Annals of R.S.C.B., ISSN:1583-6258, Vol.28, Issue 1, 2024, Pages. 179 - 185
Received 15 February 2024; Accepted 30March 2024.

# Biochemical changes in thalassemia patients infected with *Toxoplasma gondii*

**May Naji Alkhanaq [1]*, Ikhlas Abbas Marhoon [2], Shifaa Ali Abdulmohsin Al-Hachami [3], Maryam Mohsin Naser [4]**

[1] Department of Biology, College of Science, University of Wasit, Wasit, Iraq
[2] Department of Biology, College of Education, University of Al-Qadisiyah, Al-Qadisiyah, Iraq
[3] Department of Biology, College of Education for Pure Sciences, University of Wasit, Wasit, Iraq
[4] Ministry of Health, Wasit Health Department, Wasit, Iraq
* Corresponding author

## Abstract

The current study aimed to determine the prevalence rate of *Toxoplasma gondii* infection in Betathalassemia Major Patients and detection the effect of toxoplasmosis on biochemical changes in patients, in Wasit province. Serum samples from two groups were patients (175 people with beta-thalassemia) and control (35 healthy individuals), were obtained and used to test for anti *T. gondii* (IgM and IgG) antibodies using the ELISA technique. Then, in *T. gondii* seropositive patients and controls, the levels of (T3,T4, TSH) were determined using the Mini Vidas technology and urea, creatinine, ALP, ALT, AST using the Cobas c111 technique. According to the findings, the patient had the percentages of anti-*T. gondii* IgM and IgG antibodies were 2.28% and 10.85%, respectively. Additionally, the concentrations of T3, T4, TSH, urea, creatinine, ALP, ALT and AST respectively in *T. gondii* seropositive patients were 2.15 mlU/ml, 101.27 nmol/L, 4.70 µIU/ml, 23.95 mg/dL, 0.37 mg/dL, 181.22U/L, 40.18U/L and 48.73U/L; while in control, they were 9.72 mlU/ml, 11.14 nmol/L, 7.17 µIU/ml, 4.40 mg/dL,-20.13 mg/dL, 9.47 U/L, 0.15U/L and 1.30U/L. In conclusion, statistical analysis results suggest that high significant differences between patients and control individuals.

**Keywords:** Beta-thalassemia, *T. gondii*, ELISA, Mini Vidas, Cobas c111

## Introduction

One of the most prevalent epidemic diseases in the world, beta-thalassemia has a multitude of hemoglobin synthesis abnormalities, including those brought on by a decreased globin protein production. There are three different forms of thalassemia that can be distinguished clinically: major, minor, and intermediatethalassemia [1,2,3]. Patients with β -thalassemia major recurrently get blood transfusions for their treatment, and each one ml of bursting red cells increases the body's iron overload by one mg. Patients develop iron overload as a result of excessive number of blood transfusion [4]. The transfusion-transmitted an infection problem which is proportional to the rate of infection in the blood donors as well, may lead to an increase of morbidity and mortality rate among patients with thalassemia such as Toxoplasmosis caused by *T. gondii,* has been described as a very important obligatory intra-cellular protozoan parasitic organism in humans and veterinary animals, classified in the phylum Apicomplexan [5,6]. According to serologic research, a one-third of the world's population is infected by *T. gondii*, which is one of most common pathogens of infection in humans [7]. Tachyzoite, bradyzoite, and sporozoite are stages of the *T. gondii* life cycle that can infect cells: these are exclusively formed in the final host during sexual reproduction and

Annals of R.S.C.B., ISSN:1583-6258, Vol.28, Issue 1, 2024, Pages. 179 - 185
Received 15 February 2024; Accepted 30March 2024.

are released in the oocysts by felid feces [8]. The parasite *T. gondii* is spread through contact with cats and their excrement, eating undercooked meat, consuming unpasteurized milk, blood transfusion, and organ transplantation and can danger in immune compromised people [9, 10, 11].

Thalassemia patient has a higher risk of major opportunities for infections like toxoplasmosis due to a fundamental defect in the host defense, and this risk may be related to chronic immunological stimulation from recurrent blood transfusions, iron overload, splenectomy, and immune deficit [12, 13].

Many researches have been conducted on the various clinical manifestations of the diseases, including those on the relationship between toxoplasmosis and hepatomegaly and certain abnormalities of liver function, also the effect of infection in the kidney function [14]. However, *T. gondii* examination program prior to blood transfusion has not yet been taken into consideration, additionally, children with various blood disorders such thalassemia will have a compromised immune system, making them more vulnerable to opportunistic *T. gondii* infection [15]. Three various complications caused by this disease including growth retardation, endocrine dysfunction hypothyroidism, [16] progressive liver failure and abnormal kidney function [17].

Thyroxine (T4) and triiodothyonine (T3) are two essential thyroid hormones that the thyroid gland secretes into the bloodstream [18]. Iodine and tyrosine are used to make T3 and T4, which promote optimal growth, development, function, and preservation of all body tissues. Thyroid-Stimulating Hormone (TSH), a hormone released by the pituitary gland, influences the synthesis and secretion of these hormones [19].

There are many different systemic disorders that can have an impact on the pituitary gland, In some cases, a disease process that affects other organs, such as an inflammatory, autoimmune, and infectious disorder, may directly impact the pituitary, This could lead to altered pituitary function with or without obvious structural alterations to the gland, Other times, the main illness process has remote or indirect effects on how the hypothalamus and pituitary glands produce hormones [20].

The donors in the Iraqi centers of blood transfusion aren't screening to *T. gondii* . The aim of this study was the serological evaluation of Toxoplasmosis infection in thalassemia major patientsand detection the effect of toxoplasmosis infection on biochemical changes in patients, in Wasit province.

## Materials and methods

### *Design of Experiment*

In this study, 175 Beta-thalassemia patients of both sexes (males and females), ranging in age from 1 to 78 years, were enrolled in the Thalassemia Center at the Al-Kut hospital for Pediatrics & Gynecology Obstetrics. A control group of 35 healthy people in the same age range and of both sexes was also included.

### *Collection of Samples*

Five ml of venous blood from patients and control were collected, put in a gel tube, and spun down to generate a serum that was then stored at -20°C until examination was done [21].

Annals of R.S.C.B., ISSN:1583-6258, Vol.28, Issue 1, 2024, Pages. 179 - 185
Received 15 February 2024; Accepted 30March 2024.

***Examination of T. gondii antibodies***

ELISA tests and the Toxo-IgG Kit and Toxo-IgM Kit (Human, Germany firm) were used to test serum samples from 175 patients for anti-*T. gondii* IgM and IgG antibodies. Both assays for IgG and IgM were carried out in accordance with the manufacturer's instructions. The ELISA reader (optical absorbance, OD = 450) recorded the final data.

***Detection the levels of some Biochemical Parameters***

Serum samples from 23 *T. gondii* seropositive patients and 35 from control were tested for T3, T4, TSH using the Mini Vidas technique (Marcy-l' Étoile, French company) and (Urea, Creatinine, ALP, ALT, AST) by using Cobas c111 technique(Roche®, Germany company) were carried out in accordance with the manufacturer's instructions **StatisticalAnalysis using** SPSS computer software (version 26) was used to analyze the data for findings percentages (%) and numbers, also the T-test was used to compare the percentages. Statistics were considered significant for P values less than 0.05.

## Results and Dissection

### Seroprevalence of anti-*Toxoplasma* IgM and IgG antibodies

According to the findings of the current study, the overall prevalence of toxoplasmosis infection was 13%; this included anti-*T. gondii* IgM were 4/23 (2.28%) and anti-*T. gondii* IgG were 10.82% antibodies in patients samples by using ELISA test as shown in table (1)

**Table 1: Seroprevalence of anti-*Toxoplasma* IgM and IgG antibodies by Using ELISA Test**

| Antibodies | Patients (No.=23) | |
|---|---|---|
| | No. | % |
| **IgM** | 4 | 2.28 |
| **IgG** | 19 | 10.85 |
| **Total (IgM and IgG)** | 23 | 13.12 |

The results of current study agree with the results of other researchers [22] in Iran, which record the prevalence of *Toxoplsma*-IgG, and *Toxoplsma*-IgM antibodies in patients with thalassemia were 16%, and 0%, respectively. Also, results of current study agree with the results of other study [23] in Aydin's province of turkey, which record that *Toxoplsma*-IgG antibody were 19.4% in beta-thalassemia major patients whereas, *Toxoplsma*-IgM antibody were 5.5%.

Thalassemic patients may have higher anti-*Toxoplasma*IgM and IgG antibody titers than the control group in this study because of the frequent blood transfusions they receive, which puts them at higher risk for contracting the parasite than healthy people [21]. Toxoplasmosis is a serious problem because it has long been recognized as an opportunistic infection in immunocompromised patients and as the third leading cause of death in AIDS patients after *Pneumocystis* and *Cryptosporidium* [24, 25].

Annals of R.S.C.B., ISSN:1583-6258, Vol.28, Issue 1, 2024, Pages. 179 - 185
Received 15 February 2024; Accepted 30March 2024.

**Detection the levels of T3, T4 and TSHin *T.gondii* Seropositive Patients and Control**

According to the findings of the current study, patients with *T. gondii*seropositivity had a total rate of T3, T4 and TSH using the Mini Vidas technique 2.15 mlU/ml, 101.27 nmol/L, 4.70 µIU/ml), compared to 9.72 mlU/ml, 11.14 nmol/l,-7.17 µIU/ml)in control serum samples. The statistical analysis revealed significant variations between patient and control groups, as shown in table (2).

**Table 2: T3, T4 and TSH concentration in T.gondii Seropositive Patients and Control**

| Parameters | Patients | Control |
|---|---|---|
| T3 (mlU/ml) | 2.15 | 9.72 |
| T4 (nmol/l) | 101.27 | 11.14 |
| TSH (µIU/ml) | 4.70 | -7.17 |

This finding demonstrated how toxoplasmosis affects thyroid hormones, High levels of TSH and T3 were seen, and patients with toxoplasmosis who experience a drop in serum T4 may be suffering from primary thyroid dysfunction.Whenever *T. gondii* enters the hypothalamus, it causes an alteration in the region's stimulation, which distracts the release of TSH and causes aberrant T3 and T4 produces [26, 27]. T3 hormone is releases to the blood stream as a result of neurological stimulation of the hypothalamus-pituitary thyroid axis brought on by *T. gondii* [28]. In addition to the fact that parasites can exacerbate thyroid issues, some individuals with thyroid and autoimmune thyroid disorders also have parasitic infections [29]. According to [30], the thyroid gland helps the body regulate both growth and the rate of chemical processes.

Due to *T. gondii'*s direct involvement in the thyroid gland, its multiplication and propagation in thyroid tissue changes, and the ensuing alteration of thyroid hormones, there is a strong correlation between thyroid gland destruction and the infection by *Toxoplasma* [31].

**Detection the levels of Urea and Creatinine in T.gondii Seropositive Patients and Control**

According to the findings of the current study, serum samples from *T. gondii* seropositive patients were found to have a total rate of Urea and Creatinine was 23.95, 0.37 mg/dL, compared to 4.04, 20.13 mg/dL in serum samples from controls. Table 3 shows the statistically analyzed results, which show significant variations between the patient and control groups.

**Table 3: Urea and creatinine concentration in *T.gondii* Seropositive Patients and Control**

| Parameters | Patients (mg/dL) | Control (mg/dL) |
|---|---|---|
| Urea | 23.95 | 4.04 |
| Creatinine | 0.37 | -20.13 |

The findings of the present research agree with those of [5] in AlSamawah province, which record that urea creatinine in thalassemia patients with toxoplasmosis (13.49 ± 8.183 mg/dl) and (0.66 ± 0.2608 mg/dl), whereas control group were 10.42 ± 4.573 mg/dl and 0.56 ± 1.0134 mg/dl. This study showed increase in both urea and creatinine levels in patients

compared with control group, The *T. gondii* parasite produces glomerular damage and urinary irregularities that result in failure of the kidneys, which may be the reason of the rise in urea and creatinine rates in patients.

**Detection the levels of ALP, ALT and AST in T.gondii Seropositive Patients and Control**

According to the findings of the current study, serum samples from *T. gondii* seropositive patients were found to have a total rate of ALP, ALT and AST using the Cobas c111 technique were 181.22, 40.18 and 48.73 U/L; whereas, they were 9.47, 0.15, 1.30)U/L in control serum samples, Table (4) shows the statistically analyzed results, which show significant variations between the patient and control group.

**Table 4: Liver enzymes activity in *T.gondii* Seropositive Patients and Control**

| Parameters | Patients (U/L) | Control (U/L) |
|---|---|---|
| ALP | 181.22 | 9.47 |
| ALT | 40.18 | 0.15 |
| AST | 48.73 | 1.30 |

The current study therefore concentrated on measuring the levels of some enzymes in Beta thalassemia patients, These included ALT, ALP and AST which present in liver cells, because it is a biomarker of functioning of the liver. The findings of the present research agree with those of [5]in Al-Samawah province, which record that AST and ALT in thalassemia patients with toxoplasmosis were (77.9 ±3.017IU/L ) and (89.36 ± 6.377IU/L),whereas control group were (70.04± 1.291IU/L) and (23.28 ± 5.447IU/L).

The biological functions of every living creature are controlled by enzymes, because the quantity of a particular enzyme differs from tissue to tissue, any harm to these tissues results in the leakage of their constituent parts into the circulatory system, increasing the levels of those constituent parts in blood serum [32, 33]. According to [34], *T. gondii* infection causes severe and progressive liver damage. Patients with *T. gondii* infections had higher serum levels of the liver enzymes AST and ALT, which are excellent indicators of hepatocellular damage [22].

**References**

1. Rund, D. and Rachmilewitz, E. 2005. "β-thalassemia". N. Engl. J. Med., 353:1135- 46.
2. Anchalee, T., Sumalee J., Suporn, C. and Punnee, B. 2012."Multiplex ARMS-PCR Analysis for Nineteen b-Thalassemia Mutations". J HematolTransfus Med, 22: 31-40.
3. Ali, S., Mumtaz, S., Shakir, H. A., Khan, M., Tahir, H. M., Mumtaz, S., & Khan, M. A. (2021). Current status of beta-thalassemia and its treatment strategies. Molecular Genetics & Genomic Medicine, 9(12), e1788.
4. Ali, B. A., & Mahmoud, A. M. (2014). Frequency of glomerular dysfunction in children with beta thalassaemia major. Sultan Qaboos University Medical Journal, 14(1), e88

5. Mousa, N. M., &Nahab, H. M. (2020). Serological detection and hematological changes associated with Toxoplasmosis in thalassemia patients in Al-Samawah province. International Journal of Pharmaceutical Research, 12(4).
6. Al-Hadraawy,S.K., Alzeyadi, M., Shakir,A.,and Al-dujaili,A. (2019). Detection of *Toxoplasma gondii* genotypes in abortion women by RFLP-PCR in Al-Najaf Al-Ashraf province. IOP Conf. Series: JPhy, 5: 1-12.
7. Tenter, A.; Heckeroth, A. and Weiss, L. (2000). *Toxoplasma gondii*: from animals to humans. Int J parasitol; 30: 1217 – 1258.
8. Attias, M., Teixeira, D. E., Benchimol, M., Vommaro, R. C., Crepaldi, P. H., & De Souza, W. (2020). The life-cycle of *Toxoplasmagondii*reviewed using animations. Parasites & vectors, 13(1), 588.
9. Fromont EG, Riche B, Rabilloud M. *Toxoplasma*seroprevalence in a rural population in France: detection of a household effect. *BMC infectious diseases.* 2009; 9(1):76.
10. Shin D-W, Cha D-Y, Hua QJ, Cha G-H, Lee Y-H. Seroprevalence of *Toxoplasma gondii* infection and characteristics of seropositive patients in general hospitals in Daejeon, Korea. *The Korean journal of parasitology.* 2009; 47(2):125–30.
11. Xiao Y, Yin J, Jiang N, Xiang M, Hao L, Lu H, *et al*. Seroepidemiology of human *Toxoplasma gondii*infection in China. *BMC infectious diseases.* 2010; 10(1):4.
12. Ferreira MN, Bonini-Domingos CR, Fonseca Estevão I *et al*. Anti-*Toxoplasmagondii* antibodies in patients with beta-hemoglobinopathies: the first report in the Americas. BMC Res Notes. 2017; 10(1):211.
13. Ghaffari J, Vahidshahi K, Kosaryan M *et al*. Humeral immune system state in β thalassemia major. Med Glas (Zenica). 2011; 8(2): 192-6.
14. Flegr J, Preiss M, Klose J, Havlıček J, Vitáková M, and Kodym P. Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite *Toxoplasmagondii* Dopamine, a missing link between schizophrenia and toxoplasmosis Biological psychology. 2003; 63(3):253-68.
15. Elmore SA, Jones JL, Conrad PA, Patton S, Lindsay DS, and Dubey J. *Toxoplasmagondii*: epidemiology, feline clinical aspects, and prevention. Trends in parasitology. 2010; 26(4):190-6.
16. Hokeleck, M.; Kiling, M.; Erturt, M. and Yyar, Y. (2001). Production of *Toxoplasmagondii* in human amnion cell cultures. Turt. Parasite. Derg; 25(4):323-325.
17. Henry L. and I. Davidson. (1974). Clinical diagnosis by lab. Methods, Saunders Company. Landon.
18. Boelaert K. & Franklyn J. A. “Thyroid hormone in health and disease”. J. Endocrinol. 187, 1, 2005.
19. Iglesias P. and Diez J. J. “Thyroid dysfunction and kidney disease. European Journal of Endocrinology”. 160(4), 503- 515, 2009.
20. Marques, P., &Korbonits, M. (2022). Pituitary Dysfunction in Systemic Disorders. In *The Pituitary* Content Repository Only. (pp. 385-412).
21. Hanifehpour, H., Shariat, S. K. S., Ghafari, M. S., Kheirandish, F., Saber, V., &Fallahi, S. (2019). Serological and molecular diagnosis of *Toxoplasmagondii* infections in thalassemia patients. *Iranian Journal of Parasitology*, *14*(1), 20.

22. Moghimi, M., Doosti, M., Vahedian-Ardakani, H. A., Talebi, A., Akhavan-Ghalibaf, M., Najafi, A., Aminorroaya, M. M., Yazdani, S.h, Shayestehpour, M., Bahrami, H., &Khodayari, F. (2015). Serological Study on Cytomegalovirus and *Toxoplasma gondii* in Thalassemia Major Patients of Yazd, Iran. *Iranian journal of pediatric hematology and oncology*, *5*(3), 149–154.
23. Karakas S, Ozlem S, Tellioglu AM, Ertabaklar H, Ertug S. (2012). Investigation of Anti *Toxoplasma gondii*IgG and IgM Antibodies in Beta Thalaseamia Major Patients in Aydin Province. TürkiyeParazitolojiiDergisi, 36(3): 133-6.
24. Dubey JP. Toxoplasmosis of animals and humans, 2nd ed CRC Press, Boca Raton, Florida: 2010; 313.
25. Rashno M.M., FallahiSh, Kheirandish F, et al. Seroeprevalence of Toxoplasma gondii infection in patients with Alzheimer's disease. Arch Clin Infect Dis. 2016; 11:e37205.
26. Ain K. B., Mori Y. &Refetoff, S. “Reduced clearance rate of thyroxinbinding globulin (TBG) with increased sialylation, a mechanism for estrogeninduced elevation of serum TBG concentration”. J. Clin. Endocrinol. Metab. 65, 689, 1987.
27. Al-Terihy F. A., Shemran K., AbdulsamieAltaee A. H. &Jabuk, Sh. K. “The association between thyroid hormones and lipid profile in patients with primary hypothyroidism”. Med. J. Babylon. 9(4), 721-727, 2012.
28. Razooqi, M. A., Gharban, H. A., and Al-Kaabi, M. A. (2022). Molecular and Seroprevalence of Toxoplasmosis in Goats’ Blood and Milk in Iraq. *Archives of Razi Institute*, *77*(5), 1749-1755.
29. Thierry Hertoghe M. D. “The Hormone Solution, Stay Younger Longer With Natural Hormone & Nutrition Therapies”. Three Rivers Press. New York. 170, 2002.
30. Melmed S., Polonsk, K. S., Larsen P. R. &Kronenberg H. M. “Williams Textbook of Endocrinology”. 12th ed. Saunders. 331, 2011.
31. Salman Y. “Serological cross reaction among some causative agents of women (*Toxoplasma gondii*& Cytomegalovirus & Rubella virus), with the incidence of Hepatitis virus (B&C)”. Tik J Pharm Sci. 3, 102-111, 2007.
32. Murry, R., Bender, D., Botham, K., Kennelly, P., Rodweel, V. and Weil, P. 2012."Harper's Illustrated Biochemistry". 29 th ed., a Lang medical book/ McGraw - Hill Companies, New York.
33. Nelson, D. L. and Cox, M. M. 2013."Lehninger, Principles of Biochemistry". 6 th ed., W. H. Freeman and Company, New York.
34. Flori P, Bellete B, Crampe C, *et al*. A technique for dating toxoplasmosis in mpregnancy and comparison with the Vidas anti-*Toxoplasma* IgG avidity test. ClinMicrobiol Infect. 2008; 14:242– 249.