD)) and non-AITD people with or without seropositivity to *T. gondii* antibodies (TG) by Real-time PCR. Data are indicated as Mean±SD. * $p< 0.05$ A statistically significant difference was observed in comparison to the control group; + $p< 0.05$ A statistically significant difference was observed in comparison to the GD/Non-TG; # $p< 0.05$ A statistically significant difference was observed in comparison to the HD/Non-TG.

T. gondii antibodies was 3.76 and 1.96-fold, respectively. Statistical analysis showed that the gene expression level of IFN-γ was significantly higher among AITD patients seropositive for *T. gondii* compared with AITD patients who were seronegative for *T. gondii* antibodies ($p < 0.05$).

Discussion

Thyroid disease is the second most common endocrine condition after diabetes mellitus and a significant global public health concern [19]. Thyroid hormones are essential for the body's growth, development, and metabolism, as well as the regulation of other bodily activities. HD and GD are the most common examples of AITD [20]. Here, we aimed to assess whether individuals infected with *T. gondii* could experience direct impacts on the thyroid gland through increased levels of IFN-γ. The current study showed that among AITD patients, 33 patients (33.00%) exhibited seropositivity for anti-T. gondii IgG antibodies, whereas 9 (12.85%) of the samples collected from the healthy control group demonstrated the presence of anti-*T. gondii* IgG antibodies. This was due to exposure to risk factors such as eating contaminated food oocysts and undercooked meat.

Consistent with our findings, Tozzoli et al. reported a 65.50% prevalence of latent toxoplasmosis in patients with AITD in north-western Italy [21]. Conversely, Kankova et al. found that only 27.10% of 127 pregnant women with AITD in Prague tested positive for latent toxoplasmosis. The differences in our results compared to other studies on the prevalence of toxoplasmosis in AITD patients may be due to geographical differences in autoantibody production patterns [21].

IFN-γ is a particular type of the protein which is implicated in the functioning of the human immune system. It is produced by natural killer cells (NK) during innate immunity and T cells (CD8+ and CD4+ cells) during adaptive immunity. Various interleukins are released locally to activate these cells

in response to different infections and antigens, including intracellular parasites such as *T. gondii.* [9]. The results showed that the mean serum levels of IFN-γ among GD patients with or without seropositivity to *T. gondii* antibodies were 614.17 and 507.12 pg/mL, respectively. In addition, the mean serum levels of IFN-γ among HD patients with or without seropositivity to *T. gondii* antibodies were 581.15 and 435.82 pg/mL, respectively. Statistical analysis showed significant differences among AITD patients seropositive for *T. gondii* compared with AITD patients who were seronegative for T. gondii antibodies ($p < 0.05$). The results showed that the gene expression level of IFN-γ among GD patients with or without seropositivity to *T. gondii* antibodies was 3.82 and 2.21-fold, respectively. In addition, the gene expression level of IFN-γ among HD patients with or without seropositivity to *T. gondii* antibodies was 3.76 and 1.96-fold, respectively.

The current study's findings regarding the estimation of INF-γ levels by using an ELISA test were widely agreed upon. The results of the present study are consistent with those reported by Sadoon et al. (2022) in Thi-Qar Province, Southern Iraq. Their research indicated that the mean level of INF-γ in diabetic patients with toxoplasmosis was 37.40 pg/mL, which is significantly higher than the mean level observed in diabetic patients without *T. gondii*, recorded at 16 pg/mL. In comparison, healthy controls exhibited a mean INF-γ level of 33.80 pg/mL, while those without toxoplasmosis had a mean level of 18.20 pg/mL [22]. A survey conducted by Abdullah et al. (2011) demonstrated that a cohort of 270 women who experienced abortion and were diagnosed with toxoplasmosis exhibited elevated levels of IFN-γ [23]. The findings were also in agreement with those of another study conducted in the Iraqi Province of Al-Najaf, which reported that women with toxoplasmosis who had experienced abortions had high levels of IFN-γ (52.66 ± 24.33 pg/mL) and other cytokines [24]. Abdul-Lateef et al. (2012) also showed that the IFN-γ level in patients with toxoplasmosis was higher than in the control group [25]. The current study's findings were also compatible with an Egyptian study, which found that cancer patients who were seropositive for Toxoplasma had higher levels of IFN-γ than those who were not seropositive for Toxoplasma [26]. In addition, a study conducted in the United States revealed that women who had abortions and toxoplasmosis had elevated levels of IFN-γ [27]. The current investigation is consistent with a study conducted in the Thi-Qar Province of Iraq, which sought to identify parasitic, viral, or bacterial diseases in patients diagnosed with COVID-19. The findings indicated that individuals diagnosed with toxoplasmosis demonstrated increased levels of IFN-γ [28]. Conversely, research conducted in China in 2022 reported a reduction in IFN-γ levels among patients with toxoplasmosis [29]. The results of the current study were in agreement with those of the study conducted in Thi-Qar province, Iraq, which estimated the level of IFN-γ in individuals with and without *T. gondii* infection among 120 women who had experienced abortions. Their results showed that the mean levels of IFN-γ among toxoplasmosis-positive women who had experienced abortions were 38.6% compared to 28.80% in the control groups [30]. The findings of the present study are consistent with those reported by Azab and Khalaf (2024), which indicated that the mean level of IFN-γ in breast cancer patients with toxoplasmosis was 47.66 pg/mL, compared to 0.00 pg/mL in those without the infection. In contrast, the mean IFN-γ level in the healthy control group was recorded at 0.57 pg/mL [31]. The main limitations and confounders of

this study include the limited sample size. Moreover, the lack of tissue biopsy analysis for detecting parasite presence and the uncertainty surrounding the exact time to start breast cancer treatment to adjust for toxoplasmosis are other limitations that are planned to be investigated in subsequent research.

Conclusion

The current study showed the high prevalence *T. gondii* antibodies among AITD patients in Iraq. It was also found that the level of IFN-γ was notably higher among AITD patients who were seropositive for *T. gondii* antibodies. However, it is essential that these studies are conducted with a larger sample size. These results indicated that individuals infected with *T. gondii* may experience direct impacts on the thyroid gland through elevated levels of IFN-γ. It may be advisable to implement screening programs for the detection of toxoplasmosis, particularly in relation to the reactivation of chronic infections, as a standard follow-up procedure for patients diagnosed with breast cancer. However, further studies are required to confirm these findings.

Acknowledgements

The authors express their gratitude to all patients and medical personnel who participated in this study.

Ethics Approval and Consent to Participate: Patient enrollment was conducted in accordance with the principles outlined in the 1964 Helsinki Declaration. The study received review and approval from the ethical committee of the University of Thi-Qar, Thi-Qar, Iraq (Approval No. 2022237). Additionally, written informed consent was obtained from all participants prior to their registration. The authors affirm that all procedures were executed in compliance with applicable guidelines and regulations.

Author contributions: AKK planned the tests and supervised; AHH performed tests and collected data. AKK, and AHH, prepared the draft and edited the manuscript. All authors have reviewed and approved the final version of the manuscript for publication.

Competing interests: None declared by Authors.

Fundings: None declared by Authors.

References

1. Dardé, M. L.; Ajzenberg, D.; Smith, J. Population structure and epidemiology of Toxoplasma gondii". In Weiss, L. M.; Kim, K. (eds.). Toxoplasma Gondii: The Model Apicomplexan. Perspectives and Methods. Amsterdam, Boston, Heidelberg, London, New York: Elsevier.2011; . 49–80
2. Havelaar AH, Kemmeren JM, Kortbeek LM . Disease burden of congenital toxoplasmosis. Clin Infect Dis.2007; 44: 1467–1474.
3. Cook TB, Brenner LA, Cloninger CR, Langenberg P, Igbide A, Giegling I, Hartmann AM, Konte B, Friedl M, Brundin L, Groer MW, Can A, Rujescu D, Postolache TT ""Latent" infection with Toxoplasma gondii: association with trait aggression.2015;
4. Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: from animals to humans. International journal for parasitology. 2000 Nov 1;30(12-13):1217-58.
5. Cappa M, Bizzarri C, Crea F. Autoimmune thyroid diseases in children. Journal of thyroid research. 2011;2011(1):675703.
6. Taylor PN, Albrecht D, Scholz A, Gutierrez-Buey G, Lazarus JH, Dayan CM, Okosieme OE. Global epidemiology of hyperthyroidism and hypothyroidism. Nature Reviews Endocrinology. 2018 May;14(5):301-16.
7. Al-Khamesi MB. Effect of Toxoplasmosis on lipid profile and thyroid hormones in aborted women. Al-Nahrain Journal of Science. 2016;19(4):122-6.
8. Alvarado-Esquivel C, Ramos-Nevarez A, Guido-Arreola CA, Cerrillo-Soto SM, Pérez-Álamos AR, Estrada-Martínez S, Gutierrez-Martinez VD, Sifuentes-Alvarez A, Ramírez-Valles EG, Contreras-Cisneros E. Association between Toxoplasma gondii infection and thyroid dysfunction: a case-control seroprevalence study. BMC Infect Dis. 2019 Sep 18;19(1):826. doi: 10.1186/s12879-019-4450-0. PMID: 31533667; PMCID: PMC6751844.
9. Clark JT, Christian DA, Gullicksrud JA, Perry JA, Park J, Jacquet M, Tarrant JC, Radaelli E, Silver J, Hunter CA. IL-33 promotes innate lymphoid cell-

dependent IFN-γ production required for innate immunity to Toxoplasma gondii. Elife. 2021 Apr 30;10:e65614.
10. Tozzoli R, Barzilai O, Ram M, Villalta D, Bizzaro N, Sherer Y, Shoenfeld Y. Infections and autoimmune thyroid diseases: parallel detection of antibodies against pathogens with proteomic technology. Autoimmun Rev. 2008;8(2):112–115. doi: 10.1016/j.autrev.2008.07.013. [PubMed] [CrossRef] [Google Scholar]
11. Shapira Y, Agmon-Levin N, Selmi C, Petríková J, Barzilai O, Ram M, Bizzaro N, Valentini G, Matucci-Cerinic M, Anaya JM, Katz BS, Shoenfeld Y. Prevalence of anti-Toxoplasma antibodies in patients with autoimmune diseases. J Autoimmun. 2012;39(1–2):112–116. doi: 10.1016/j.jaut.2012.01.001. [PubMed] [CrossRef] [Google Scholar
12. Wasserman EE, Nelson K, Rose NR, Rhode C, Pillion JP, Seaberg E, Talor MV, Burek L, Eaton W, Duggan A, Yolken RH. Infection and thyroid autoimmunity: a seroepidemiologic study of TPOaAb. Autoimmunity. 2009;42(5):439–446. doi: 10.1080/08916930902787716. [PubMed] [CrossRef] [Google Scholar]
13. Kaňková Š, Procházková L, Flegr J, Calda P, Springer D, Potluková E. Effects of latent toxoplasmosis on autoimmune thyroid diseases in pregnancy. PLoS One. 2014;9(10):e110878. doi: 10.1371/journal.pone.0110878. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
14. STAHL W., KANEDA Y. Impaired thyroid function in murine toxoplasmosis. Parasitology. 1998;117(3):217–222. doi: 10.1017/S003118209800300X. [PubMed] [CrossRef] [Google Scholar]
15. Dubey JP, Newell TK, Verma SK, Calero-Bernal R, Stevens EL. Toxoplasma gondii infection in llama (Llama glama): acute visceral disseminated lesions, diagnosis, and development of tissue cysts. J Parasitol. 2014;100(3):288–294. doi: 10.1645/13-427.1. [PubMed] [CrossRef] [Google Scholar]
16. Basso W, Hartnack S, Pardini L, Maksimov P, Koudela B, Venturini MC, Schares G, Sidler X, Lewis FI, Deplazes P. Assessment of diagnostic accuracy of a commercial ELISA for the detection of Toxoplasma gondii infection in pigs compared with IFAT, TgSAG1-ELISA and Western blot, using a Bayesian latent class approach. International journal for parasitology. 2013 Jun 1;43(7):565-70.
17. Moshfe A, Arefkhah N, Sarkari B, Kazemi S, Mardani A. Toxoplasma gondii in blood donors: a study in Boyer-Ahmad County, Southwest Iran. Interdisciplinary perspectives on infectious diseases. 2018;2018(1):3813612.
18. Bergallo M, Gambarino S, Loiacono E, Vergano L, Galliano I, Montanari P, Astegiano S, Tavormina P, Tovo PA. Evaluation of IFN-γ polymorphism+ 874 T/A in patients with recurrent tonsillitis by PCR real time mismatch amplification mutation assay (MAMA real time PCR). Cytokine. 2015 Feb 1;71(2):278-82.
19. Anandkumar, S., Chacko, J., and Usha, M. Thyroid Disorder: An Overview. Research Journal of Pharmacology and Pharmacodynamics.2020; 12(1), 1-4.
20. Jenkins D, Penny MA, Fletcher JA, Jacobs KH, Mijovic CH, Franklyn JA0Sheppard MC. HLA class II gene polymorphism contributes little to Hashimoto's thyroiditis. Clin. Endocrinol (Oxf).1992; 37(2):141-5.
21. Tozzoli R, Barzilai O, Ram M, Villalta D, Bizzaro N, et al. (2008) Infections and autoimmune thyroid diseases: Parallel detection of antibodies against pathogens with proteomic technology. Autoimmun Rev 8: 112–115
22. Sadoon, M. M. & Khalaf, A. K . Estimation the Level of Interferon Gamma for Diabetic Patients Infected with Toxoplasmosis in Thi-Qar Province/ Southern Iraq. University of Thi-Qar Journal Of Medicine.2022; 24(2), 112-117.
23. Abdullah D. A. The level of cytokines IL-4, IL-12p40, IFNy during acute toxoplasmosis. Journal of the Faculty of Medicine Baghdad.2011; 53(4), 408-413
24. Rufash H. N., & Yousif J. J. effective of genotypes of Toxolplasma gondii Parasite on the level of some cytokine and marker in aborted women in Najaf governorate. Biochem. Cell. Arch.2018; 18 (1) 293-297.
25. Abdul-Lateef H. I., AL-Najar S. A., & Majeed N. G. A. The levels of IFN-, IL-12 and testosterone hormone in persons with asymptomatic toxoplasmosis. Journal of the Faculty of Medicine Baghdad.2012; 54(1), 79-82.
26. Ali M. I., Abd El Wahab W. M., Hamdy D. A., & Hassan A. Toxoplasma gondii in cancer patients receiving chemotherapy: seroprevalence and interferon gamma level. Journal of Parasitic Diseases.2019; 43, 464-471
27. Arranz-Solís D., Mukhopadhyay D., & Saeij J. J. Toxoplasma effectors that affect pregnancy outcome. Trends in parasitology.2021; 37(4), 283-295.
28. Abed. A. A & Khalaf, A. K . Serological detection of some parasitic, viral and bacterial infection associated with COVID-19 patients in Thi- Qar province,thesis collage medicin university of Thi –Qar.2022;110
29. Yan X., Sun Y., Zhang G. and et al. Study on the antagonistic effects of koumiss on Toxoplasma gondii infection in mice. Frontiers in

Nutrition.2022; 2260.

30. Ibtisam Al Aboosi, Amall Y.AL-Mulla, Mustafa mudhafar, Qais R. Lahhob, Moamin Ibraheem Kate, Mohammed Hassib Ali, Murtadha A Al-khegane, Mustafa Jawad Kadham, & Abdulrahman Abbas Jasim. Toxoplasmosis and Relation with Some Immunological Markers for Aborted Women in Thi-Qar Province. Journal of Population Therapeutics and Clinical Pharmacology, 30(2), 309-316.
31. Azab Hameed, F., & Khalaf, A. K. The effect of infection with Toxoplasma gondii in inducing interferon-gamma