RESEARCH PAPER

The Influence of ZINC on Humoral and Cellular Immunity in a Toxoplasmosis Patient

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ABSTRACT
Toxoplasmosis infection can influence complex immune responses that increase both humoral and cell-mediated immunity. Zinc is a very important material that increases the function of the immune system and has been linked to immune response modulation. In this study, the role of zinc on humoral (IgG, IgM) and cellular (CD4, CD8) immunity was investigated in patients with different serological status for Toxoplasma gondii. 102 samples of serum and EDTA were collected from different labs in Erbil and Duhok cities. Toxoplasmosis was enrolled, and zinc was evaluated with a spectrophotometer. T-cell subset (CD4 and CD8) test determined by flow cytometry. Jamovi was used to perform the statistical analysis such as descriptive statistics, T-test, Pearson's, and linear regression. The results showed that zinc was greater in patients with higher IgG levels, the P-value was less than 0.005. The correlation between zinc and IgG was moderate (r =0.461, R^2 = 0.212), demonstrating that zinc accounted for 21.2% of the disparity in IgG levels, and the P-value was less than 0.005. Furthermore, there was no relation between zinc and IgM; the P-value was 0.930, the CD4 p-value was 0.016, and the CD8 p-value was 0.002. Zinc plays a significant role in increasing the latent IgG levels, which means it increases memory against Toxoplasma gondii. These findings are important in demonstrating the role of increasing humoral immunity against the disease by zinc.

Keywords: *Toxoplasma, Zinc, immune system, Humeral, Cellular, IgG, IgM*
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INTRODUCTION

One-third of the world's population is infected with Toxoplasmosis, its widespread zoonotic disease caused by the obligatory intracellular protozoan parasite called Toxoplasma gondii(1). Eating undercooked meat carrying cysts, vertical transmission during pregnancy, and oocysts in food or water can transmit the disease. While most immunocompetent patients are asymptomatic. T. gondii poses serious hazards to immunocompromised hosts, including HIV/AIDS patients or those undergoing immunosuppressive treatment, and to the fetus in congenital infection, in which case it can cause severe neurological damage or death. The host immune response to T. gondii is difficult, cellular and humoral immunity are both involved(2, 3). The immune system differs from person to another depending on the genetic background and Singal nucleotide polymorphism(4). The humoral immune response represents the formation of specific antibodies. The immunoglobulin IgM is the first antibody to be produced by the immune system and used as an indicator of acute infection of toxoplasmosis(5). On the other hand, immunoglobulin IgG is produced in the latent response and can stay for a long period, staying for years, providing immunity against the disease. Toxoplasma IgG

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and IgM antibodies are serological blood tests to detect toxoplasmosis in suspected patients. In addition, the cell-mediated immunity production of T-cell CD4 and CD8 plays an important role in fighting against toxoplasmosis(6). T helper cell CD4 activates macrophages and stimulates the production of B-cells to produce antibodies through the secretion of cytokines. CD8 cytotoxic T cells play a significant role in identifying and killing infected cells, as well as controlling the spread and replication of the parasite(5). Therefore, cell-mediated immunity is necessary for the prevention of recurrent infections and reactivation of latent antibodies(7). Zinc is an essential mineral that stimulates the immune system, enhancing both innate and adaptive immune responses(8). Zinc is significant for the production of proteins, T cell maturation, antioxidant protection, and promoting the inflammatory response(9). In addition, it has a significant role in transcription factors, enzymes, and enhances cellular signaling processes. Studies predicted that zinc deficiency decreases the production of antibodies and the activity of thymic hormones that affect the host defense mechanism against toxoplasmosis(10). Despite zinc's well-established role in the immune system, little is known about how it affects immunological parameters in people infected with T. gondii(11). The study of the relation between zinc and the immune system in toxoplasma patients is important because of the prevalence of zinc deficiency in the majority of developing nations and the global significance of toxoplasmosis(12). Different levels of zinc concentrations that influence the humoral and cell-mediated immunity in patients with toxoplasmosis are uncertain. This study will compare and evaluate zinc levels, IgG, and IgM antibodies with CD4 and CD8 in toxoplasmosis patients.

METHODS AND MATERIALS

Study Design and Sample Collection

Totally 102 samples of serum and EDTA were collected from patients (male and females) from clinical laboratories in Erbil and Duhok cities, Amer, King, Smart, Ariyo, and Floria labs. The duration of collection sample was about two months, the samples were enrolled for toxoplasmosis screening IgG and IgM by Cobas E411 device (Roche, Germany) and for T-cell subset test by Flowcytometery in Amer lab.

Inclusion and Exclusion Criteria

Patients aged bigger than 18 years were either positive or negative for toxoplasmosis, serum were collected for toxoplasmosis IgG, IgM and EDTA for T-cell subset test (CD4 and CD8), pregnant women, and patients with autoimmune disease, chronic infection, cancer, receiving zinc supplement or immunotherapy, and hemolyzed samples were excluded from this study.

Laboratory Analysis

Toxoplasmosis IgG and IgM antibodies were enrolled on Cobas E411(Roche, Germany). Zinc level test was measured in a spectrophotometer (EMC Lab) using Lab Kit reagents (China). Furthermore for CD4 and CD8 test EDTA samples were sent to Amer lab to analyze T-cell subset test by flocytometery device. Other data such as age and gender were recorded for all patients.

Material and Equipment

Table 1: shows the devices and materials with brand and source.

Equipment	Brand	Source
Centrifuge	Hettich	Germany
Micropipettes	Physio Care	USA
Water Bath	GFL	Germany
Freeze	–	–
EDTA Tubes	ISMS	China
Serum Tubes	ISMS	China
Cobas E411	Roche	Germany
Spectrophotometer	EMC Lab	–
Zinc Reagent	LABKIT	China
Yellow Pipette Tips	ISMA	China
Blue Pipette Tips	ISMS	China
Khan Tubes	Physio Care	USA

Zinc and T Cell Subset Analysis Procedures

Zinc procedure

Zinc levels were enrolled manually by Lab Kit reagent and results were measured by spectrophotometer device.

1- Three khan tubes were prepared by adding 1 ml of the zinc reagent. First tube was used as bland, second tube for standard and last tube for the patient's serum.

2- In blank tube 50µL of distilled water was added, in standard tube 50µL of standard solution was added, and in patient tube 50µL of patient serum was added to the khans' tubes.

3- The three tubes were left in incubation for 5 minutes at 37°C or in room temperature for 10 minutes

4- The three tubes were measured by spectrophotometer at 580 nm.

5- . Zinc level was calculated using the formula: (Sample Absorbance / Standard Absorbance) × 200 (µg/dL).

T-cell subtest test procedure

For measuring T-cell subset specifically CD4 and CD8 count by flowcytometry, EDTA samples were sent for Dr, Amer lab in Duhok city

Statistical Analysis

The statistical analysis of this study was measured by Jamovi (version X.X) program which provide functions same to SPSS program for linear, logistic regressions and

correlation analysis(13). The descriptive statistics which include mean. median and ± SD for IgG and IgM and zinc levels were analyzed (Table 2). Pearson correlation coefficient (r), T-test, and linear regression was analyzed to assess the relation between zinc and IgG, IgM, CD4, CD8 levels, R^2 is the proportion of variance in IgG accounted for by zinc was also analyzed.

RESULTS

Table 2, shows the descriptive data mean, median, and ± SD of IgG, IgM, and zinc levels.

Descriptive statistic	IgG	IgM	Zinc Level
Mean	134	8.58	92.1
Median	0.456	0.206	88.9
Standard Deviation	290	45	22.7
minimum	0.100	0.0100	54.0
maximum	2014	342	168

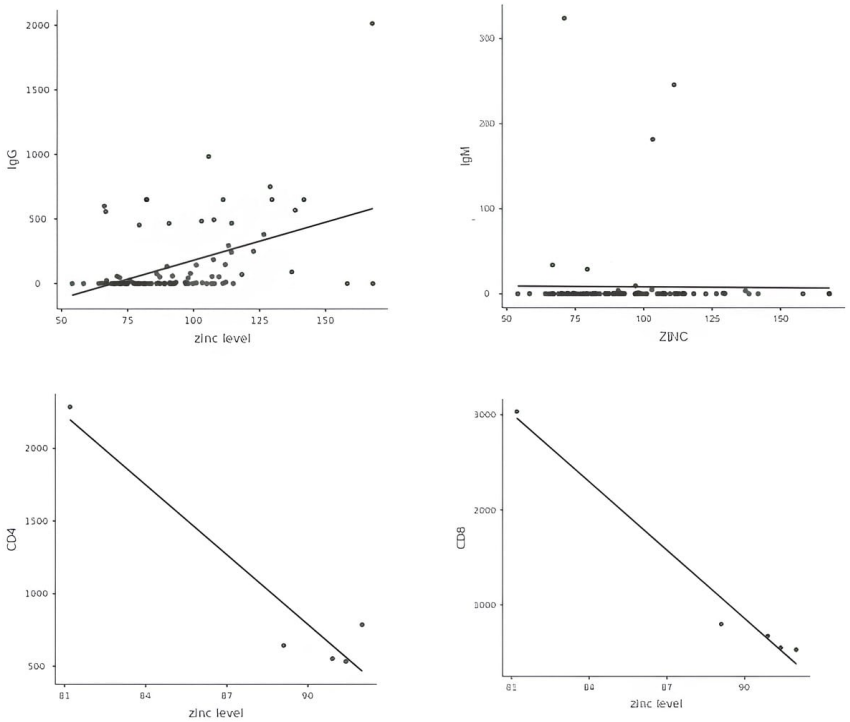

Figure 1. Shows correlation in a scatter plot between zinc serum levels and IgG antibody titers in Toxoplasma gondii-infected subjects ($p < 0.05$). correlations between zinc levels and IgM p = 0.596, CD4 p-value=0.016, and CD8 p-value =.0.002

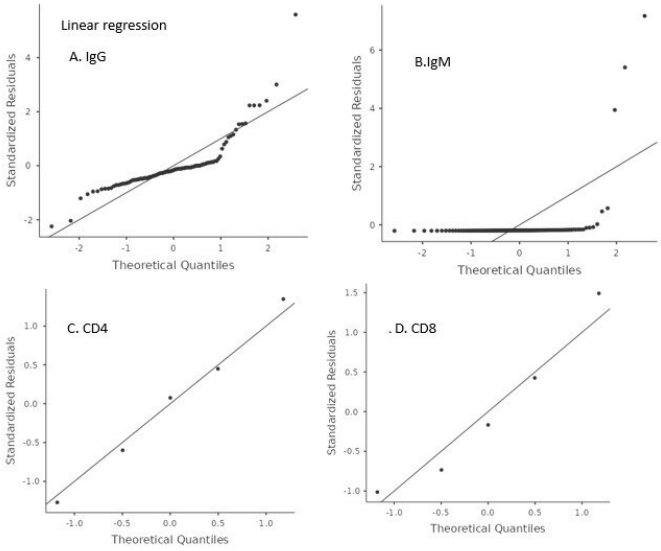

Figure 2, A/ shows Linear regression analysis between serum zinc levels and IgG concentrations B/ shows the linear regression between zinc levels and IgM. C/ shows the linear regression between zinc and CD4, and D/ shows the linear regression between zinc level and CD8.

Table 3. shows the statistical data (T-Test, correlation matrix, and linear regression) between zinc and immune cells.

Correlation	T-test p-value	Correlation matrix	Linear regression	
Zinc and IgG	<0.001	R=0.461	R=0.461	AIC= 1426
			R^2= 0.212	BIC=1434
		P= <0.001	P= >0.005	
Zinc and IgM	0.596	R=0.009	R=0.0088	AIC=1056
			R^2= 0.000078	
		P= 0.930	P= 0.931	BIC=1064
zinc and CD4	…….	R=0.943	R=0.0045	
		P=0.016	R^2= 0.000845	……
			P= 0.001	
zinc and CD8	……..	R=0.987	R=0.0079	
		P=0.002	R^2= 0.000467	……..
			P= 0.032	

DISCUSSION

In this study, 102 samples were analyzed, and the mean levels of IgG, IgM, and zinc were 134 IU/mL, 8.58 IU/mL, and 92.1 µg/dL, respectively; in addition, the median levels were of IgG, IgM, and zinc were 0.456 IU/mL, 0.206 IU/mL and 88.9 for zinc level. For the standard deviation of IgG = 290, IgM = 45, and zinc levels = 22.7. In general, the median was lower, which reflects a deviation in the distribution, which means a very small population had increased antibody responses (Table 2). This pattern is common in groups with predominantly seronegative or latent infections, where a small fraction has current or recent immunity to Toxoplasma gondii. The zinc level ranged from 54 to 168 µg/dL. As zinc is a very important component in enhancing humoral and cellular immunity, especially T and B lymphocytes. This variation could affect individual immune status. The wide range of observed IgG and zinc levels points to a possible biological relationship between immunological response and zinc status(14-16). Furthermore, scatter plot analysis showed a very strong relationship between zinc levels and IgG levels. This finding would suggest a connection between higher zinc levels and stronger IgG-mediated immunity against Toxoplasma gondii(17). However, there were no relationships detected between zinc and IgM, $CD4^+$, or $CD8^+$ levels(18). This suggests that zinc is unlikely to have a significant impact on acute-phase antibody levels or T-cell subpopulation counts in this population, which is consistent with the findings of this study. These findings support the theory that zinc does not impact every component of the immune system and instead selectively increases humoral immune memory (IgG)(19). According to these results, zinc selectively boosts humoral immune memory (IgG) rather than affecting all immune system components (Figure 1).An independent t-test was performed to confirm the relationship between zinc levels and IgG Participants with

high and low IgG levels had significantly different zinc levels ($p < 0.001$), supporting the scatter plot's results The idea that zinc status may affect or reflect the strength of long-term humoral immunity against exposure to Toxoplasma gondii is supported by the finding that rats with elevated IgG levels also have significantly higher zinc levels(20). On the other hand, the analysis showed no significant differences between zinc levels and IgM concentration ($p=0.596$), which would suggest that zinc is not responsible for the acute phase antibody response within this study. These results support the idea that IgG represents a longer-lasting, more stable immunological memory that may be maintained by micronutrients like zinc, while IgM is more likely to be temporary and less reliant on nutrition(21) (Table 3). The correlation matrix test revealed some associations between immunological markers and zinc concentrations. Zinc concentrations and IgG showed a substantial positive association ($r = 0.461$, $p <.001$) according to the test, but zinc levels did not significantly correlate with IgM ($r = -0.009$, $p = 0.930$), suggesting that zinc may not play a significant role in the acute phase antibody response. In addition, significant negative associations between zinc levels and CD4 ($r = -0.943$, $p = 0.016$) and CD8 ($r = -0.987$, $p = 0.002$) T-cell counts were discovered within cellular immunological markers, this result stands against other studies, which suggest that zinc is related to increasing CD4 and CD8 cells(22). This inverse correlation between zinc and CD4, CD8 is due to the small sample size (n=5), this low sample size restricts the validity of the results. Furthermore, rather than distinct actions by zinc, the highly significant correlation between CD4 and CD8 ($r = 0.983$, $p = 0.003$) may suggest a synergistic immunoregulatory mechanism. (Table 3). Due to this inverse relation between zinc and CD4, CD8, this needs more study on a larger sample size, because our findings are against the hypothesis of other studies that suggest zinc increases levels of IgG, CD4, and CD8, which means zinc affects not only humoral immunity but also increases cell-mediated immunity(23-25). Serum zinc levels and IgG levels were found to be significantly correlated by linear regression analysis. With a statistically significant p-value <.001 and a moderate effect size ($R = 0.461$, $R2 = 0.212$), the model indicates that zinc concentration accounts for roughly 21.2% of the variability in IgG levels. The AIC (1426) and BIC (1434) results indicated that the model fit was considered adequate. This study's results provide credence to the idea that zinc maintains humoral immune responses, specifically IgG synthesis, In those who have been exposed to Toxoplasma gondii. In contrast, linear regression analysis between zinc levels and IgM showed an insignificant relationship ($R = 0.0088$, $R^2 = 0.000078$, $p = 0.930$), with considerably poorer indicators for model fit (AIC = 1056, BIC = 1064) (Figure 2) (Table 3). According to this study, zinc does not affect the acute-phase antibody response, which is the production of IgM, but it may have an impact on long-term immunity, IgG. These opposing effects demonstrate zinc's selective immunomodulatory role, which is in immunological memory preservation rather than the induction of early immune responses.

CONCLUSION

The study found a favorable relationship between serum zinc levels and IgG, suggesting that zinc may be responsible for the improvement of humoral immunity over the long term in people who had previously been exposed to Toxoplasma gondii. The lack of a link between zinc and IgM levels indicates that zinc's role in acute-phase antibody responses is restricted. Zinc levels were also found to have inverse correlations with T-cell markers (CD4 and CD8); however, due to the limited number of samples examined for cell-mediated immunity, these results should be interpreted cautiously. The limited sample size would have diminished the results' validity and statistical power, underscoring the need for more extensive data to draw definitive conclusions on zinc's effect on cellular immune function.

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Authors' Declaration

Conflicts of Interest: None. We confirm that all the figures and tables in the manuscript are ours. No animal studies are present in the manuscript. No human studies are present in the manuscript. The ethical approval was agreed by the ethics committee of Cihan university under the approval number CUE-REC:2025-3. All the samples used in this study were collected with prior informed consent to ensure patient confidentiality.

REFERENCES.

1. Saleh MA. Determination of antibodies (IgG, IgM) against Toxoplasma gondii in some Iraqi individuals by using ELISA technique. Baghdad Science Journal. 2011;8(4):11.
2. AL-Mossawei MT, AL-Mossawei HM, AL-Dujaily KY. Serological study of toxoplasmosis spread among unmarried female university students using LAT, ELISA and IgG avidity. Baghdad Science Journal. 2016;13(4):0714-.
3. Al-Hassnawi ATS, Al-Morshidy KAH, Al-Harbi NY. Milk tumor necrosis factor alpha and interleukin-1beta among Toxoplasma gondii-free and infected women. Baghdad Science Journal. 2022;19(1):3.
4. Khalil Baqer S, Ali Ameen Shwan N. PPARG and FTO gene variants and their association with type 2 diabetes in the Kurdish population. Passer Journal of Basic and Applied Sciences. 2023;5:171-7.

5. Ahmed ZaA, Idan EM, Ardalan NM. Role of IL-37 and Dectin-1 during Toxoplasmosis. Baghdad Science Journal. 2023;20(3):24.

6. Saheb EJ, Al-Issa YA, Mussa IS, Zghair KH. Incidence of toxoplasmosis in psoriasis patients and possible correlation with tumor necrosis factor-α. Baghdad Science Journal. 2020;17(1 (Suppl.)):0214-.

7. Salah HA, Ahmed AS. Coronavirus disease diagnosis, care and prevention (COVID-19) based on decision support system. Baghdad Science Journal. 2021;18(3):23.

8. Maywald M, Rink L. Zinc in human health and infectious diseases. Biomolecules. 2022;12(12):1748.

9. Costa MI, Sarmento-Ribeiro AB, Gonçalves AC. Zinc: from biological functions to therapeutic potential. International Journal of Molecular Sciences. 2023;24(5):4822.

10. Sarhan MH, Felemban SG, Alelwani W, Sharaf HM, Abd El-Latif YA, Elgazzar E, et al. Zinc oxide and magnesium-doped zinc oxide nanoparticles ameliorate murine chronic toxoplasmosis. Pharmaceuticals. 2024;17(1):113.

11. Baghdadi HB, Albalawi AE, Shater AF, Almohammed H, Alanazi AD. Linalool-zinc oxide nanocomposite controls Toxoplasma gondii infection through inhibiting inflammation, oxidative stress, and pathogenicity. Journal of Basic Microbiology. 2024;64(8):e2400039.

12. Hatem Hameedi Z, Kesmati M, Hammadi Muttlaq Alawadi HM, Shafiei M. Evaluation of Age, Education, Trace Elements and Vitamins in Men with Toxoplasma Gondii in Maysan, Iraq. Journal of Advanced Biomedical Sciences. 2024;14(4):274-81.

13. de Souza RS, Sequeira CA, Borges EM. Enhancing statistical education in chemistry and STEAM using JAMOVI. Part 1: descriptive statistics and comparing independent groups. Journal of Chemical Education. 2024;101(11):5027-39.

14. El-Kady AM, S. Hassan A, Mohamed K, Alfaifi MS, Elshazly H, Alamri ZZ, et al. Zinc oxide nanoparticles produced by Zingiber officinale ameliorates acute toxoplasmosis-induced pathological and biochemical alterations and reduced parasite burden in mice model. PLOS Neglected Tropical Diseases. 2023;17(7):e0011447.

15. Szewczyk-Golec K, Pawłowska M, Wesołowski R, Wróblewski M, Mila-Kierzenkowska C. Oxidative stress as a possible target in the treatment of toxoplasmosis: perspectives and ambiguities. International journal of molecular sciences. 2021;22(11):5705.

16. Alanazi AD, Alghabban AJ. Therapeutic effects of zinc nanoparticles green synthesized by Ferula macercolea extract against chronic toxoplasmosis in mice. Journal of Global Antimicrobial Resistance. 2025;42:195-203.

17. Babekir A, Mostafa S, Obeng-Gyasi E. The association of Toxoplasma gondii IgG antibody and chronic kidney disease biomarkers. Microorganisms. 2022;10(1):115.

18. Fang EE, Nyasa RB, Ndi EM, Zofou D, Kwenti TE, Lepezeu EP, et al. Investigating the risk factors for seroprevalence and the correlation between CD4+ T-cell count and humoral antibody responses to Toxoplasma gondii infection amongst HIV patients in the Bamenda Health District, Cameroon. PLoS One. 2021;16(12):e0256947.

19. Kazemi Arababadi M, Abdollahi SH, Ramezani M, Zare-Bidaki M. A review of immunological and neuropsychobehavioral effects of latent toxoplasmosis on humans. Parasite Immunology. 2024;46(7):e13060.

20. Caudet J, Trelis M, Cifre S, Tapia G, Soriano JM, Rodrigo R, Merino-Torres JF. Do Intestinal unicellular parasites have a role in the inflammatory and redox status among the severely obese? Antioxidants. 2022;11(11):2090.

21. Isreal O, Alonge M. Maternal Immunity in Response to Toxoplasmosis: Implications for Pregnancy Loss and Fetal Health in Thi-Qar Province.

22. Marhoon S, Hamad SS. Evaluation of some immunological and physiological parameters in patients with chronic kidney disease infected with Toxoplasma gondii. Journal Port Science Research. 2025;8(1):. 36-40.

23. Khalaf A. The effect of infection with Toxoplasma gondii in inducing interferon-gamma in breast cancer patients. Archives of Razi Institute. 2024;79(1):138.

24. Arafa FM, Mogahed NM, Eltarahony MM, Diab RG. Biogenic selenium nanoparticles: trace element with promising anti-toxoplasma effect. Pathogens and Global Health. 2023;117(7):639-54.

25. El Shanawany EE, Abdel-Rahman EH, Nemr WA, Hassan SE, Hassan NM, Desouky HM, et al. Comparative evaluation of live attenuated and killed tachyzoites as vaccine candidates for toxoplasmosis. AMB Express. 2025;15(1):102.