

Physiological Characterization for Autoimmune Thyroiditis Patients with Latent Toxoplasmosis

Akram Hadi Haddad ¹, Amal Khudair Khalaf ¹ and Dheyaa Kadhim Al-Waeli ²

akram-h22@utq.edu.iq

aml-kh@utq.edu.iq¹

1. Department of Microbiology / College of Medicine / University of Thi-Qar / Iraq

2. Department of internal medicine /College of Medicine /University of Thi-Qar / Iraq

Abstract: The most prevalent parasite infection in worldwide is toxoplasmosis that associated with a number of problems such as thyroid diseases. The aim of the following study was to investigate relationship between basophil and eosinophil in patients with autoimmune thyroid diseases (AITD) who had *T.gondii* and estimation its association body mass index. The following study was included 100 patients with (AITD) and 70 as healthy control whom entered Thi-Qar specialized diabetes endocrine & metabolism center in Nasiriyah city, southern Iraq. The samples were collected during period extended from July to November (2023) and tested by using serological method (ELISA) to determine the presence of anti *T.gondii* (IgG) antibodies and complete blood count(CBC) and determine BMI of patients. The results showed 33% of (AITD) patients infected with *T. gondii* and did not show significant differences in basophil and eosinophil results and elevated of BMI in hashimotos patients while decreased in Gravis patients.

Keywords: Toxoplasmosis, Autoimmune thyroid disease, BMI

Introduction: Toxoplasmosis is parasitic protozoan that caused by *T. gondii*. It infect the warm-blooded animals [1]. *T. gondii* is spread through undercooked meat that contains tissue cysts, while oocysts shed and contaminate soil, water, vegetables, and other surfaces contaminated by an infected animal's excrement. Additionally, organ transplants, blood transfusions, and transplacental transfers between a mother and her fetus can also spread the infection. [2]. Due to *T. gondii* complex lifecycle, which includes producing sporozoites in feline epithelial intestinal cells through a sexual cycle and forming tachyzoites and bradyzoites through an asexual cycle that affects birds and mammals like humans, it is still a global threat to both human and animal health [3]. The thyroid gland, which secretes the thyroid hormones triiodothyronine (T3), tetraiodothyronine (T4), and calcitonin—which are essential for regulating metabolism as well as heart, brain, and bone functions—is one of the many organs and glands that *T. gondii* can attack [4]. Hypothyroidism and hyperthyroidism are two different forms of thyroid gland disorders hypothyroidism causes symptoms such as weight gain, sensitivity to cold, hair loss, and slow heart rates while hyperthyroidism causes symptoms such as sweating, anxiety, fatigue, and weight loss [5]. When there is an imbalance in immunological tolerance, the body's immune system attacks the thyroid gland and its hormones, disrupting the hormone system, autoimmune thyroid disease results from this. AITD causes the generation of specific autoantibodies that are directed against thyroid antigens that result disorders characterized by

hyper- or hypothyroidism are linked to these antibodies [6]. An example of AITD is Hashimoto's thyroiditis, a frequent cause of hypothyroidism mostly caused by T cell-mediated autoimmune responses. Alternatively, Graves' disease, an AITD, causes hyperthyroidism due to humoral autoimmune reactions. [7].

A few recent studies have demonstrated a specific correlation between *T. gondii* and AITD with greater autoantibody levels. AITD is also thought to be associated with a family history, other autoimmune illnesses, and molecular similarities between thyroid autoantigens and *T. gondii* pathogen components [8]. A white blood cell, especially eosinophils, is one type that supports the immune system. They are involved in the body's defensive response against allergies, parasites, and fungal infection. [9] Elevated eosinophil levels can be caused by many medications and disorders, whereas basophils are a type of leukocyte that works with the immune system to protect the body against infections, allergies, and parasites. In order to promote blood flow and prevent blood clots, basophils secrete enzymes.[10,11].

Eosinophils have long been linked to innate immunity, acting as a pro-inflammatory factor by defending against parasites. Moreover, eosinophils aid in the release of cationic granules that are produced from them, including neurotoxins, cationic proteins, and eosinophil peroxidase. These granules damage cells by adhering to charged cell membranes, breaking down the lipid bilayer, and changing how tissues' enzymes function [12,13].

Material and method:

Blood collection: 5 ml of blood collected from 100 patients with AITD and 70 healthy controls, drawn from the veins of patients at Thi-Qar specialized diabetes endocrine & metabolism center in Nasiriyah city, southern Iraq. during period extended from July to November (2023), whole blood placed in a tube containing ethylene diamine tetra acetic acid (EDTA), in order to use it for estimation of complete blood count (CBC) for detection basophil and eosinophil [14].

Estimation of eosinophil and basophil: Eosinophil and basophil detected in (AITD) patients and healthy control by using CBC kit from China (Genrui Biotech).

Results: The results of the current study showed 33% of (AITD) patients infected with *T.gondii* (IgG) and eosinophil and basophil in gravis patients were did showed a significant results as shown in table (1).

Table (1): Basophil and eosinophil among Graves' patients with latent toxoplasmosis (IgG)

Cases	Basophil($10^3/ \text{MI}$) Mean \pm SD	Eosinophil($10^3/ \text{MI}$) Mean \pm SD
Graves' Disease (+Toxo Igg)	0.52 \pm 0.04	3.9 \pm 0.86
Graves' Disease (-Toxo Igg)	0.4 \pm 0.02	2.60 \pm 0.28
Control (+Toxo Igg)	0.71 \pm 0.08	5.08 \pm 1.22
Control (-Toxo Igg)	0.39 \pm 0.03	2.65 \pm 0.28

$X^2 = 1.26$ df = 3 p-value=0.73 (Non-Significant differences $P > 0.05$)

Table (2) the level of basophil and eosinophil among Hashimoto's patients with and without *T.gondii*(IgG) compared to a control group. The statistical analysis did not revealed significant differences in level of basophil and eosinophil ($X^2 = 1.94$, p-value = 0.73).

Table (2): Basophil and eosinophil among Hashimoto's patients with latent toxoplasmosis (IgG)

Cases	Basophil($10^3/ \text{MI}$) Mean \pm Sd	Eosinophil($10^3/ \text{MI}$) Mean \pm Sd
Hashimoto's Disease (+Toxo Igg)	0.49 \pm 0.6	5.73 \pm 0.98
Hashimoto's Disease(-Toxo Igg)	0.31 \pm 0.04	2.28 \pm 0.22
Control (+Toxo Igg)	0.71 \pm 0.08	5.08 \pm 1.22
Control (-Toxo Igg)	0.39 \pm 0.03	2.65 \pm 0.28

$X^2 = 1.94$ df = 3 p-value=0.58 (Non-Significant differences $P > 0.05$)

The results of the current study showed an association between the body mass index of patients with Gravis disease and latent toxoplasmosis (IgG) when the body mass index of patients decreased with latent toxoplasmosis (18 ± 2.64) kg/m^2 when compared with control groups were (22.68 ± 3.82) kg/m^2 as it was listed in the following table:

Table (3): BMI among Gravis patients with latent toxoplasmosis (IgG)

Cases	BMI of Male(Kg/M^2) Mean \pm Sd	BMI of Female (Kg/M^2) Mean \pm Sd
Graves' Disease (Toxo+)	18 \pm 2.64	22.68 \pm 3.82
Graves' Disease (Toxo -)	20.06 \pm 3.64	25.81 \pm 4.48
Control (Toxo+)	22.13 \pm 4.22	23.91 \pm 2.86
Control(Toxo)	21.44 \pm 3.66	22.52 \pm 3.42

$X^2 = 28.65$ df = 3 p-value=0.00 (high significant difference $P \leq 0.05$)

The results showed significant differences ($X^2 = 34.15$, $p\text{-value}=0.00$) between the body mass index of Hashimoto's thyroiditis patients with and without latent toxoplasmosis (IgG) were (29.21 ± 6.82) and (29.66 ± 4.68) kg/m^2 respectively for male and female when compared with control groups (22.13 ± 4.22 , 23.91 ± 2.86) respectively. The following table showed the association between BMI of hashimotos patients with latent toxoplasmosis and control groups :

Table (4):BMI among Hashimoto's patients with latent toxoplasmosis(IgG)

Cases	Bmi Of Male (Kg/M ²) Mean± Sd	Bmi Of Female(Kg/M ²) Mean± Sd
Hashimoto's Disease (Toxo+)	29.21 ± 6.82	29.66± 4.68
Hashimoto's Disease (Toxo -)	29.3± 5.24	33.61± 6.42
Control (Toxo +)	22.13±4.22	23.91± 2.86
Control (Toxo -)	21.44±3.66	22.52± 3.42

$X^2 = 34.15$ df = 3 $p\text{-value}=0.00$ (high significant difference $P \leq 0.05$)

Discussion: One type of white blood cell that supports the immune system is the eosinophil. They are involved in the body's defensive response against allergies, parasites, and fungal infection it can be measured as a part of the complete blood count(CBC).This illness frequently suggests that parasites are present. [15]. The current study did not show significant differences of basophile counts in gravis patients compared with healthy control subjects. Basophils have been recognized as important players for protective immunity against a variety of different endo- and ecto parasites [16]. The current study revealed a slightly increases in eosinophil, may be due to the ability of the parasite to stimulate the host's humoral and cellular immune system. [15]. Eosinophil have long been associated with innate immunity helping to promote pro-inflammatory responses through anti-parasitic defense Furthermore, eosinophils contribute to the release of cationic granules derived from eosinophils, such as neurotoxins, cationic proteins, and eosinophil peroxidase, which cause harm to cells by attaching to charged cell membranes, disrupting the lipid bilayer and altering the function of tissues' enzymes [17,18].

This study disagree with study in Australia (2020) that showed lower eosinophil counts that detected in the seropositive subjects when compared to the seronegative subjects[19].

The current study did not show significant difference in the level of basophil and eosinophil parameters between infected Hashimotos patients and those who were free of infection in all patients. Eosinophils are an essential part of the innate and adaptive immune response[20]. Its plays a harmful and pro-inflammatory role in the Th2 immune response that triggered by parasitic infection. Additionally non-professional antigen-presenting cells in reaction to parasites or by directing and coordinating the actions of dendritic cells and T lymphocytes[18].

Eosinophils are effector cells that the body uses to fight against parasite infections; the specific function of these cells varies based on the type of parasite. The observed relationship between T.

gondii and eosinophil counts is not unexpected, as eosinophils are primarily thought to help cells in the body fight off parasitic infections[21].The innate immune response against parasites depends on eosinophils mainly because they secrete pro-inflammatory cytokines such as TNF α and IL-6 [22]

Basophil did not showed significant differences between *T.gondii* infection and hashimotos patients, these results agree with study by Petrasch *et al* (1993) in patients with Hashimoto's thyroiditis and explained that there are no differences in relative or total basophil counts between hypothyroidism patients and healthy controls[23].

BMI is a person's weight in kilograms divided by the square of height in meters. High BMI may be a sign of high body fatness. BMI searches for weight ranges that may be connected to health issues. Because thyroid hormones have significant impacts on the regulation of thermogenesis, glucose metabolism, and lipid metabolism, they play a major role in regulating the dynamic energy balance of mammals [24]. The effect of hypo- or hyperthyroidism on body weight was found to be that hypothyroidism's low thyroid hormone concentrations are associated with lower energy expenditure and fluid retention, while hyperthyroidism is typically linked to increased energy expenditure and weight loss [25].

Regarding to the role of BMI in Gravis patients shows an decrease mean of BMI in patients compared with healthy control with highly significant difference.

The current study agree with study in Iraqi by Lghewish *et al.* (2020) that determine the mean BMI of hyperthyroid patients was 22.51 kg/m² [27] while hyperthyroidism associated with underweight or weight loss[26].Because of increased basal metabolism and thermogenesis, increased adrenergic hyperstimulation, and increased total energy expenditure, people with hyperthyroidism are more likely to lose weight. Hyperthyroidism can also result in faster gastrointestinal transit and, occasionally, anorexia due to the anorexigenic effects of T₃[27].

Thyroid hormones affect basal metabolism, food intake, lipid and glucose metabolism, thermogenesis, and fat oxidation. Thyroid problems are therefore associated with variations in body temperature, body weight, resting energy expenditure, and overall energy expenditure, regardless of physical activity[28].

The data of current study showed a significant relationship between BMI and Hashimotos patients where the results explained an elevated mean of BMI in Hashimotos patients compared with healthy control .

The current study agree with study in Iraqi that achieved by Lghewish *et al.* (2020) that showed an increase in mean of BMI of hypothyroidism patients at 29.35 kg/m² [26].

The study by Doaa A. *et al* (2021) were aimed to study the role of latent *T. gondii* infection in the pathogenesis of metabolic syndrome in obese adolescents that explained the prevalence of metabolic syndrome was significantly higher within obese *T.gondii*-seropositive subjects compared to obese *T.gondii*-seronegative group[29]. Hypothyroidism is characterized by a lower

metabolic rate and impaired thermogenesis; it has also been connected to an increased body mass index and a higher prevalence of obesity. According to some clinical data, even moderate thyroid conditions, like subclinical hypothyroidism, can cause noticeable changes in body weight and increase the risk of obesity and overweight [30].

Conclusion: These findings indicated elevated BMI in patients with Hashimoto's thyroiditis and a decrease BMI in patients with Graves disease, while showing non-significant differences in levels of eosinophils and basophils for patients with AITD.

Acknowledgments: we express our gratitude to the personnel of the Thi-Qar specialist diabetes endocrine and metabolism center in Nasiriyah City for their cooperative efforts in sample collection.

Conflict of interest: No conflict of interest

References :

1. Martorelli Di Genova B, Wilson SK, Dubey JP, Knoll LJ. Intestinal delta-6-desaturase activity determines host range for *Toxoplasma* sexual reproduction. *PLoS biology*. 2019 Aug 20;17(8):e3000364.
2. Sakikawa M, Noda S, Hanaoka M, Nakayama H, Hojo S, Kakinoki S, Nakata M, Yasuda T, Ikenoue T, Kojima T. Anti-*Toxoplasma* antibody prevalence, primary infection rate, and risk factors in a study of toxoplasmosis in 4,466 pregnant women in Japan. *Clinical and Vaccine Immunology*. 2012 Mar;19(3):365-7
3. Dubey JP, Lindsay DS, Speer C. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clinical microbiology reviews*. 1998 Apr 1;11(2):267-99..
4. Pappachan JM, Buch HN. Endocrine hypertension: a practical approach. *Hypertension: from basic research to clinical practice*. 2017:215-37.
5. Wu F, Xu Y, Xia M, Ying G, Shou Z. Hookworm anemia in a peritoneal dialysis patient in China. *The Korean journal of parasitology*. 2016 Jun;54(3):315.
6. McLeod DS, Cooper DS. The incidence and prevalence of thyroid autoimmunity. *Endocrine*. 2012 Oct;42:252-65.
7. De Leo S, Pearce EN. Autoimmune thyroid disease during pregnancy. *The Lancet Diabetes & Endocrinology*. 2018 Jul 1;6(7):575-86.
8. Murad MA, Eassa SH. Detection of Toxoplasmosis in Association with Autoimmune Thyroid Disease During Pregnancy in Duhok, Iraq. *Journal of Pure & Applied Microbiology*. 2023 Jun 1;17(2).
9. Shapira Y, Agmon-Levin N, Selmi C, Petříková J, Barzilai O, Ram M, Bizzaro N, Valentini G, Matucci-Cerinic M, Anaya JM, Katz BS. Prevalence of anti-*Toxoplasma* antibodies in patients with autoimmune diseases. *Journal of autoimmunity*. 2012 Aug 1;39(1-2):112-6
10. Wendy Book, MD; Margaret Collins MD . eosinophil-associated disease; APFED.2022
11. William C Lloyd . Everything you need to know about basophils;Medically reviewed.2023
12. Corsi-Zuelli, F.; Marques, L.; da Roza, D.L.; Loureiro, C.M.; Shuhama, R.; Di Forti, M.; Del-Ben, C.M. The independent and combined effects of cannabis use and systemic inflammation during the early stages of psychosis: Exploring the two-hit hypothesis. *Psychol. Med.*2022, (52)3874–3884. .
13. Silver, J.S.; Stumhofer, J.S.; Passos, S.; Ernst, M.Hunter, C.A. IL-6 Mediates the Susceptibility of Glycoprotein 130 Hypermorphs to *Toxoplasma gondii*. *J. Immunol.*2011, (187) 350–360

14. Seydel G.S. , Guzelgu F. The effect of hemoglobin variants on high-performance liquid chromatography measurements of glycated hemoglobin. *Int J Med Biochem.*2021 ;4(1):25-28
15. Chen, H., Chen, G., Zheng, H. & Guo, H..Induction of immune responses in mice by vaccination with Liposome-entrapped DNA complexes encoding *Toxoplasma gondii* SAG1 and ROP1 genes. *Chin. Med. J.*2003, 116: 1561
16. Kang, H., Remington, J.S. & Suzuki, Y.Decreased resistance of B cell-deficient mice to infection with *Toxoplasma gondii* despite unimpaired expression of IFN γ , TNF- α , and inducible nitric oxide synthase. *J Immunol*, 2000. 164: 2629–2634.
17. Corsi-Zuelli, F.; Marques, L.; da Roza, D.L.; Loureiro, C.M.; Shuhama, R.; Di Forti, M.; Del-Ben, C.M. The independent and combined effects of cannabis use and systemic inflammation during the early stages of psychosis: Exploring the two-hit hypothesis. *Psychol. Med.*2022, (52)3874–3884. .
18. Silver, J.S.; Stumhofer, J.S.; Passos, S.; Ernst, M.Hunter, C.A. IL-6 Mediates the Susceptibility of Glycoprotein 130 Hypermorphs to *Toxoplasma gondii*. *J. Immunol.*2011, (187) 350–360
19. Aus Molan a, Kazunori Nosaka a, Michael Hunter b c, Wei Wang a . Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in a representative Australian human population:Elsevier.2020, (8) 808-814
20. Long, H.; Liao, W.; Wang, L.; Lu, Q. A Player and Coordinator The Versatile Roles of Eosinophils in the Immune System. *Transfus. Med. Hemotherapy.*2016, (43) 96–108
21. Kuang, F.L. Approach to Patients with Eosinophilia. *Med. Clin. N. Am.*2020, (104) 1–14.
22. Aceves, S.S.; Ackerman, S.J. Relationships Between Eosinophilic Inflammation, Tissue Remodeling, and Fibrosis in Eosinophilic Esophagitis. *Immunol. Allergy Clin. North. Am.*2009, (29) 197–211
23. S G Petrasch et al . Basophilic leukocytes in hypothyroidism, pubmed; .1993,71(1):27-30
24. Ranran Xu, Fei Huang, Shijie Zhang, Yongman Lv & Qingquan Liu. Thyroid function, body mass index, and metabolic risk markers in euthyroid adults, *BMC endocrine disorder*. 2019,(58)
25. Villabona C, Sahun M, Roca M, Mora J, Gomez N, Gomez JM, Puchal R, Soler J. Blood volumes and renal function in overt and subclinical primary hypothyroidism. *Am J Med Sci*. 1999;318:277–80
26. Lghewish, R. A., Alshibly, I. K., and Hasan, K. C. Role of Cytokines in The Pathogenesis of Thyroid Disease Among Iraq Patients. *Plant Archives.*2020, 20(2), 6101-6108.
27. Karmisholt, J., Andersen, S., and Laurberg, P. Weight loss after therapy of hypothyroidism is mainly caused by excretion of excess body water associated with myxoedema. *The Journal of Clinical Endocrinology and Metabolism.*2011,96(1), E99-E103.
28. Sanyal, D., and Raychaudhuri, M. . Hypothyroidism and obesity: An intriguing link. *Indian journal of endocrinology and metabolism.*2016, 20(4)
29. Doaa A Salem , Nanees A Salem , Shimaa R Hendawy . Association between *Toxoplasma gondii* infection and metabolic syndrome in obese adolescents: A possible immune-metabolic link;NIH.2021,10.1016
30. Abdulrazaq, H. Y., and Hussein, A. F. Obesity and Graves' Disease, Possible Association. *Journal of Pharmaceutical Negative Results.*2022, 226-229.