

Conducting Hormonal, Biochemical, and Serological Tests in Autoimmune Thyroid Patients

Abstract:

Background: One of the most frequent autoimmune illnesses affecting the thyroid gland is immunological thyroid disease. Antibodies against the enzyme thyroglobulin and thyroid-stimulating hormone receptors are produced by the immune system. Hashimoto's disease and Graves' disease are the two primary forms of autoimmune diseases. **Methods:** 91 blood samples were taken, including 71 for thyroid disease patients and 20 for healthy persons as control samples, from both sexes and of various ages. The tests were performed using a variety of methodologies. **Results:** Some of autoantibodies were found in varying quantities in 44 individuals. While TPO antibodies were found in 75% of 33 individuals, Anti-TG Ab was found in 72.72% of the patients, 15.90% in seven patients, and anti-TSHR Ab and antinuclear antibodies were found in 22.72% of the patients compared to the control samples. Any type of autoantibody, as well as the results of hormonal testing, revealed disease in a variety of individuals at varying rates. Because the patients were on therapy, the highest rate was 59.09% for thyroid-stimulating hormones. Chemical analyses revealed a sugar and fat imbalance with no discernible link. Interleukin 4 was detected in 4 individuals (9.09%), including one patient with autoimmune hypothyroidism (2.27%) and three patients in C3 18 with immunological hyperthyroidism (6.82%). The findings revealed an increase in complement molecule concentration in 40.90% of patients, including 15 patients with autoimmune hypothyroidism (34.09%) and 3 patients with immune thyroid gland C3 hyperactivity (6.71%). The statistical study revealed a $P < 0.01$ level of significance correlation between the presence of sugar and triglycerides and the complement molecule. Interleukin 4 and antibodies to receptors have a substantial correlation at the $P < 0.01$ TSHR Ab level TSH. **Conclusions:** The C3 gland patients for thyroid peroxidase had the largest amount of antibodies, lipids, and sugar, according to the study. Interleukin-4 occurred in certain individuals who had antibodies to thyroid-stimulating hormone receptors, according to the research.

Keywords: autoimmune, hypothyroidism, Interleukin-4, complement, TSHR.

Introduction:

Autoimmunity is the breakdown of autoimmune tolerance that can impact a single organ or the entire body. Organ-specific autoimmunity is defined as an uncontrolled immune response to self-antigens across the body, whereas systemic autoimmunity is defined as an unregulated immunological response to self-antigens throughout the body [1].

Thyroid hormones are well-known for their capacity to regulate metabolism, development, and a variety of other bodily activities. Triiodothyronine and thyroxine are the two primary hormones

produced by the thyroid gland <https://www.sciforce.org/index.php/IJIM/article/view/137>. The stimulating hormone for thyroid hormone synthesis, which is released by the anterior pituitary gland, is another crucial hormone. Several disorders, including autoimmune thyroid diseases, are caused by a deficiency in the production of these hormones [5].

One of the most frequent autoimmune illnesses affecting the thyroid gland is autoimmune thyroid disease. Antibodies against the enzyme thyroglobulin, thyroglobulin, and thyroid-stimulating hormone receptors are produced by the immune system. Hashimoto's disease, which causes hypothyroidism, and Graves' disease, which causes hyperthyroidism, are the two primary forms [1, 2].

Several variables influence the incidence and spread of the condition, including environmental and genetic factors, immune system imbalances, and deficiencies in other components including iodine, vitamin D, and selenium. Women are also more affected than males by the condition.

Several autoantibodies appear in Hashimoto's disease and Graves' disease, including:

Anti-thyroid peroxidase Ab: This enzyme is responsible for the production of thyroid hormones and is used to diagnose anti-TPO (anti-TPO) illness.

Anti-thyroglobulin Ab: This antibody is directed against the protein thyroglobulin, which is a key starter of thyroid hormone synthesis [3].

Anti-thyroid-stimulating hormone receptors Ab (TRABs): Thyroid-stimulating hormone receptor antibodies, some of which enhance thyroid gland function and others which block it. In the same patient, these antibodies may exist with various roles [4].

Thyroid hormones affect glucose metabolism through a variety of mechanisms, including halving the insulin life in hyperthyroidism due to increased glycolysis and increased secretion of biologically inactive insulin precursors, resulting in a decrease in the ratio of c-peptides to insulin, resulting in a defect in the use of insulin as a treatment [citation](#).

When an excess of thyroid hormones causes glucose to be absorbed by the gut. It causes the liver to produce excess quantities of glucose transporter from the plasma membrane, increasing in hepatic glucose production and aberrant glucose metabolism [6].

Thyroid hormones control the formation, mobilization, and breakdown of lipids in the circulation. Obesity and thyroid hormones are intimately linked. Any problem with these hormones' production causes local fat buildup and an increase in body mass [7].

C3 is a key component of the human body's innate immunity. It is released by fatty tissues and the liver and has a direct link to insulin resistance and plasma fat content. During sleep, it might alter metabolism and thyroid function [8].

IL-4 is a kind of cytokine that helps B cells make antibodies and is involved in allergic reactions. Th-2 cells manufacture IL-4. IL-4 activates a variety of immunoglobulin-secreting cell types that are linked to the severity of Graves' disease and thyrotropin receptor antibody levels [9].

Materials and methods:

A total of 91 blood samples were taken, with 71 samples from patients with thyroid issues and 20 samples from healthy people serving as controls for age groups ranging from 12 to 71 years old and both sexes.

Name, age, gender, past viral or bacterial infections, type of job, psychological condition, family history of the patients, smoking, medications utilized, and other clinical symptoms were all documented in a unique form.

Medical syringes were used to extract 5 ml of venous blood, which was then deposited in anticoagulant-free tubes and centrifuged at 3000 rpm for 10 minutes.

Estimation of thyroid hormone concentrations T3 and T4:

The amounts of thyroid hormones were estimated using a Japanese Tosho AIA 360 hormone meter and a Japanese Tosoh Bioscience assay kit. This is a competitive enzymatic immunoassay that takes place entirely inside the examination vessel and uses magnetic beads to cover fixed antibodies with enzyme-tagged antibodies. The enzyme in the vessel competes with the enzyme in the test sample, and the unbound labeled enzymes are eliminated after washing. The luminous base material should then be added. The amount of enzyme bound to the beads is proportional to the amount of enzyme in the serum [10].

Estimation of the concentration of thyroid stimulating-hormone (TSH):

The TSH in the serum binds to a monoclonal antibody coupled to magnetic beads and another monoclonal antibody conjugated with alkaline phosphatase in a dual-site immunoassay test done fully inside the examination vessel. The monoclonal antibody that is not attached to the enzyme is washed away from the magnetic beads. After that, the substrate is incubated with the sample. The enzyme concentration attached to the beads is exactly proportional to the enzyme concentration in the serum. A pre-prepared graph with established standard concentrations is used to compute the outcome [11].

Biochemical tests (Cholesterol, Triglyceride, HDL, LDL, VLDL, and Glucose):

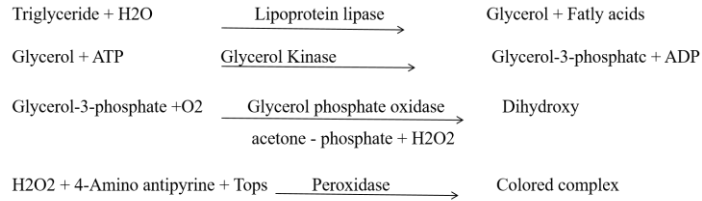
An American Chemistry Analyzer Smart-150 was used to conduct these tests and using an examination kit from the Italian company Giese-diagnosics.

Determination of Cholesterol level in serum:

Cholesterol ester analysis to free cholesterol and fatty acids by the enzyme Cholesterol esterase CHE in the presence of the enzyme Cholesterol oxidase CHOD. Chromatic reflects the level of total cholesterol [12].

Determine the Triglyceride:

This method depends on the enzymatic hydrolysis of triglycerides present in the blood serum to cholesterol according to the following equations [13]:



Determination of High-Density Lipoprotein (HDL) level:

This method involves precipitating fatty acids of lipoproteins (Chylomicrons), very low-density lipoproteins (VLDL), and low-density lipoproteins (LDL) in the blood serum by adding phosphotungstic acid ions in the presence of magnesium ions. The centrifugal separation process contains HDL [14].

Determination of Very Low-Density Lipoprotein VLDL:

The value of VLDL is extracted according to the researcher's method [15] according to the following equation: $\text{TG}/5 = \text{VLDL}$.

The concentration of triglycerides divided by 5 equals the concentration of low-density lipoproteins.

Determination of Low Density-Lipoprotein LDL:

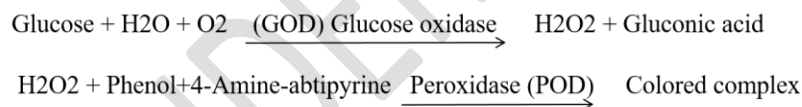
The value of LDL is extracted according to the method [16] according to the following equation:

$$\text{CHO} - (\text{VLDL} + \text{HDL}) = \text{LDL}$$

$$\text{Cholesterol concentration minus (HDL + very LDL)} = \text{LDL}.$$

Determination of Glucose Level:

The glucose present in the sample was determined as shown by the following equations [13]:



Determination of the concentration of IL-4:

A microplate reader was used to determine the quantity of cytokine (interleukin 4) produced by Labtech, a British company. The pits in the microplate are covered with IL-4 antibodies using the sandwich approach. After incubation, the complex is created by adding HRP, serum, and standard solution, followed by the conjugated reagent. All substances unrelated to the washing process are removed, and the enzyme's base material is introduced. When the reaction solution is added to stop the reaction, the color becomes blue, then yellow. At a wavelength of 450 nm, the optical density is measured, and the results are computed using a particular standard curve.

Determination of C3:

A test kit was used to determine the complement concentration from the LTA (Italy). The procedure is based on the radial diffusion of the antigen contained in the sample's serum (complement) after it is added to the pits of the complement test plate, C3, which is made up of an agarose gel with a specific antibody against a molecule. After incubation, an immunological complex forms around the pits in the form of a sedimentary ring whose diameter is proportional to the protein content (antigen) [17].

Statistical Analysis:

A statistical analysis tool called SPSS VS 25 was used to calculate the percentage, average, and standard deviation. T-test for regression was applied the statistical function to correlation statistics.

Results and discussion:

Patients and control samples were among the 91 blood samples tested. With a prevalence of 61.97%, autoimmune thyroid illness was found in 44 persons. Only individuals with autoimmune thyroid illness had their hormone, biochemical, and serological testing conducted.

The percentages of autoantibodies found in autoimmune thyroid patients are shown in Table 1.

Table 1: Autoantibodies and their percentages in patients with AITD.

Autoantibodies	Normal values	Total no.	Positive samples	Percentage %
ANA	≤ 0.572	44	10	22.72
TPO	UP to 35 IU/ml	44	33	75
TG	UP to 115 IU/ml	44	32	72.72
TSHR	(230-550) pg/ml	44	7	15.9

Antinuclear antibodies were found in 10 of the 44 patients, at a rate of 22.72 percent, and this proportion matched that of studies conducted in the United States, where 343 AITD patients out of a total of 1671 patients with thyroid problems had antinuclear antibodies [18].

Anti-TPO antibodies were found in 33 individuals (75%) and anti-TG anti-thyroglobulin antibodies were found in 32 patients (72.72%), according to the research. These percentages were similar to those found in Indian research, which found a high percentage of peroxidase antibodies in thyroid gland patients, as well as the greatest percentage of IgM antibodies in 35 autoimmune thyroid patients [19].

Anti-TSHR Ab was found in 15.90% of the patients, and the analysis revealed the existence of antibodies against thyroid-stimulating hormone receptors. This percentage matched that of research conducted in Germany, which found TSH receptor antibodies in 10 of 133 patients, or 7.5% [20].

The study showed a difference in the concentrations of thyroid hormones, as shown in Table (2).

Table 2: Hormonal tests for immune thyroid patients with percentages

Test type	Normal Values	Total no.	Positive sample	%	Immune Hypothyroidism		Immune Hyperthyroidism		Negative sample	%
					Positive sample	%	Positive sample	%		
T3	(0.92-2.8)nmol/l	44	9	20.45	5	11.36	4	9.09	35	79.55
T4	(60-140) nmol/l	44	10	22.73	6	13.64	4	9.09	34	77.27
TSH	(0.25-4.5)mIU/ml	44	26	59.09	20	45.45	6	13.64	18	40.91

Nine of the 44 autoimmune thyroid patients had T3 hormone disturbances at a rate of 20.45%, with five of them being immunocompromised hypothyroid patients with a rate of 11.36% and four being immunocompromised hyperthyroid patients with a rate of 9.09%. Ten patients out of 44 had a T4 hormone concentration abnormality, representing a 22.73% prevalence, with six patients having immune hypothyroidism at 13.63% and four having immune hyperthyroidism at 9%. TSH levels reached 59.09% in 26 individuals, including 20 patients with immunological hypothyroidism 45.45%, and 6 patients with hyperthyroidism 13.64%, all of whom were being treated. This supports the theory that infection has a role in the disruption of thyroid gland activities in those who have autoimmune thyroid disease.

Thyroid hormones T3, T4, and TSH can be used to distinguish between hypothyroidism and hyperthyroidism, according to studies conducted in India [19, 21].

As demonstrated in Table 3, several patients had issues with sugar and fat metabolism. A deficiency in sugar metabolism was found in 7 individuals out of 44 with immunological hypothyroidism, or 15.91%. In 12 patients with immune hypothyroidism, the proportion of elevated cholesterol was 27.27%, whereas, in one patient with immune hyperthyroidism, it was 2.27%. In 12 patients with immune hypothyroidism, the proportion of elevated LDL was 27.27%, while in one patient with immune hyperthyroidism, the rate was 2.27%. The proportion of high-density lipids dropped by 27.27% in 12 immune hypothyroidism patients and 13.64% in 6 immunological hyperthyroidism patients.

Six immunocompromised hypothyroid individuals with high triglycerides 13.64% and two immunocompromised hyperthyroid patients with high triglycerides of 4.44% were found in the research. In six patients with immune hypothyroidism and two patients with immune hyperthyroidism, the percentage of high-ultra-low HDL fat was of 13.64% and 4.54%, respectively. The rise in glucose, cholesterol, triglycerides, LDL, and HDL cholesterol in individuals matched the results of a Croatian investigation [22].

Table 3: Biochemical tests for immunocompromised thyroid patients with percentages.

Test type	Normal Values	Total no.	Immune Hypothyroidism		Immune Hyperthyroidism		Normal sample	%
			Abnormal sample	%	Abnormal sample	%		
Glucose	(70 - 110) mg/dl	44	7	15.91	-	-	37	84.09
Cholesterol	< 200 mg/dl	44	12	27.27	1	2.27	31	70.45
Triglyceride	< 150 mg/dl	44	6	13.64	2	4.54	36	81.82
HDL	Male: 35- 80 mg/dl Female: 42 -88 mg/dl	44	12	27.27	6	13.46	26	59.09
VLDL	< 30 mg/dl	44	6	13.46	2	4.54	36	81.82
LDL	< 130 mg/dl	44	12	27.27	1	2.27	31	70.45

As stated in Table 4, the research was also interested in conducting several serological tests, such as interleukin-4 and complement C3 molecule.

Table 4: Serological tests in infected patients with AITD.

Test type	Total no. Patients	Positive sample	%	Normal Values	Immune Hypothyroidism		Immune Hyperthyroidism	
					Positive sample	%	Positive sample	%
IL-4	44	4	9.09	(20- 48) pg/ml	1	2.27	3	6.82
C3	44	18	40.9	(91 – 156)mg/dl	15	34.09	3	6.81
Control	13	1	7.69	(91 – 156)mg/dl	-	-	-	-

IL-4 levels were high in 4 individuals at a rate of 9.09%, with one patient having hypothyroidism at a rate of 2.27% and three patients having hyperthyroidism at a rate of 6.82%. The function of IL-4 in the development of autoimmune thyroid illness has been proven in studies conducted in Tunisia and the United Kingdom [23, 24].

The results showed an increase in the concentration of C3 molecule in 18 patients by 40.90%, including 15 patients with immune hypothyroidism by 34.09% and 3 patients with immune hyperthyroidism by 6.81% compared to the control samples of 13 samples, where the percentage of increase in complement was 7.69% in one sample. The percentages were in the pathological cases among people with high fat and sugar, which confirms the close link between the disease and the concentration of complements in the body.

A study registered in Tehran dealt with the relationship of the complement molecule C3 with thyroid hormones and fats, and there was a significant correlation between the complement molecule and fats from a statistical point of view, as shown in Table 5 [8].

In terms of the association between complement and IL-4 and autoimmune thyroid disease AITD, we couldn't uncover anything comparable.

Table 5 shows that there is a substantial positive correlation between IL-4 and TSH receptor antibodies, indicating that there is a significant correlation between IL-4 and TSHR Ab at the $P < 0.01$ value. The complement molecule has a strong positive correlation at the $P < 0.01$ value. There is a correlation coefficient between C3, sugar, and triglycerides, proving that they have a direct relationship at the $P < 0.01$ value. The data revealed a less significant between C3, cholesterol, and extremely low-density lipids. Other significant correlations between the variables investigated in this study were not discovered.

Table 5: Statistical analysis values.

Variables	r	P
IL4-TSHR Ab	**0.864	0.01
C3- Glucose	**0.468	0.01
C3-CHO	*0.338	0.05

C3-TRI	**0.4	0.01
C3-VLDL	*0.307	0.05

Conclusions:

The greatest percentage of thyroid peroxidase antibodies, TOP Ab, was revealed in the study. In individuals with AITD, the complement C3 molecule, sugar, and fat had a substantial significant correlation. The lack of high levels of disruption in hormonal and chemical testing, as well as a significant association between IL-4 and TSHR Ab in certain patients, might be related to the fact that patients were treated throughout the research.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References:

1. Kristensen B. (2016). Regulatory B and T cell responses in patients with autoimmune thyroid disease and healthy controls. *Dan Med J* 2016; 63(2):B5177.
2. Kim S, Myong JP, Jee H. (2016). Combined effect of Hashimoto's thyroiditis and BRAFV600E mutation status on aggressiveness in papillary thyroid cancer. *Head Neck*. 2016; 38(1):95-101.
3. Tipu H.N, Ahmed D, Bashir M.M, and Asif N. (2018) Significance of Testing Anti-Thyroid Autoantibodies in Patients with Deranged Thyroid Profile. *Journal of Thyroid Research*, 9610497.
4. Napolitano G, Bucci I, Dalmazi G.D, and Giuliani C. (2021) Non-Conventional Clinical Uses of TSH Receptor Antibodies: The Case of Chronic Autoimmune Thyroiditis. *Front. Endocrinol*, 12:769084.
5. Shahid M, Ashraf M, and Sharma S. (2021) Physiology, Thyroid Hormone. *StatPearls [Internet]*. Treasure Island (FL).

6. Ogbonna SU, Ezeani1 IU, Okafor CI, Chinenye S. (2019) Association between glycemic status and thyroid dysfunction in patients with type 2 diabetes mellitus. *Diabetes, Metabolic Syndrome and Obesity: Targets and therapy*.
7. Sun X, Chen L, Wu R, Zhang D, and He Y. (2021) Association of thyroid hormone with body fat content and lipid metabolism in euthyroid male patients with type 2 diabetes mellitus: a cross-sectional study. *BMC Endocrine Disorders* (2021) 21:241. <https://doi.org/10.1186/s12902-021-00903-6>.
8. Karkhaneh M, Qorbani M, Ataie-Jafari A , Mohajeri-Tehrani M, Asayesh H, and Hosseini S. (2019). Association of thyroid hormones with resting energy expenditure and complement C3 in normal weight high body fat women. <https://doi.org/10.1186/s13044-019-0070-4>.
9. Raveinal R, Darwin E, Decroli E, Jamsari J.(2020) Correlation between Interleukin-4 Gene Promoter Polymorphisms with Thyrotropin Receptor Antibody and Transforming Growth Factor- β . *Open Access Maced J Med Sci*. 2020 Oct 14; 8(A):793-796. <https://www.id-press.eu/mjms/index>.
10. Howanitz J, Howanitz P, and Henry J. (1991). Evaluation of Endocrine Function, in Henry, J. (ed), *Clinical Diagnosis and Management by Laboratory Methods* (18th ed). pp.308-320.
11. Taimela E, Tahtela R, Koskinen P, Nuutila P, Forsstrom J, Taimela S, et al. (1994) Ability of two new thyrotropin (TSH) assays to separate hyperthyroid patients from euthyroid patients with low TSH. *Clin. Chem.* 40, 101-105.
12. Kaplan LA, and Pesce AJ. (1989) *Clinical Chemistry*, Mosby Ed.
13. Vassault A. et al. (1986). *Ann. Biol. Clin.*, 44, 686.
14. Trinder P. (1969) *Ann. Clin. Biochem.* 6, 24.
15. Friedwald W, Levy R. I, and Fredrickson D. S. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, without use of Preparative Ultracentrifuge. *Clinical Chemistry*, 18(6): 499- 502.
16. Tietz W, et al. *Clinical Guide to Laboratory Tests*, 3rd ed AACC 1995.
17. Mancini & coll. (1965). *Immunochemistry*. 2:235.
18. Siriwardhane T, Krishna K, Ranganathan V, Jayaraman V, Wang T, Bei, et al. (2018). Exploring Systemic Autoimmunity in Thyroid Disease Subjects. *Journal of Immunology Research*, 6895146.
19. Sultana Q, Anjum A, Fathima N, Siraj M, and Ishaq M. (2016). Seropositivity to anti-thyroid peroxidase and anti-thyroglobulin autoantibodies in hypo and hyper-thyroidism: Diagnostic and epidemiological significance. *Indian J Microbiol Res*, 3(4):368-372.
20. Diana T, Krause J, Olivo P.D, Konig J, Kanitz M, Decallonne B, et al. (2017). Prevalence and clinical relevance of thyroid stimulating hormone receptor-blocking antibodies in autoimmune thyroid disease. *Clin Exp Immunol*, 189(3):304 –309.
21. Jantikar A. (2020). A study on relationship between thyroid peroxidase antibodies (Anti-TPO antibodies) and thyroid dysfunction patients. *International Journal of Clinical Biochemistry and Research*; 7(2):238–242.

22. Blaslov K, Gajski D, Vucelić V, Gaćina P, Mirošević G, Marinković J, et al. (2020). The association of subclinical insulin resistance with thyroid autoimmunity in euthyroid individuals. *Acta Clin Croat*, Vol. 59, No. 4, 2020.
23. Zaaber I, Mestiri S, Hammedi H, Marmouch H, Mahjoub S, Tensaout B, and Said K. (2016) Association of Interleukin-1B and Interleukin-4 Gene Variants with Autoimmune Thyroid Diseases in Tunisian Population. *Immunological Investigations*, Vol. 45, No. 4, 284–297.
24. Shen X, Yan X, Xie B, Xu D, Wang B, Zhu J, et al. (2015) Genetic variants of interleukin-4 gene in autoimmune thyroid diseases: An updated meta-analysis. *Autoimmunity*, Early Online: 1–7, 2014 Informa UK Ltd.