

Evaluation of Caspase-8 Level in Serum of Patients with Autoimmune Thyroid Disorders

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Abstract:

Autoimmune thyroid diseases (AITDs), including Graves' disease and Hashimoto's thyroiditis, are characterized by lymphocytic infiltration of the thyroid and the production of thyroid autoantibodies. While the etiology of AITDs is still not fully understood, a combination of genetic susceptibility and environmental triggers is believed to contribute to the breakdown in immune tolerance. This study aimed to evaluate the levels of thyroid hormones (T3, T4, TSH), Anti-thyroid peroxidase Abs (Anti-TPO Abs), Anti-Thyroglobulin Abs (Anti-TG), and apoptosis marker (Caspase-8) in Iraqi patients with AITDs compared to healthy controls.

A total of 1000 patients participated in the study 820 were excluded from the study because it was non-immune thyroid disease, and 180 subjects were recruited, including 60 Graves' disease patients, 60 HT patients, and 60 healthy controls. Serum levels of T3, T4, TSH, Anti-TPO Abs and Anti-TG, caspase 8, and inhibin were measured. T3 and T4 levels were significantly decreased in Hashimoto's thyroiditis patients and increased in Graves' disease patients compared to controls. TSH was significantly elevated in Hashimoto's thyroiditis and reduced in Graves' disease patients. Caspase-8 was significantly higher in Hashimoto's groups compared with the control group, and Significantly lower in Graves group compared with the control group.

In conclusion, an altered balance in thyroid hormones, Anti-TPO Abs, Anti-TG, and apoptosis markers occurs in AITDs. Elevated caspase-8 suggests that increased apoptosis may contribute to thyroid damage in AITDs. Further research is needed to clarify the roles of these mediators in AITD pathogenesis.

Keywords: Apoptosis, Caspase-8, Graves' disease, Hashimoto's thyroiditis, Thyroid hormones.

1.Introduction:

Autoimmune thyroiditis (AIT) is the predominant autoimmune disorder affecting the endocrine system [1]. Hashimoto's thyroiditis (HT) is a condition characterized by inflammation of the thyroid gland caused by the body's immune system attacking its thyroid tissue. It is also referred to as lymphocytic thyroiditis and chronic autoimmune thyroiditis [2]. Graves' Disease (GD) is named after Robert J. Graves, the first person to identify this condition in the 19th century. It is characterized by an enlarged and hyperactive thyroid gland (hyperthyroidism caused by circulating autoantibodies), a rapid heart rate, and abnormalities in the eyes[3,4]. Both Graves' disease and Hashimoto's thyroiditis are autoimmune disorders that affect the thyroid gland. Graves' disease is often identified by the presence

of autoantibodies in the bloodstream that activate the TSH receptor, leading to the development of hyperthyroidism and goiter. The development of AITD is influenced by both genetic and environmental factors, contributing to its complex etiology. AITD is a multifaceted condition caused by the development of autoimmunity against thyroid antigens within a particular genetic framework, facilitated by exposure to environmental factors. The thyroid gland is the primary target of autoimmune reactions with the highest frequency. While AITDs are recognized as organ-specific autoimmune disorders, the exact cause of these autoimmune responses is still uncertain [5,6]. Thyroid peroxidase (TPO) and thyroglobulin (Tg) are the primary autoantigens in Hashimoto's disease. However, these antibodies (TPO-Ab and Tg-Ab) are also present in around 70% of individuals with Graves' disease. Like Graves' illness, where the thyroid-stimulating hormone receptor (TSHR) is the main autoantigen, a small percentage of individuals with Hashimoto's disease possess these antibodies [7].

Caspases involved in mammalian apoptosis and inflammation have traditionally been categorized according to their respective cellular functions. Inflammatory caspases, including caspase 1, 4, 5, and 11, stimulate the inflammasome and pyroptosis, an inflammatory form of programmed death, which subsequently initiates inflammation. Apoptotic caspases, on the other hand, are involved in a mode of programmed cell death that inhibits the immune system. They consist of two types of caspases—initiator caspases, caspase-2, caspase-8, -9, and -10—and effector caspase-3, caspase-6, and -7 [8]. Among these, caspase-8 stands out due to its multifaceted roles in a variety of inflammatory processes. Caspase-8 is an apical enzyme that is responsible for initiating the extrinsic apoptotic cell death pathway when death receptors, including tumor necrosis factor receptor 1, Fas cell surface death receptor (FAS), and death receptors (DRs), are activated [9]. Zymogen, an inactive precursor comprising two death effector domains (DEDs) at the N-terminus and a protease domain at the C-terminus, which comprises a large subunit (p18) and a small subunit (p10), is utilized in the synthesis of caspase-8. Caspase-8 is dimerized and auto-processed via a proximity-driven mechanism to become active [10,11]. When caspase-8 is in an active state, it is capable of cleaving effector caspases and additional substrates [12].

2. Methods and instruments

Between August 2022 and April 2023, a sample of a total of 1000 patients participated in the study 820 were excluded from the study because it was non-immune thyroid disease, and 180 subjects were recruited, including hyperthyroidism 60 Graves' disease patients, 60 hypothyroidism (Hashimoto's disease) patients, and 60 healthy controls was randomly chosen from Al-Yarmouk Teaching Hospital and Al-Jawda Private Laboratory in the Baghdad governorate.

This study was conducted on newly diagnosed patients with Hashimoto thyroiditis and Graves' disease who were referred to endocrine healthcare facilities in the Baghdad governorate. The method of successive sampling was used to constantly enroll persons who were at least 15 years old. The control group included euthyroid individuals who were referred for routine check-up examinations. Autoimmune thyroid illness was not documented in their familial or personal medical records. The case groups and control groups were matched according to age, and sex.

The inclusion criteria included patients who had recently been diagnosed with either Hashimoto thyroiditis or Graves' disease. The research excluded individuals exhibiting signs of acute or chronic infectious illness, malignancies, or those who had recently taken medications for dyslipidemia, hypertension, non-immune thyroid problems, or oral contraceptives during the last six months.

After filling out a questionnaire that asked about their age, gender, medical history, medication use history, and family history.

Patients with Graves' disease who have low levels of thyroid-stimulating hormone (TSH) and/or have greater levels of tetraiodothyronine (T4) and/or triiodothyronine (T3) with positive anti-thyroid peroxidase antibodies and/or Anti thyroglobulin antibodies. On the other hand, patients with Hashimoto's thyroiditis (HT) who have high levels of thyroid-stimulating hormone (TSH) and/or have low levels of tetraiodothyronine (T4) and/or triiodothyronine (T3) with positive anti-thyroid peroxidase antibodies and/or Anti thyroglobulin antibodies.

Participants underwent phlebotomy between 8 and 9 AM after abstaining from food for 12 hours. Serum samples were obtained and kept at a temperature of -70 °C before testing. The study assessed the levels of T3, T4, TSH, Anti-TPO, Anti-TG, and Caspase-8 in the serum of patients diagnosed with Graves' disease and Hashimoto's thyroiditis, as well as in the control group. The Human Caspase-8 ELISA Kit was used to assess the concentrations of Caspase-8 in both the group of patients and the group of controls. The Roche Cobas e411 automated analyzer employs immunochemiluminescent assays to measure levels of T3, T4, and TSH for diagnostic reasons. VIDAS is used to measure the levels of Anti-TPO, and Anti-TG.

The study methods were carried out by the ethical standards established by the national or organizational research council. We adhered to the principles delineated in the Helsinki Declaration of 1964 and its later amendments. Each participant was granted their informed consent.

The research variable was characterized using descriptive statistics, percentage, mean, and standard deviation. T-test and Kappa test was used to examine the differences in means of a quantitative variable across the three cohorts of patients with GD, HT, and a healthy control group. The statistical significance was established with a significance level of $P < 0.05$.

3. Results and Discussion

3.1. Distribution of patients and control groups according to age and gender

The research investigated the association between age, gender, and autoimmune thyroiditis. Table (1) shows the distribution of patients and control groups based on age and gender. Results in Table (1) showed that the percentage of Hashimoto's disease in males is (10.0) % in all age groups, while their percentage in females is (90.0) % in all age groups. The percentage of Graves in males is (23.3) % in all age groups, while their percentage in females is (76.7) % in all age groups.

Table (1) Distribution of Studied groups according to Gender

Groups		Male	Female	Total
Control	No.	30	30	60
	%	50 %	50 %	100 %
Hashimotos	No.	6	54	60
	%	10.0%	90.0%	100 %
Graves	No.	14	46	60
	%	23.3%	76.7%	100 %

The result of this study is consistent with the scientific opinions in the precedent referred to by many researchers, which stipulated Hashimoto’s disease is 4 to 10 times more common in females than males [13].

3.2.Comparison between Studied groups according to T3, T4, and TSH levels.

The results of this study in Table (2) revealed that (Mean± Std.) of serum T3 decreased highly significantly (p<0.01) in Hashimoto's hypothyroidism patients’ group (0.79±0.40 ng/ml) as compared to (Mean± Std.) of control group (1.00±0.19 ng/ml), while its level increased highly significantly (p<0.01) in Graves hyperthyroidisms patients’ group (2.11±0.09 ng/ml) by comparing with control group (1.00±0.19 ng/ml).

Table (2) shows, (Mean± Std.) of serum T4 decreased highly significantly(p<0.01) in the Hashimotos hypothyroidism patients’ group (59.83±23.47 nmol/l) as compared to (Mean± Std.) of the control group (95.07±13.99 nmol/l). At the same time, its level increased highly significantly (p<0.01) in Graves Hyperthyroidisms (159.13±50.46 nmol/l) by comparing with the control group (95.07±13.99 nmol/l).

Table (2) shows, (Mean± Std.) of serum TSH increased highly significantly (p<0.01) in Hashimoto's hypothyroidism patients’ group (19.07±20.17 μIU/ml) as compared to (Mean± Std.) of the control group (1.39±0.51 μIU/ml), while its level decreased highly significantly (p<0.01) in Graves hyperthyroidisms patients group (0.07±0.16) by comparing with the control group (1.39±0.51 μIU/ml).

Table (2): Comparison between Studied groups according to T3, T4, and TSH

Groups	(Mean± Std.)	Compared with control		Hashimotos with Graves	
		t-test	P-Value	t-test	P-Value
Control	T3(ng/ml)				
	1.00±0.19				
Hashimotos	0.79±0.40	3.642	.000 (P<0.01 HS)		
Graves	2.04±1.41	5.678	.000 (P<0.01 HS)	6.608	.000 (P<0.01 HS)
Control	T4(nmol/l)				
	95.07±13.99				
Hashimotos	59.83±23.47	9.989	.000 (P<0.01 HS)		
Graves	159.13±50.46	9.478	.000 (P<0.01 HS)	13.823	.000 (P<0.01 HS)
Control	TSH(uIU/ml)				
	1.39±0.51				
Hashimotos	19.07±20.17	6.786	.000 (P<0.01 HS)		
Graves	0.07±0.16	19.225	.000 (P<0.01 HS)	7.296	.000 (P<0.01 HS)

The current study, as in Table (2), showed a decrease in T4 hormone in Hashimoto's patients and an increase in Grave's patients. The findings of the present research align with well-established scientific evidence that demonstrates a reduction in T4 hormone levels in individuals with Hashimoto's disease due to impaired thyroid gland function, leading to insufficient release of thyroid hormones. Hypothyroidism occurs due to insufficient synthesis of thyroid hormones. Hypothyroidism occurs when

there is insufficient synthesis of thyroid hormones [14]. The findings of the present research are consistent with well-established scientific evidence, which suggests that Graves patients exhibit elevated levels of T4 hormone due to excessive synthesis of thyroid hormones. Grave's disease is an autoimmune condition characterized by creating antibodies that target the TSH receptors in the thyroid gland. This leads to excessive synthesis of T3 and T4 hormones. When hyperthyroidism is manifested, signs and symptoms will likely be accompanied by reduced TSH and elevated T3 and T4 levels.

The current study, as in Table (2), showed an increase in TSH hormone in Hashimoto's patients and a decrease in Grave's patients. The result of the current study is consistent with established scientific facts that indicate an increase in the level of the TSH hormone in Hashimoto's patients because of a defect in the functioning of the thyroid gland, which results in a lack of secretion of thyroid hormones. The result of the current study also agrees with established scientific facts that indicate a low level of TSH hormone in Graves patients because of a defect in the functioning of the thyroid gland, which increases the secretion of thyroid hormones. Hyperthyroidism occurs due to an inappropriately high synthesis and secretion of thyroid hormone by the thyroid [15].

3.3.Distribution of Studied groups according to Anti-TPO Abs

Table (3) shows the distribution of studied groups according to Anti-TPO Abs. The percentage of Anti-TPO Abs positivity showed a highly significant correlation in Hashimoto hypothyroidism (63.3%) and Graves hyperthyroidism (86.7%) at the (P<0.03) in comparison with the negativity percentage which been in Hashimoto's hypothyroidism (36.7%)and Graves hyperthyroidism (13.3%).

Table (3) Distribution of Studied groups according to Anti-TPO Abs

Anti-TPO Abs		Study groups		Total
		Hashimotos	Graves	
Positive	No.	38	52	90
	%	63.3%	86.7%	75.0%
Negative	No.	22	8	30
	%	36.7%	13.3%	25.0%
Total	No.	60	60	120
	%	100.0%	100.0%	100.0%
Kappa Test		P=.003 (HS)		

The results of this study are consistent with previous scientific opinions, which showed that thyroid peroxidase antibodies are a marker of autoimmune thyroid disease that is significantly high in patients with Hashimoto’s thyroiditis and, in patients with hyperthyroidism during Graves’ disease [16].

3.4. Distribution of Studied Groups according to Anti-TG Abs

Table (4) shows the distribution of Studied groups according to Anti TG Abs. The percentage of Anti TG Abs positivity showed no significant correlation in Hashimoto's hypothyroidism (96.7%) and Graves hyperthyroidism (90.0%) at the (P=0.143) is compared with the negativity percentage which in Hashimoto hypothyroidism (3.3%)and Graves hyperthyroidism (10.0%).

Table (4) Distribution of Studied groups according to Anti TG Abs

Anti TG		Study groups		Total
		Hashimoto's	Graves	
Positive	No.	58	54	112
	%	96.7%	90.0%	93.3%
Negative	No.	2	6	8
	%	3.3%	10.0%	6.7%
Total	No.	60	60	120
	%	100.0%	100.0%	100.0%
Kappa Test		P=.143 (NS)		

The study showed that the percentage of Anti TG Abs positivity has no significant correlation with Hashimoto's hypothyroidism (96.7%) and Graves hyperthyroidism (90.0%) at the (P=0.143) in comparison with the negativity percentage which been in Hashimoto's hypothyroidism (3.3%) and Graves hyperthyroidism (10.0%).

The results of this study are consistent with previous scientific opinions, which showed that antibodies against thyroglobulin are present in(97%)of patients with Hashimoto's thyroiditis, and Graves hyperthyroidism (90.0%)

The group with Hashimoto's thyroiditis exhibited the highest proportion of positive patients in this investigation, potentially indicative of autoimmune devastation of the thyroid organs in those individuals. This ratio is comparatively elevated in light of analogous inquiries conducted by other scholars [17]. Our data align with those of a study conducted in Oman [18], which reported the greatest incidence of positive anti-TG in the group diagnosed with Hashimoto's thyroiditis, and with studies conducted in Sudan [19], which reported the highest incidence of positive anti-TG in the group diagnosed with Graves disease.

3.5. Comparison between Studied groups according to Caspase-8 enzyme (ng/ml) levels.

Table (5) shows the Comparison between Studied groups according to the Caspase-8 enzyme. Plasma levels of the Caspase-8 enzyme showed a highly significant positive relation in Hashimoto's hypothyroidism (3.25±0.57) by comparing with the control group (0.89±0.12) at the (P<0.01). Plasma levels of the Caspase-8 enzyme showed a highly significant negative relation in Graves hyperthyroidism (0.73±0.14) by comparison with the control group (0.89±0.12) at the (P<0.01).

Table (5): Comparison between Studied groups according to Caspase-8 enzyme (ng/ml) levels.

Groups	(Mean± Std.) Caspase-8 enzyme (ng/ml)	Compared with control		Hashimotos with Graves	
		t-test	P-Value	t-test	P-Value
Control	0.89±0.12				
Hashimotos	3.25±0.57	31.164	.000 (P<0.01 HS)		
Graves	0.73±0.14	6.852	.000 (P<0.01 HS)	33.077	.000 (P<0.01 HS)

Mounting evidence indicates that apoptosis contributes to the pathogenesis of the autoimmune thyroid disorders Hashimoto's thyroiditis and Graves' disease. Although apoptosis is involved in the development of both Hashimoto's thyroiditis and Graves' disease, the specific mechanisms that regulate these processes seem to be distinct. Apoptosis induction in Hashimoto's thyroiditis causes the elimination of thyrocytes, while apoptosis in Graves' disease results in harm to lymphocytes that infiltrate the thyroid [20]. In our study, we found serum levels of the Caspase-8 enzyme showed a highly significant positive relation in Hashimoto's hypothyroidism by comparing with the control group. Serum levels of the Caspase-8 enzyme showed a highly significant negative relation in Graves hyperthyroidism by comparison with the control group which means that caspase-8 (apoptosis) has a role in autoimmune thyroid disease.

4. Conclusions

The current investigation showed autoimmune thyroiditis affects females more than males. An altered balance in thyroid hormones, Anti-TPO Abs, and Anti-TG occurs in AITDs. The current investigation showed that serum Caspase-8 levels were up in patients with Hashimoto's and reduced in those with Graves hyperthyroidism compared to the control group. Additional research is necessary to ascertain the precise mechanism by which thyroid hormones and leptin interact.

5-Authors' Declaration

-Conflicts of Interest: None.

- We affirm that all the Figures and Tables in the text are our work. In addition, any external figures and pictures used in the text have been obtained with the requisite permission for re-publication, which is provided with the manuscript.

-Ethical Approval: The proposal received approval from the local ethics committee at the University of Middle Technical University.

6-Authors' Contribution Statement

Mohammed Ali Mohammed Al-Badri, Issam Jumaa Nasser, and, Mahdi, Ali A.A. contributed to the design and implementation of the research, the analysis of the results, and the writing of the manuscript.

7. Acknowledgement

The Laboratory Clinical Chemistry Unit at Al Yarmouk Teaching Hospital and Al-jawda Laboratory played a crucial role in the success of this investigation by helping and supporting it.

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