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Seroprevalence of *Toxoplasma gondii* among Children with Beta-thalassemia Major and its Effect on Selected Immune Parameters

Beta-Talassemi Major Hastası Çocuklarda Toxoplasma gondii Seroprevalansı ve Bazı Bağışıklık Parametreleri Üzerindeki Etkisi

Ghufran Gubari Shannoon, Hussain A. Mhouse Alsaady, Ahmed A. Khalifa

University of Misan College of Science, Department of Biology, Maysan, Iraq

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ABSTRACT

Objective: The seroprevalence of anti-*Toxoplasma gondii* antibodies among children with beta-thalassemia major is not explored yet. The effect of *T. gondii* infection on the immune response of patients and to analyse its potential association with selected immune parameters, namely interleukin (IL)-10, IL-12, IL-17, interferon-gamma (IFN-γ), and the concentrations of $CD4^+$ and $CD8^+$ cluster of differentiation markers in serum is examined. The study aimed to investigate the seroprevalence of anti-*T. gondii* antibodies and the immunological impact in children with β-thalassemia major.

Methods: The study included 90 children (71 BTM and 19 controls), aged 6-15 years. Serum, obtained from 5 mL blood samples, was used to detect anti-*T. gondii* immunoglobulin (Ig)G and IgM antibodies by rapid tests, ELISA, and VIDAS, while cytokine levels were measured by ELISA. After serological testing, participants were classified into 4 groups: thalassemia + parasite, thalassemia only, parasite only, and healthy control. Data were analysed using chi-square and Kruskal-Wallis tests ($p<0.05$), with results expressed as mean ± standard error for statistical comparison between study groups.

Results: The total seroprevalence of *T. gondii* was 30.0% (27/90); it was 35.21% among thalassemia patients and 10.53% (2/19) in the control group. Among thalassemia patients, IgM and IgG seropositivity rates were 8.45% (6/71) and 26.76%, respectively; in controls, they were 0.0% and 10.53% (2/19). BTM children showed significantly lower levels of IL-10, IL-12, IL-17, $CD4^+$, and $CD8^+$ ($p<0.05$), whereas IFN-γ ($p>0.12$).

Conclusion: Children with BTM showed a significantly higher seroprevalence of *T. gondii* than healthy controls. Infection was associated with significant alterations in immune parameters, indicating increased immune dysregulation in these patients.

Keywords: *Toxoplasma gondii*, beta-thalassemia major, interleukins, IFN-γ

ÖZ

Amaç: Majör beta-talasemi hastası çocuklarda anti-*Toxoplasma gondii* antikorlarının seroprevalansı henüz araştırılmamıştır. Bu çalışmada, *T. gondii* enfeksiyonunun hastaların bağışıklık tepkisi üzerindeki etkisi ve seçilmiş bağışıklık parametreleri, yani interlökin (IL)-10, IL-12, IL-17, interferon-gamma (IFN-γ) ile serumdaki $CD4^+$ ve $CD8^+$ farklılaşma kümesi belirteçlerinin konsantrasyonları arasındaki potansiyel ilişki analiz edilmiştir. Çalışma, β-talasemi majör hastası çocuklarda anti-*T. gondii* antikorlarının seroprevalansını ve bunun immünolojik etkisini araştırmayı amaçlamıştır.

Yöntemler: Çalışmaya 6-15 yaş aralığındaki 90 çocuk (71'i β-talasemi majör hastası, 19'u sağlıklı kontrol grubu) dahil edildi. 5 mL kan örneklerinden elde edilen serum, hızlı test, ELISA ve VIDAS yöntemleriyle anti-*T. gondii* immünoglobulin (Ig)G ve IgM antikorlarını saptamak için kullanılırken, sitokin düzeyleri ELISA ile ölçüldü. Serolojik testlerin ardından, katılımcı çocuklar dört gruba ayrıldı: talasemi + parazit, sadece talasemi, sadece parazit ve sağlıklı kontrol. Veriler, ki-kare ve Kruskal-Wallis testleri ($p<0.05$) kullanılarak analiz edildi ve sonuçlar, çalışma grupları arasında istatistiksel karşılaştırma amacıyla ortalama ± standart error olarak ifade edildi.

Sonuç: *T. gondii*'nin toplam seroprevalansı %30,0 (27/90) olarak saptandı; talasemi hastalarında bu oran %35,21 iken, sağlıklı kontrol grubunda %10,53 idi. Talasemi hastalarında. IgM ve IgG seropozitifliği sırasıyla %8,45 (6/71) ve %26,76 iken, kontrol grubunda bu oranlar %0,0 ve %10,53 (2/19) idi. BTM'li çocuklarda IL-10, IL-12, IL-17, $CD4^+$ ve $CD8^+$ düzeyleri istatistiksel olarak anlamlı derecede daha düşüktü ($p<0.05$), buna karşın IFN-γ ($p>0.12$).

Address for Correspondence/Yazar Adresi: Hussain A. Mhouse Alsaady Prof. Dr., University of Misan College of Science, Department of Biology, Maysan, Iraq
E-mail/E-Posta: hussainsaady@uomisan.edu.iq **ORCID ID:** orcid.org/0000-0003-4411-8185

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Sonuç: Majör β-talasemi hastası çocuklarda, sağlıklı kontrol grubuna kıyasla *T. gondii* seroprevalansı istatistiksel olarak anlamlı derecede daha yüksek bulunmuştur. Enfeksiyon, bağışıklık parametrelerinde istatistiksel olarak anlamlı değişikliklerle ilişkilendirilmiş olup, bu da söz konusu hastalarda bağışıklık düzensizliğinin arttığını göstermektedir.
Anahtar Kelimeler: *Toxoplasma gondii*, majör beta-talasemi, interlökinler, IFN-γ

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects more than one-third of the world's population and causes one of the most prevalent parasitic diseases globally (1). The parasite possesses three morphological stages (tachyzoite), (bradyzoite), and (oocysts) and three common major genotypes (I, II, and III) associated with human infection (2,3) and all these strains have been registered in Iraq in Maysan province (3). Toxoplasmosis is of considerable global public health significance. While immunocompetent individuals may present with mild or no symptoms, the parasite can cause serious complications in immunocompromised patients, particularly those with thalassemia who receive regular blood transfusions (4,5). Recent regional studies have also highlighted the continuing importance of parasitic infections in Maysan Province and southern Iraq. A previous sero-epidemiological study in Maysan reported the presence of *T. gondii* antibodies among men and pregnant women, indicating that toxoplasmosis remains an important local public health issue (6).

Beta-thalassemia major is the most prevalent hereditary haemoglobin disorder, resulting from mutations in the genes encoding beta-globin chains, leading to severe haemolytic anemia and immune dysfunction (7,8). The tachyzoite stage remains viable in stored blood at 4 °C for up to 50 days (9), and the seroprevalence of *T. gondii* among blood donors is estimated at 33% (10). Cytokines constitute a central axis of the immune response against *T. gondii*. Infection induces notable changes in interleukin (IL)-10, IL-12, IL-17, IFN-γ, $CD4^+$, and $CD8^+$ levels (11,8). The chronic immune suppression resulting from repeated blood transfusions has been linked to increased susceptibility of thalassemia patients to *T. gondii* infection (12). Despite the recognized susceptibility of patients with transfusion-dependent beta-thalassemia major to infection with *T. gondii*, data on its seroprevalence and associated immune responses in children, particularly in southern Iraq, remain scarce. Therefore, the present study aimed to determine the seroprevalence of *T. gondii* among children with BTM major and to evaluate its association with selected immune parameters.

METHODS

Sample Collection and Study Design

A total of 90 venous blood samples (5 mL each) were collected from beta-thalassemia major patients (n=71). Samples were obtained from selected children with beta-thalassemia major who had documented medical histories; cases with conditions other than *T. gondii* infection were excluded. Healthy controls (n=19) have no medical history. Aged 6-15 years, between November 2025 and mid-February 2026, from the Center for Hereditary and Haemorrhagic Blood Diseases (Thalassemia Center), from Maysan Teaching Children's and Maternity Hospital, Maysan, Iraq. Because antibodies to toxoplasmosis were detected in two children in the control group of children without thalassemia, the study population was divided into four groups: (i) thalassemia with *T. gondii* (thalassemia + parasite); (ii) thalassemia only; (iii) non-thalassemia with toxoplasmosis (parasite only); and (iv) non-thalassemia without toxoplasmosis (healthy control). Blood samples for thalassemia patients were drawn before transfusion, placed in gel tubes, centrifuged at 3,000 rpm for 10 minutes to obtain serum, and stored at -20°C until use.

Ethical approval for this study was obtained from the Research and Knowledge Management Unit, Training and Human Development Center, Misan Health Directorate, Ministry of Health, Republic of Iraq (approval no: 709; date: 27.11.2025). Written informed consent was obtained from the parents or legal guardians of all participating children prior to their inclusion in the study.

Serological Diagnosis of T. gondii Infection

Three diagnostic techniques are employed according to the manufacturer's instructions:

(a) Rapid antigen detection test: an immunochromatographic flow assay for initial qualitative detection of immunoglobulin (Ig) G and IgM,

(b) VIDAS is an automated system employing enzyme-linked fluorescent assay,

(c) Enzyme-linked immunosorbent assay (ELISA): indirect sandwich ELISA for qualitative detection of IgG and IgM (Nipigon Co., Canada) and for quantitative measurement of the concentrations of human interleukins and cluster of differentiation markers (IL-10, IL-12, IL-17, IFN-γ, $CD4^+$ and $CD8^+$). Uniform procedures were used in the ELISA protocol for all serological and immunological markers in this study. Results were obtained using a semi-automatic ELISA system (Bio kit, USA), with optical density (OD) measured at 450 nm.

Assessment of Immune Parameters: IL-10, IL-12, IL-17, IFN-γ, $CD4^+$, $CD8^+$

Sandwich-ELISA was used to quantify the levels of certain cytokines. It was performed according to the manufacturer's instructions provided with each kit (SunLong Biotech Co., China). Each Microelisa strip plate provided in this kit was pre-coated with one of the human interleukins or cluster differentiation markers (IL-10, IL-12, IL-17, IFN-γ, $CD4^+$, and $CD8^+$). The cells were loaded at the bottom of the wells with 50 µl of the serum samples diluted 1:5 (10 µl serum: 40 µl diluent), mixed well, and shaken. Then each microtiter plate was sealed with Closure plate membrane and incubated at 37 °C for 30 minutes. T he membrane was carefully removed from each microtiter plate and washed five times with a washing solution. Then, 50 µl of horseradish peroxidase-conjugated antibodies specific for human interleukins or cluster differentiation markers (IL-10, IL-12, IL-17, IFN-γ, $CD4^+$, and $CD8^+$) were added to each well except the blank control well, and the microtiter plates were incubated at 37 °C for 30 minutes. The microtiter plates were washed as described above. Then, 50 µl of chromogen A and 50 µl of chromogen B were added and carefully mixed and incubated at 37 °C for 15 minutes in the dark. Finally, the reaction was terminated by adding 50 microliters of stop solution.

The OD of the samples was measured spectrophotometrically at a wavelength of 450 nm using the ELISA reader. The OD value was proportional to the concentration of each parameter. You can estimate the concentration of each parameter in the samples by comparing sample ODs to the standard curve.

Statistical Analysis

Statistical analyses were performed using SPSS version 27. Chi-square test (χ^2) and Kruskal-Wallis test (H statistic) were applied using a significance level of 0.05 to assess statistical significance. Results are expressed as mean ± standard error.

RESULTS

T. gondii Infection Among Beta-thalassaemia Major Patients

The current results (Table 1) show a seroprevalence of *T. gondii* of 35.21% (25/71) among children with beta-thalassemia major, which is higher than the seroprevalence recorded in non-infected children with thalassemia (control group), 10.53% (2/19). A statistically significant relationship was found between beta-thalassemia major and toxoplasmosis (χ^2=4.349, p<0.05).

Distribution of IgM and IgG Antibodies among Children Population

The results of the current study (Table 2) showed that the overall seroprevalence of anti-*T. gondii* IgM and IgG antibodies among the children participating in the study was 30% (27/90). The participating children with thalassemia recorded the highest seroprevalence of the two antibodies (anti-*T. gondii* IgM + anti-*T.* gondii IgG*)*, at 35.21% (25/71), compared with 10.53% (2/19) among children without thalassemia. Table 2 also shows that the seroprevalence of the anti-*T. gondii* IgM antibody among children with thalassemia was 8.45%, which is lower than the seroprevalence of the anti-*T. gondii* IgG antibody (26.76%). On the other hand, the seroprevalence of the anti-*T. gondii* IgG antibody among children without thalassemia was 10.53%; in contrast, no seropositivity for anti-*T. gondii* IgM was detected in sera from children without thalassemia. This study did not record the presence of both antibodies in any of the children's sera.

Effect of T. gondii on the Levels of Some Cytokines, IL-10, IL-12, IL-17, and IFN-γ, Among Children with Thalassemia

The mean serum IL-10 levels of the study groups are shown in Table 3: IL-10 levels were 18.342±1.423 pg/mL in the thalassemia + parasite group and 20.461±2.063 pg/mL in the thalassemia only group, values that were close to the healthy control group (19.876±1.053 pg/mL), but all were lower than the parasite only group, which showed the highest mean level (31.700±1.700 pg/mL).

The distribution of the IL-10 levels among participating children in the four groups is shown in Figure 1. There are statistically significant differences in mean IL-10 levels among the four groups (H=8.896, p<0.05). The study also found (Table 3) that the mean of IL-12 levels in the serum of the children group: "thalassemia + parasite", and the children group: "thalassemia only" were 10.074±1.473 and 13.202±2.172 pg/mL, respectively, which are close to each other but lower than the level of 22.123±2.359 pg/mL in "healthy control". In contrast, the mean of IL-12 level in the children group: "parasite only" of 45±5.700 pg/mL, is higher than among the other three groups of children (see Table 3). The distribution of the IL-12 levels among participating children across the four groups was shown in Figure 2, which confirms these results. There are statistically significant differences in mean IL-10 levels among all groups. Statistically significant differences in mean IL-12 levels were observed among the groups (H=24. 217, p<0.001).

Table 1. Relationship between *Toxoplasma gondii* and beta-thalassemia major

		Toxoplasma gondii				**Total**
		Infected		**Non-infected**		
		No.	**%**	**No.**	**%**	
Thalassemia	**Infected**	25	35.21	46	64.8	71
	Non-infected	2*	10.53	17	89.47	19
	Total	27	30	63	70	90
		χ^2=4.349, p<0.05				

*: From a group of healthy control children with no history of genetic or other diseases, only two cases of toxoplasmosis were detected

Table 2. Distribution of anti-*Toxoplasma gondii* IgM and IgG antibodies among participated children in this study

		***Toxoplasma gondii* seropositive**											
		anti-*Toxoplasma gondii* IgM				**anti-*Toxoplasma gondii* IgG**				**Total**			
		Yes	**No**	**Total**	**Infected %**	**Yes**	**No**	**Total**	**Infected%**	**Yes**	**No**	**Total**	**Infected %**
Thalassemia	Yes	6	65	71	8.45	19	52	71	26.76	25	56	71	35.21
	No	0	19	19	0.00	2	17	19	10.53	2	17	19	10.53
	Total	6	84	90	6.67	21	69	90	23.33	27	63	90	30

IgM: Immunoglobulin M, IgG: Immunoglobulin G

The results also showed (Table 3) that the mean of IL-17 levels in the serum of the children group: "thalassemia + parasite", and the group: "thalassemia only" were 112.944±6.886 and 127.021±8.624 pg/mL, respectively, which are lower than the mean level of 194.647±13.162 pg/mL in the "healthy control". In contrast, the mean level of IL-17 of 305±12 pg/ml in the children group: "parasite only" is higher than those in all three other groups of children. The distribution of IL-17 levels among the participating children across the four groups is shown in Figure 3 and supports these results. There are statistically significant differences in mean IL-17 levels among all groups of children in this study (H=27.38, p<0.001). The results of the study also showed (Table 3) that the mean of IFN-γ level in the serum of the children group: "thalassemia + parasite", "healthy control" and "parasite only", are 90.271±17.988, 103.200±2.200 and 101.17±9.712 pg/mL, respectively, are lower than that of 129.578±33.233 pg/mL of the children group: "thalassemia only". The distribution of the IFN-γ levels among participating children in the four groups is shown in Figure 4. There are no statistically significant differences between the mean IFN-γ levels for the different groups of children (H=10.936, p>0.12).

CD4⁺ and CD8⁺ T-cell Levels Across Study Groups

The current study (Table 4) shows the means of the levels of the cluster differentiation markers of $CD4^+$ and $CD8^+$ in serum of both children thalassemia groups: thalassaemia + parasite and thalassaemia only, are 0.825±0.055 and 0.790±0.067ng, for $CD4^+$ respectively, and 4.136±0.369 and 3.800±0.308ng for $CD8^+$ respectively are lower than their means among control children groups who were not infected with thalassemia, whether infected with toxoplasma (parasite only) or not (healthy control), which were 1.064±0.048, 1.064±0.048 ng for $CD4^+$ and 4.450±0.650 and 4.517±0.247 ng for $CD8^+$ respectively. The distribution of the $CD4^+$ and $CD8^+$ levels among participating children in the four groups was shown in Figures 5 and 6, respectively, There are statistically significant differences between the means of the $CD4^+$ and the means of the $CD8^+$ levels of the four children groups, respectively [H=20.634 and H=13.281, (p<0.05 and 0.05)] respectively.

Table 3. The mean of cytokine levels (mean ± SE) pg/mL among different groups of participating children

Group	No.	IL-10 mean ± SE	IL-12 mean ± SE	IL-17 mean ± SE	IFN-γ mean ± SE
Thalassaemia + parasite	25	18.342±1.423	10.074±1.473	112.944±6.886	90.271±17.988
Thalassaemia only	46	20.461±2.063	13.202±2.172	127.021±8.624	129.578±33.233
Parasite only	2	31.700±1.700	45.000±5.700	305.000±12.000	103.200±2.200
Healthy control	17	19.876±1.053	22.123±2.359	194.647±13.162	101.170±9.712
Total	90	20.012±1.155	14.725±1.416	139.840±6.714	112.716±17.989
H statistics value		8.896	24.217	27.380	10.936
p		<0.05	<0.001	<0.001	>0.12

H: Kruskal-Wallis test value, SE: Standard error, IL: Interleukin, IFN-γ: Interferon-gamma

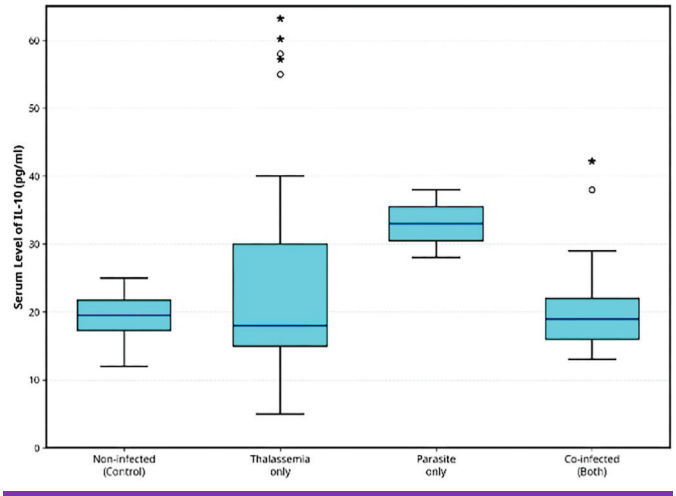

Figure 1. Show the distribution of interleukin-10 levels among the four study groups

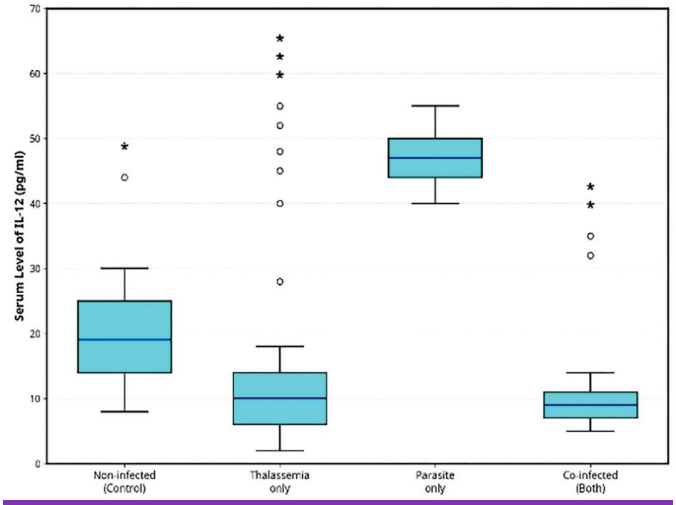

Figure 2. Show the distribution of interleukin-12 levels among the four study groups

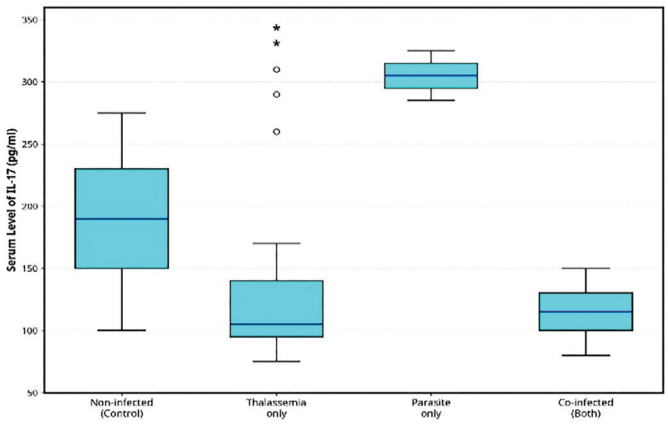

Figure 3. Show the distribution of interleukin-17 levels among the four study groups

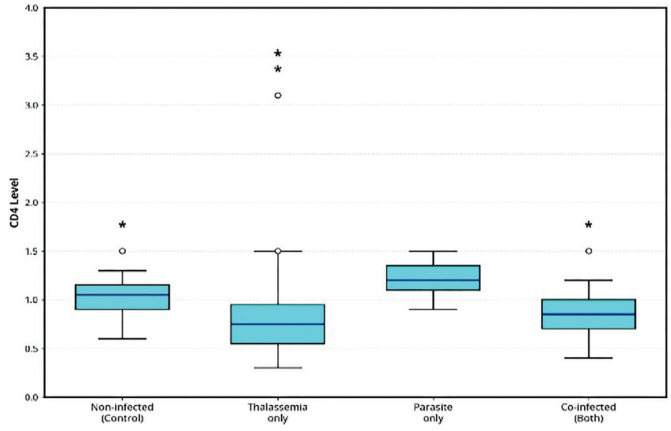

Figure 5. Show the distribution of CD4⁺ levels among the four study groups

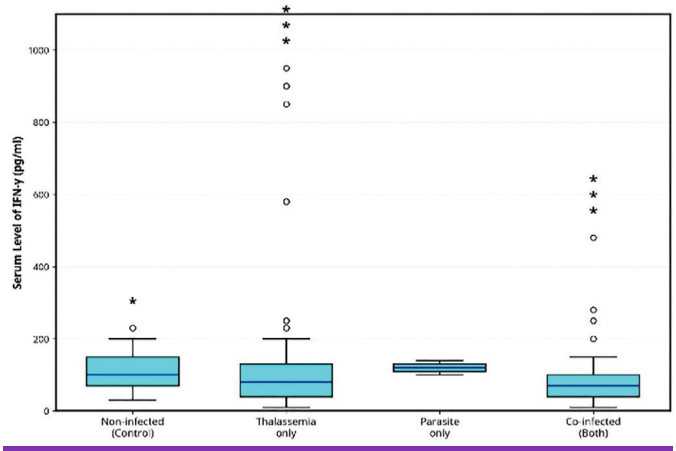

Figure 4. Show the distribution of IFN-γ levels among the four study groups
IFN-γ: Interferon-gamma

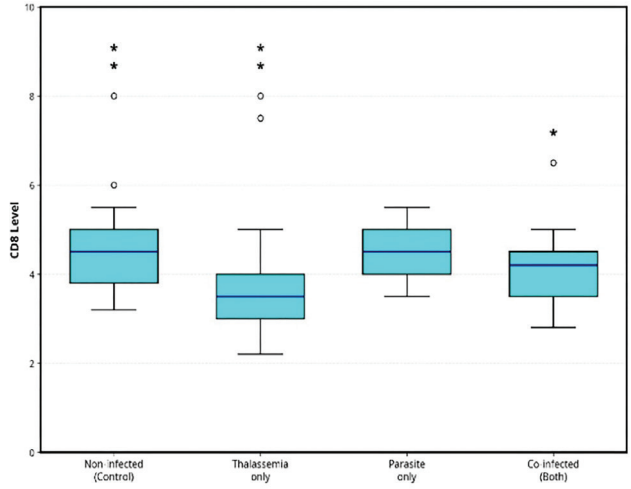

Figure 6. Show the distribution of CD8⁺ levels among the four study groups

Table 4. CD4⁺ and CD8⁺ T-cell levels (ng/mL, mean ± SE) across study groups

Groups	No.	CD4⁺ mean ± SE ng/mL)	CD8⁺ mean ± SE (ng/mL)
Thalassaemia + parasite	25	0.825±0.055	4.136±0.369
Thalassaemia only	46	0.790±0.067	3.800±0.308
Parasite only	2	1.170±0.270	4.450±0.650
Healthy control	17	1.064±0.048	4.517±0.247
Total	90	0.860±0.040	4.043±0.194
H statistic		20.634	13.281
p		<0.05	<0.05
Normal range		0.08-4 ng/mL	3-20 ng/mL

H: Kruskal-Wallis test value, SE: Standard error

DISCUSSION

T. gondii Infection in Beta-thalassaemia Major Patients

The significant elevation of seroprevalence in thalassemia patients (35.2%) relative to controls (10.5%) is consistent with numerous studies confirming heightened toxoplasmosis susceptibility in this cohort owing to immune deficiency and repeated transfusions (13-15). An immunohistochemical study from Maysan Province documented the highest IgG seroprevalence (66.7%) in placental tissue of women who had experienced at least one abortion (3). An Egyptian study reported IgG seropositivity at 53.6% in beta-thalassemia major children and 18% in healthy controls (13). The elevation rate in thalassemia patients likely reflects the partial immune dysregulation induced by chronic transfusion dependency, which raises the probability of acquiring or reactivating *T. gondii* infection (12,8).

Distribution of IgM and IgG Antibodies

The predominance of chronic (IgG-positive) over acute (IgM-positive) infection is consistent with an Iranian study reporting 30.67% IgG seropositivity using ELISA (14). with a Mosul-based study recording IgG positivity at 28% (15). and The current findings showed higher seroprevalence of anti-*T. gondii* IgG and IgM antibodies than those reported in an Egyptian study, where 10% (10/100) and 2% (2/100) of thalassemia patients were positive for IgG and IgM antibodies, respectively (16). The absence of cases with both antibodies simultaneously is noteworthy, suggesting that patients are either in the acute or the chronic phase of infection, with no concurrent transition stage observed. The higher proportion of chronic infection among thalassemia patients reflects the persistent immunosuppression characteristic of this disease, which permits the parasite to establish and maintain latent infection in tissue cysts (17). This pattern aligns with a Turkish study reporting 19.4% IgG and 5.5% IgM seropositivity in beta-thalassemia major patients (18). The current findings showed higher anti-*T. gondii* IgG seroprevalence than that reported by an Iranian study, in which IgG positivity was 13.6% (15/110) among thalassemia patients and 26.3% (29/110) among healthy controls (19).

Effect of T. gondii Infection on IL-10

T-cell-derived IL-10 contributes to limiting excessive inflammation and supporting host survival. Therefore, chronic *T. gondii* infection, together with the immunosuppressive state associated with beta-thalassemia major, may impair T-cell function and consequently reduce IL-10 production (20-22). The reduction in IL-10 may also be related to immune dysregulation caused by repeated blood transfusions, iron overload, and splenectomy in patients with beta-thalassaemia major. Recurrent transfusions can alter cytokine profiles and impair dendritic cell function, thereby weakening cytokine regulation. In addition, splenectomised thalassaemic patients have been reported to produce significantly lower IL-10 concentrations than healthy controls, with values of 7.5±4.1 pg/mL compared with 9.2±3.4 pg/mL, supporting the role of splenectomy in reducing anti-inflammatory cytokine output (23,24). These findings are further supported by an Iraqi serological-genetic study conducted among children attending a thalassemia centre in Baghdad, which reported that children carrying seven to eight high-risk genetic variant alleles had a markedly increased disease risk, estimated at 12.35-fold (95% confidence interval: 7.18-21.25; $p<0.001$). This finding suggests that genetic susceptibility may contribute to impaired IL-10 regulation in children with beta-thalassemia major and may partly explain the lower IL-10 levels observed in the present study (25).

These discrepancies may reflect differences in disease stage, inflammatory burden, transfusion history, splenectomy status, and the degree of immune exhaustion among the studied cohorts (26,27). The discrepancy between the present study and reports of elevated IL-10 in active toxoplasmosis may also be attributed to differences in the phase of infection. Acute *T. gondii* infection is usually associated with active immune regulation and increased cytokine production, whereas chronic infection may lead to progressive immune exhaustion and reduced regulatory cytokine output. This interpretation is supported by the finding that women with active toxoplasmosis showed higher IL-10 concentrations than controls, with values of 10.9±1.7 pg/mL and 5.11±0.23 pg/mL, respectively. Thus, the lower IL-10 level in the present thalassemic groups may reflect the combined effects of chronic infection, immune suppression, and thalassemia-related immune dysfunction rather than toxoplasmosis (28).

Effect of T. gondii Infection on IL-12

The reduced IL-12 observed in the present thalassemic groups may not reflect the usual immune response to *T. gondii* alone, but rather the combined effect of parasitic infection and beta-thalassemia-related immunosuppression. The contrast between the present findings and the elevated IL-12 reported in recent toxoplasmosis studies suggests that iron overload, chronic transfusion-related immune disturbance, and impaired macrophage function may override the expected IL-12-mediated Th1 activation in beta-thalassemia major patients (8,29,30).

This interpretation is supported by recent studies in non-thalassemic or immunologically distinct populations, in which *T. gondii* infection was associated with increased, rather than decreased IL-12. In an Iraqi study conducted in Thi-Qar province, IL-12 levels were higher in cardiac patients infected with toxoplasmosis than in controls, reaching 82.85 pg/mL in patients compared with 60.97 pg/mL in controls, indicating immune activation during infection. Similarly, a serological-immunological study from Al-Najaf reported increased IL-12 among breast cancer patients infected with *T. gondii*, with levels of 21±2.4 pg/mL compared with 8±1.04 pg/mL in controls (30).

Effect of T. gondii Infection on IL-17A

IL-17A, primarily produced by Th17 cells, contributes to host defence against infectious agents by promoting neutrophil recruitment and proinflammatory responses. The significant decrease in IL-17A in both thalassemia groups may reflect impaired Th17-mediated immunity during chronic *T. gondii* infection and beta-thalassemia-associated immune suppression (31). This finding agrees with the chronic-phase pattern reported by (32), where IL-17A was much lower in chronic toxoplasmosis (12.96±6.039 pg/mL) than in acute infection (202.0±24.80 pg/mL). This supports the interpretation that the reduced IL-17A in the current study may be related to the predominance of chronic infection and immune exhaustion (32) and not agree with (33). Who reported higher IL-17A levels in women with recurrent abortion associated with toxoplasmosis (6.584±0.854 pg/mL) compared with healthy controls (4.284±0.728 pg/mL). This variation may be related to differences in study population, clinical condition, and immune status (33).

Effect of T. gondii Infection on IFN-γ

IFN-γ is a key cytokine in cellular immunity against *T. gondii*. The non-significant increase observed in the present study may reflect a modest immune response to the infection in children with beta-thalassemia major (34).

The present findings agree with a recent Iraqi study reporting higher IFN-γ levels in thalassaemic groups, with the highest concentration recorded in beta-thalassemia patients only (23.900±0.218 pg/mL), followed by beta-thalassaemia patients infected with *T. gondii* (23.404±0.235 pg/mL). Lower levels were recorded in *T. gondii*-positive controls (14.394±0.264 pg/mL) and healthy controls (10.958±0.178 pg/mL), supporting the role of IFN-γ activation in thalassaemia-associated immune responses (35). In a complementary context related to *T. gondii* infection, the

present findings agree with an Iraqi study that reported elevated serum IFN-γ levels in women with toxoplasmosis-associated miscarriage, reaching 47.080±4.213 pg/mL compared with 25.298±4.751 pg/mL in healthy controls. This increase supports the role of IFN-γ in activating cell-mediated immunity against *T. gondii* infection (36). The results also agree with a recent Brazilian study showing no significant difference in serum IFN-γ among pregnant women with acute toxoplasmosis (29.00±15.25 pg/mL), chronic toxoplasmosis (33.61±28.79 pg/mL), and uninfected controls (29.79±19.16 pg/mL). This suggests that IFN-γ may remain elevated or relatively sustained across infection phases, unlike cytokines that decline clearly during chronic infection (32).

Effect of T. gondii Infection on CD4⁺ and CD8⁺ T-cells

Chronic *T. gondii* infection may further aggravate immune suppression through exhaustion of CD4⁺ and CD8⁺ T-cells. This immune exhaustion can reduce the ability of T-cells to maintain effective anti-parasitic responses and may contribute to parasite persistence or reactivation, particularly in immunocompromised thalassemia patients exposed to chronic immune stimulation from repeated transfusions (21).

The present findings are consistent with a recent Iraqi study that reported altered T-cell counts in patients with beta-thalassemia major infected with *T. gondii*. CD4⁺ T-cells were lower in infected thalassemia patients, with a mean of 530.63±84.15, compared with 804.62±1.71 in controls. However, the reported CD8⁺ T-cell values appear higher in infected thalassemia patients, 870.67±1.25, compared with 597.78±3.96 in controls (37).

Clinical significance: The increased seroprevalence of *T. gondii* and associated immune alterations observed in children with beta-thalassemia major support routine serological screening and immunological monitoring as part of their clinical management to reduce infection-related complications. Approval of a pre-transfusion blood test for toxoplasmosis as a routine screening test for thalassemia patients

Scientific contribution: This study provides novel evidence from Maysan Province that children with beta-thalassemia major exhibit a higher seroprevalence of *T. gondii,* accompanied by significant immune alterations, thereby advancing current understanding of the immunological impact of toxoplasmosis in this vulnerable population and supporting routine serological and immunological monitoring.

Study Limitations

We encountered considerable difficulty convincing parents of healthy children to participate in this study, and at times even persuading the patients themselves.

CONCLUSION

The findings of the present study suggest that beta-thalassemia major may increase children's susceptibility to *Toxoplasma gondii* infection through transfusion-related immune dysregulation and impaired cellular immunity. The observed changes in cytokine profiles and T-cell responses also indicate weakened immune control of the parasite and possible immune exhaustion, highlighting the need to strengthen blood screening and preventive measures to reduce the risk of infection in this immunocompromised group.

*Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Research and Knowledge Management Unit, Training and Human Development Center, Misan Health Directorate, Ministry of Health, Republic of Iraq (approval no: 709; date: 27.11.2025).

Informed Consent: Written informed consent was obtained from the parents or legal guardians of all participating children prior to their inclusion in the study.

*Footnotes

*Authorship Contributions

Surgical and Medical Practices: A.A.K., Concept: G.G.S., H.A.M.A., Design: G.G.S., H.A.M.A., Data Collection or Processing: G.G.S., A.A.K., Analysis or Interpretation: G.G.S., H.A.M.A., A.A.K., Literature Search: G.G.S., H.A.M.A., Writing: G.G.S., H.A.M.A.

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