Al-Nahrain Journal of Science
ANJS, Vol. 28(3), September, 2025, pp. 57 – 62

Immunological Evaluation of IgM and IgG Antibodies and Complement Components C3 and C4 in Thalassemia Patients with Toxoplasma Gondii Infection

Evan H. Sulaiman[1], Raghad N. Shihab[2,*], Ahmed Jabbar Obaid[1], Ahmed Abd Temur[1] , Ahlam A. Alwan[1]

[1] Department of Applied Pathological Analysis, College of Science, Al-Nahrain University, Baghdad, Iraq.
[2] Iraqi Center for Cancer and Medical Genetic Research, Mustansiriyah University, Baghdad, Iraq.

Article's Information

Received: 24.03.2025
Accepted: 02.05.2025
Published: 15.09.2025

Keywords:
Thalassemia,
Toxoplasma Gondii,
Complement system,
Antibodies,
IgM,
IgG.

Abstract

Toxoplasmosis is known to be transmissible through blood transfusion, especially from asymptomatic, seropositive individuals during the acute phase. Patients with thalassemia are particularly vulnerable to such opportunistic infections, including Toxoplasma gondii. This study aimed to evaluate the levels of Toxoplasma-specific IgG, IgM, and IgA antibodies, along with complement components C3 and C4, in thalassemia patients with and without toxoplasmosis infection. A significant difference ($P < 0.001$) was observed in all immunological markers when comparing patient groups to the negative control group. Additionally, both thalassemia groups (with and without toxoplasmosis) showed significant differences compared to the toxoplasmosis-positive control group ($P < 0.001$). Notably, thalassemia patients with toxoplasmosis exhibited higher levels of Toxoplasma-specific antibodies and altered complement levels compared to thalassemia patients without the infection ($P < 0.001$). These findings suggest an altered immunological profile in thalassemia patients, particularly when co-infected with T. gondii, highlighting the importance of monitoring such markers in this vulnerable population.

http://doi.org/10.22401/ANJS.28.3.08

*Corresponding author: raghad.shihab@uomustansiriyah.edu.iq

1. Introduction

Globally, cancer is now a health and financial load, A class of hereditary blood illnesses known as thalassemia is typified by aberrant hemoglobin synthesis, which results in anemia and red blood cells' breakdown. Hemoglobin, the protein found in red blood cells (RBCs), is responsible for transporting oxygen throughout the body. Thalassemia results from the mutations in the DNA regarding the cells that produce hemoglobin [1, 2]. A decrease in the synthesis of globin protein is one of the several hemoglobin synthesis abnormalities that define beta-thalassemia, one of the most prevalent epidemic conditions worldwide. Major, minor, and intermediate thalassemia are the three types of thalassemia that may be identified clinically [3,4]. Blood transfusions are a treatment for patients with ß-thalassemia major, and each milliliter of bursting red blood cells raises the body's iron overload by one milligram patients who receive too many blood transfusions develop iron overload [5]. Transfusion-transmitted infections, like Toxoplasmosis resulting from T. gondii, which is a very important obligatory intracellular protozoan parasitic organism in humans and veterinary animals and is classified in the phylum Apicomplexan, can increase the mortality as well as morbidity rate among patients with thalassemia. Additionally, the rate of T. gondii infection among blood donors has been linked to the overall prevalence of the parasite in the general population [6,7]. Serological studies estimate that T. gondii—one of the most widespread human pathogens—infects approximately one-third of the global population [8]. The parasite exists in three infectious stages: tachyzoites, bradyzoites, and sporozoites. These stages are generated during the parasite's life cycle, with sexual reproduction occurring exclusively in felids (cats), which serve as

the definitive host. The resulting oocysts are shed in cat feces and can contaminate soil, water, or food [9]. Immunocompromised individuals may acquire T. gondii through various routes, including contact with cat waste, consumption of unpasteurized milk or undercooked meat, blood transfusion, or organ transplantation. Figure 1 illustrates the life cycle of Toxoplasma gondii, beginning in cats (the definitive hosts), which release oocysts into the environment. These oocysts infect intermediate hosts such as humans and animals, where tachyzoites and bradyzoites proliferate and persist [10, 11]. Because of a fundamental flaw in host defense, patients with thalassemia are more susceptible to serious infections like toxoplasmosis. This risk may be linked to immune deficit, iron overload, splenectomy, and chronic immunological stimulation from frequent blood transfusions [12, 13]. One of the earliest documented cases of T. gondii transmission via blood product transfusion involved four immunocompromised individuals [14]. Similarly, Karakaş et al. [15] conducted a study in Aydin Province, Turkey, involving patients with beta-thalassemia major, to explore the possible association between frequent blood transfusions and T. gondii infection. Their findings indicated a higher seropositivity rate for anti-T. gondii antibodies in thalassemia patients compared to controls; however, the difference was not statistically significant. These findings underscore the potential risk of T. gondii exposure through repeated transfusions in thalassemia patients. Therefore, the current study aims to further investigate this relationship in an Iraqi population, with a focus on both antibody responses and complement levels.

2. Materials and Methods

Ethical approval was (2/6-RECSNU in 2025) by Ethical committee in College of Science Al-Nahrain University. The research comprised 180 patients with thalassemia and 80 controls negative who visited the Al-Karma Teaching Hospital in Baghdad, Iraq from Mar. and Jun. of 2022. The patients' ages ranged from 8 to 45 years. Following the doctor's diagnosis and the required blood tests to identify thalassemia, serum samples have been analyzed and tested for anti-Toxoplasma IgM and IgG antibodies with the use of CMIA. Each patient had five milliliters of venous blood extracted with the use of a sterile syringe. For separating the serum, 3 ml of whole blood was put into a fully labeled gel tube and centrifuged at 3000rpm for 5mins. The serum has then been stored at -20oC until it was needed for CMIA and ELISA detection. Two milliliters of whole blood were collected in a labeled EDTA tube for the leukocyte count.

2.2 T. gondii diagnosis and immunological parameters

Chemiluminescent microparticles immunoassay (CMIA) has been performed to detect both anti – Toxoplasma IgG/IgM anti-bodies in sera according to manufacturer's instruction (Architect Toxo IgM/G kit -Abbott GmbH, Germany). The sandwich enzyme linked immunosorbent assay (ELISA) This assay intended for quantitative measurement levels of total immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), and complement system compound C3, C4

2.3. Statistical analysis

All results were analyzed using SPSS program (V.20, IBM), using One Way ANOVA and obtaining Least Significant Difference (LSD). The results were presented in the form of mean±S.E, and significant difference has been considered at $p \leq 0.050$. Additionally, Pearson's correlation coefficient has been used for the purpose of finding the correlation between the studied parameters [16].

3. Results and Discussion

The current study demonstrated statistically significant differences ($P < 0.001$) in anti-Toxoplasma IgM, IgG, and IgA levels, as well as in complement components C3 and C4 among the studied groups. CMIA results (Table 1) revealed markedly elevated IgG levels in thalassemia patients co-infected with T. gondii compared to thalassemia-only and control groups. These findings suggest strong immunological stimulation in co-infected individuals, possibly due to repeated exposure via transfusions. Table 2 highlighted significantly increased IgM levels in the co-infected group, which supports the presence of recent or acute toxoplasmosis infection. These results agree with El-Tantawy et al. [18], who reported elevated IgM and IgG seropositivity among thalassemic children. However, our data revealed higher IgM responses than theirs, possibly due to differences in study population, geographical exposure, or transfusion practices. As for IgA levels, Table 6 indicates a significant rise in thalassemia patients with toxoplasmosis. Previous studies [19] noted that IgA peaks may follow IgM and serve as an additional diagnostic marker, particularly in immunocompromised hosts. Significant differences were also observed in immunoglobulin concentrations (Tables 4–5), with thalassemia patients and co-infected individuals exhibiting

higher IgM and IgG levels than controls. This immune activation may reflect continuous antigenic stimulation from transfusions, iron overload, and chronic inflammation, as supported by Saleh and Okab [26] and Javad et al. [27]. In terms of complement components, Tables 7 and 8 show significantly lower C3 and C4 levels in thalassemia and co-infected groups. These findings align with literature [28] indicating that repeated transfusions can lead to persistent complement activation, ultimately reducing synthesis or increasing consumption of C3/C4. Elevated ferritin levels in thalassemia may further impair complement production. Interestingly, some earlier studies [23, 24] questioned the necessity of routine immunoglobulin monitoring in thalassemia. However, our findings advocate for regular immune surveillance, especially in transfused patients at risk for opportunistic infections like toxoplasmosis. At the cellular level, thalassemia patients exhibit defects in innate immunity due to reduced levels of properdin, lysozyme, complement, and impaired neutrophil function [20]. This immune dysfunction, combined with splenectomy, iron chelation, and oxidative stress, may underlie the observed susceptibility to parasitic infections. Overall, our study reinforces the need for vigilant immunological monitoring and early diagnosis of T. gondii in thalassemia patients, especially those undergoing regular transfusions. Prophylactic strategies and targeted screening may reduce morbidity in this vulnerable population.

Table 1. Levels of Toxoplasma IgG antibody in the studied groups.

Groups	Toxoplasma IgG (Mean ± S.E) IU/mL	LSD	P Value
Control negative (-ve)	0.60±0.27	5.48	<0.001**
Control positive (+ve)	23.47±0.33 a		
Thalassemia patients	0.31±0.03 b		
Thalassemia patients with toxoplasmosis	56.58±3.17 abc		

** Significant differences
a significant difference vs. control negative, b vs. control positive, c vs. patients

Table 2. Specific Toxoplasma IgM antibody in the studied groups.

Groups	Toxoplasma IgM (Mean ± S.E) IU/mL	LSD	P Value
Control negative (-ve)	0.41±0.12	0.17	<0.001**
Control positive (+ve)	0.27±0.04		
Thalassemia patients	0.04±0.00 ab		
Thalassemia patients with toxoplasmosis	0.67±0.02 abc		

** Significant differences. (a) significant difference vs. control negative, (b) vs. control positive, (c) vs. patients

Table 3. Comparison between the groups in the levels of Toxoplasma IgA antibody.

Groups	Toxoplasma IgA (Mean ± S.E) IU/mL	LSD	P Value
Control negative(-ve)	0.18±0.03	0.11	<0.001**
Control positive (+ve)	1.25±0.05 a		
Thalassemia patients	0.31±0.04 ab		
Thalassemia patients with toxoplasmosis	1.34±0.02 abc		

** Significant differences. (a) significant difference vs. control negative, (b) vs. control positive, (c) vs. patients

Table 4. Levels of IgG in the sera of the studied groups.

Groups	IgG (Mean ± S.E) mg/dl	LSD	P Value
Control negative(-ve)	1418.56±2.38	40.72	<0.001**
Control positive (+ve)	1645.78±2.60 a		
Thalassemia patients	2829.05±3.61 ab		
Thalassemia patients with toxoplasmosis	2263.88±23.21 abc		

** Significant differences. (a) significant difference vs. control negative, (b) vs. control positive, (c) vs. patients

Table 5. Concentrations of IgM (mg/dl) in the sera of the studied groups.

Groups	IgM (Mean ± S.E) mg/dl	LSD	P Value
Control negative(-ve)	101.40±0.86	5.49	<0.001**
Control positive (+ve)	160.49±0.55 a		
Thalassemia patients	191.70±0.44 ab		
Thalassemia patients with toxoplasmosis	256.75±3.06 abc		

** Significant differences. (a) significant difference vs. control negative, (b) vs. control positive, (c) vs. patients

Table 6. Descriptive Comparison of IgA among groups of study.

Groups	IgA (Mean ± S.E) mg/dl	LSD	P Value
Control negative(-ve)	121.07±0.86	11.7	<0.001**
Control positive (+ve)	177.0±3.0 a		
Thalassemia patients	287.22±0.90 ab		
Thalassemia patients with toxoplasmosis	370.15±6.31 Abc		

** Significant differences. (a) significant difference vs. control negative, (b) vs. control positive, (c) vs. patients

Table 7. Concentration of C3 among groups of study.

Groups	C3 (Mean ± S.E) mg/dl	LSD	P Value
Control negative(-ve)	133.64±0.41	2.8	<0.001**
Control positive (+ve)	61.64±0.70 a		
Thalassemia patients	72.93±0.53 ab		
Thalassemia patients with toxoplasmosis	78.82±1.41 abc		

** Significant differences. (a) significant difference vs. control negative, (b) vs. control positive, (c) vs. patients

Table 8. Expressive Comparison of C4 among groups of study.

Groups	C4 (Mean ± S.E) mg/dl	LSD	P Value
Control negative(-ve)	32.69±0.27	0.11	<0.001**
Control positive (+ve)	14.20±0.30 a		
Thalassemia patients	19.43±0.29 ab		
Thalassemia patients with toxoplasmosis	26.33±0.46 abc		

** Significant differences. (a) significant difference vs. control negative, (b) vs. control positive, (c) vs. patients

4. Conclusions

This study provides substantial evidence of altered humoral immunity in β-thalassemia patients, particularly those co-infected with Toxoplasma gondii. Significant elevations in IgG, IgM, and IgA antibodies among thalassemia patients with toxoplasmosis suggest active immune response, while the observed reduction in complement components C3 and C4 may reflect underlying immune exhaustion or chronic complement activation. The findings underscore the heightened vulnerability of thalassemia patients to opportunistic infections, likely due to immune dysregulation caused by frequent transfusions, iron overload, splenectomy, and chronic inflammation. These results support the need for routine serological screening for T. gondii and regular monitoring of immunoglobulin and complement levels in transfusion-dependent patients. Future research should investigate the mechanisms behind immune impairment in thalassemia and evaluate whether early detection and prophylactic interventions can reduce infection-related complications. Integrating immunological assessment into standard care protocols may enhance patient outcomes and reduce the burden of opportunistic infections in this high-risk population.

Acknowledgments: Acknowledgment goes to individuals who participated in the research, whether patients or control group.

Conflicts of Interest: Authors declare there is no conflict of interest pertaining this work.

Funding statement: No funding is received for this work.

References

[1] Shihab, R.N.; Al-Aubaidi, I.K.; "Toxoplasmosis in thalassemic Iraqi patients: serological and hematological study". Roy. J. Infect. Dis. 27(2): 100-106, 2024.
[2] Ali, S.; Mumtaz, S.; Shakir, H.A.; Khan, M.; Tahir, H.M.; Mumtaz, S.; Khan, M.A.; "Current status of beta-thalassemia and its treatment strategies". Mol. Genet. Genomic Med. 9(12): e1788, 2021.
[3] Thein, S.L.; "Molecular basis of ß thalassemia and potential therapeutic targets". Blood Cells Mol. Dis. 70: 54-65, 2018.
[4] Lei, M.; Sun, L.; Luo, X.S.; Yang, X.; Yu, F.; Chen, X.; Wang, Z.; "Distinguishing iron deficiency anemia from thalassemia by the red blood cell lifespan with a simple CO breath test: a pilot study". J. Breath Res. 13(2): 026007, 2019.
[5] Bloch, E.M.; "Transfusion-transmitted infections". Ann. Blood 7(20): 1-19, 2022.
[6] Mousa, N.M.; Nahab, H.M.; "Serological detection and hematological changes associated with toxoplasmosis in thalassemia patients in Al-Samawah province". Int. J. Pharm. Res. 12(4): 872-876, 2020.
[7] Al-Hadraawy, S.K.; Alzeyadi, M.; Shakir, A.; Al-Dujaili, A.; "Detection of Toxoplasma gondii genotypes in abortion women by RFLP-PCR in Al-Najaf Al-Ashraf province". J. Breath Res. 5: 1-7, 2019.
[8] Attias, M.; Teixeira, D.E.; Benchimol, M.; Vommaro, R.C.; Crepaldi, P.H.; De Souza, W.; "The life-cycle of Toxoplasma gondii reviewed using animations". Parasites Vectors 13(1): 588, 2020.
[9] Silva, R.B.; Ramalho, A.S.; Cassorla, R.M.S.; "Anemia falciforme como problema de saúde pública no Brasil". Rev. Saude Publica 27(1): 54-58, 1993.
[10] Higgs, D.R.; Engel, J.D.; Stamatoyannopoulos, G.; "Thalassaemia". Lancet 379(9813): 373-383, 2012.
[11] Greene, D.N.; Vaughn, C.P.; Crews, B.O.A.; Garwal, A.M.; "Advances in detection of hemoglobinopathies". Clin. Chim. Acta 439: 50-57, 2015.
[12] Ferreira, M.N.; Bonini-Domingos, C.R.; Estevão, I.F.; "Anti-Toxoplasma gondii antibodies in patients with beta-hemoglobinopathies: the first report in the Americas". BMC Res. Notes 10(1): 211, 2017.
[13] Ghaffari, J.; Vahidshahi, K.; Kosaryan, M.; "Humoral immune system state in ß-thalassemia major". Med Glas (Zenica) 8(2): 192-196, 2011.
[14] Saeed, S.A.; Al-Aubaidi, I.K.; "Assessment of Interleukin-10 Levels in Iraqi Diabetic Type 2 Patients Infected with Toxoplasmosis". Adv. Life Sci. 10(1): 62-66, 2023.
[15] Karakaş, S.; Özlem, S.; Tellioglu, M.T.; Ertabaklar, H.; Ertug, S.; "Investigation of anti-Toxoplasma gondii IgG and IgM antibodies in beta-thalassemia major patients in Aydın province". Turk J Parasitol. 36(3): 133-136, 2012.
[16] Marasinghe, M.G.; Koehler, K.J.; "Statistical data analysis using SAS". JR Stat. Soc. Ser. A 183: 1829, 2018.
[17] Al-Aubaidi, I.K.; Saeed, S.A.; Jaaffar, A.I.; "Blood lymphocytes detection in Iraqi diabetic type 2 patients infected with chronic toxoplasmosis by using flow cytometry". Indian J Forensic Med Toxicol. 14(4): 2297-2303, 2020.
[18] El-Tantawy, N.; Darwish, A.; Eissa, A.; Eman, M.B.; "Seroprevalence of Toxoplasma gondii infection among beta-thalassemia major pediatric population: implications for transfusion transmissible toxoplasmosis". Pediatr Infect Dis J. 38(3): 236-240, 2019.
[19] Hopp, R.; Niebur, H.B.; "Persistent Hyper IgA as a Marker of Immune Deficiency: A Case Report". Antibodies (Basel) 11(2): 30, 2022.
[20] Gluba-Brzózka, A.; Franczyk, B.; Rysz-Górzyńska, M.; Rokicki, R.; Koziarska-Rościszewska, M.; Rysz, J.; "Pathomechanisms of immunological disturbances in ß-thalassemia". Int J Mol Sci. 22(18): 9677, 1-13, 2021.
[21] Zko, S.S.; Al-Naftachi, M.T.; Al-Jawadi, W.A.; Alghrer, M.M.; "Seroprevalence of Toxoplasma gondii among thalassemia patients and its relation with some biochemical parameters". J Nat Sci Biol Med. 15(1): 119-124, 2024.
[22] Hanifehpour, H.; Samsam Shariat, S.K.; Ghafari, M.S.; Kheirandish, F.; Saber, V.; Fallahi, S.; "Serological and molecular diagnosis of Toxoplasma gondii infections in thalassemia patients". Iran J Parasitol. 14(1): 20-28, 2019.
[23] Wang, H.; Kim, S.J.; Lei, Y.; "Neutrophil extracellular traps in homeostasis and disease". Signal Transduct Target Ther. 9: 235, 2024.
[24] Al-Thamir, S.N.; Al-Hussainy, S.M.M.; "Effects of iron overload and treatment methods on serum levels of IgG, IgM, and IgA in beta

thalassemia major patients". Med J Babylon. 8(3): 472-478, 2011.
[25] Daneshi, M.; "Serum immunoglobulin levels in splenectomized and nonsplenectomized patients with major beta-thalassemia". Iran J Immunol. 8(2): 95-98, 2011.
[26] Okab, H.F.; Saleh, M.B.; "Evaluation of the immune status of blood transfusion-dependent thalassemia in Thi-Qar Province, Iraq". J Educ Pure Sci. 9(2): 20-27, 2019.
[27] Javad, G.; Saeid, A.; Mohammadmehdi, N.; "Thalassemia and immune system dysfunction". Int J Curr Res. 3(12): 105-108, 2016.
[28] Asadov, C.; "Immunologic abnormalities in β-thalassemia". J Blood Disord Transfus. 5(7): 244, 2014.
[29] Goldberg, B.S.; Ackerman, M.E.; "Antibody-mediated complement activation in pathology and protection". Immunol Cell Biol. 98(4): 305-317, 2020.
[30] Qi, H.; Wei, J.; Gao, Y.; Yang, Y.; Li, Y.; Zhu, H.; Su, L.; Zhang, Y.; et al.; "Reg4 and complement factor D prevent the overgrowth of Escherichia coli in the mouse gut". Commun Biol. 3: 483, 2020.
[31] Buttari, B.; Profumo, E.; Caprari, P.; Massimi, S.; Sorrentino, F.; Maffei, L.; Gabbianelli, M.; Riganò, R.; "Phenotypical and functional abnormalities of circulating neutrophils in patients with β-thalassemia". Ann Hematol. 99(10): 2265–2277, 2020.
[32] Bazi, A.; Shahramian, I.; Yaghoobi, H.; Naderi, M.; Azizi, H.; "The role of immune system in thalassemia major: a narrative review". J Pediatr Rev. 6(2): 29-36, 2018.