Article
JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)
Vol. 34 ; No.1 | 2026

A Comparative Study of Serum Melatonin and TNF-α Levels in Patients with Thyroid Dysfunction

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التحليل المقارن لمستويات الميلاتونين وعامل نخر الورم ألفا في اضطرابات وظائف الغدة الدرقية

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Accepted: 29/3/2026 Published: 31/3/2026

ABSTRACT

Thyroid dysfunction, including hyperthyroidism and hypothyroidism, involve metabolic dysregulation, endocrine imbalance, and immune alterations. Cytokine signaling molecules such as TNF-α and regulatory hormones like melatonin may modulate pathogenic processes. Although autoimmune thyroiditis has been linked to elevated inflammatory markers, their diagnostic accuracy across broader thyroid dysfunction remains equivocal. This study aimed to assess serum melatonin and TNF-α levels among patients with thyroid dysfunction, and controls, and to assess their prospective utility as diagnostic indicators. The study involved 90 individuals, distributed proportionally into three groups including 30 hyperthyroidism, 30 hypothyroidism, and 30 controls group. Serum melatonin and TNF-α concentrations were quantified using enzyme-linked immunosorbent assay (ELISA). The results of serum melatonin concentrations were significantly elevated in both hyperthyroid (5.71 ± 0.84 ng/mL) and hypothyroid (5.57 ± 1.05 ng/mL) cohorts relative to controls (3.21 ± 0.31 ng/mL; $P < 0.001$), with no significant difference observed between the two patient groups. Receiver operating characteristic (ROC) analysis demonstrated acceptable diagnostic performance for distinguishing thyroid dysfunction from normal: hyperthyroidism, AUC = 0.761, sensitivity = 76.7%, specificity = 80.0%; hypothyroidism, AUC = 0.718, sensitivity = 73.3%, specificity = 70.0%. Mean serum TNF-α concentrations were elevated in hyperthyroid (23.56 ± 11.77 pg/mL) and hypothyroid (19.72 ± 12.05 pg/mL) patients compared with controls (15.15 ± 9.18 pg/mL), but the differences did not reach statistical significance ($P = 0.101$). Melatonin was significantly higher in both hyper- and hypothyroid patients, suggesting an adjunct biomarker for thyroid dysfunction. TNF-α showed a non-significant trend toward elevation, indicating potential inflammatory involvement that needs validation in larger studies.

Keywords: thyroid, T3, TSH, T4, melatonin.

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INTRODUCTION

Thyroid gland is one of the large organs within the human body associated with the endocrine system. The gland is responsible for producing two hormones: thyroxin (T4) and triiodothyronine (T3) [1,2]. Thyroid hormones are the main regulators of cellular growth, apoptosis, metabolism and differentiation [8]. thyroid dysfunction can range from mild to more severe form. Dynamic criteria coded thyroid dysfunctions into hypothyroidism (low T4) and hyperthyroidism (higher T4 or T3,) [3, 4].

Hyperthyroidism is the excessive production of thyroid hormone by the thyroid gland resulting in supernormal serum concentration [5]. The high level of thyroid hormone increases the metabolic state. Hyperthyroidism is the most prevalent endocrine disorder, with diverse prevalence recorded depending on the different dietary iodine intake, ethnic group and population distribution. In areas with mild to moderate iodine deficiency, hyperthyroidism is the usual cause of thyroid disease[6].

Hypothyroidism is pathologically related to a variety of disease entities (bradyarrhythmia, depression and are also independent risk factors for it). There are two dominant subtypes of hypothyroidism, clinical hypothyroidism which is characterized by increased serum TSH and decreased serum free peripheral PTHs, and subclinical hypothyroidism in which free thyroid hormone levels remain within the normal range [7]. Melatonin is an ancient molecule in numerous lines of life and it has been widely reported that melatonin performs countless actions [9]. This hormone is the principal product of the pineal gland and is produced in trace amounts by multiple other areas of the body, including the retina (darkness stimulates production) and the enterochromaffin cells of the digestive tract [10]. In addition, it is synthesized in various extra-pineal tissues such as, kidneys digestive and peripheral blood mononuclear cells [11,12]. Melatonin influences thyroid function by acting as a modulator that can decrease the production of TSH. Since TSH is responsible for stimulating the thyroid gland to produce T3 and T4, a melatonin-induced decrease in TSH levels may subsequently lead to reduced thyroid hormone production; however, melatonin acts as an antioxidant protecting thyroid cells protecting thyroid cells from oxidative damage [13]. Cytokines have a major role in regulation of thyroid dysfunction, since they have a crucial role in the regulation of immune response for patients with autoimmune diseases [14]. TNF-α is a multifunctional pro-inflammatory mediator which is primarily secreted by monocytes, macrophages and T-cells [15]. TNF-α plays an important role in the development of autoimmune thyroid disorder such as Hashimoto's and Graves' disease, that results in immune cell lysis, upregulation of adhesion molecules and inflammation. Thyroid follicular cells undergo apoptosis due to TNF-α, which is released mainly by thyroid T cells and macrophages, that cause loss of thyroid tissue and dysfunction. The producing local TNF-α, which triggers inflammation and an inflammatory response that causes further damage to the thyroid gland [16]. Although both melatonin and TNF-α have been individually implicated in thyroid pathophysiology, their concurrent evaluation in patients with thyroid dysfunction has not been systematically investigated. Therefore, this study aims to compare serum levels of melatonin and TNF-α in

hyperthyroid and hypothyroid patients relative to healthy controls, and to assess their potential as diagnostic biomarkers.

MATERIALS AND METHODS

- **Study design and population**

  A cross-sectional study was carried out on 90 participants admitted to Merjan Medical City in the period between December 2023 and March 2024. The patients were diagnosed by specialist physicians after performing standard biochemical tests. The patients equally divided into three groups: hyperthyroidism (n = 30), hypothyroidism (n = 30), and healthy controls (n = 30). Excluded the patients with any other autoimmune disease, current use of levothyroxine, anti-thyroid drugs, corticosteroids, or melatonin supplements, acute or chronic inflammatory conditions, malignancy, diabetes, hepatic or renal disease, pregnancy or recent surgery. Controls were confirmed healthy individuals with no history of thyroid disease or chronic illness. Controls were matched to patients by age and sex.

- **Sample collection and processing**

  Blood samples were collected into EDTA tubes and centrifuged at 3000 rpm for 5 minutes. Separated serum were stored at -20 until used. All samples had a one freeze-thaw cycle prior to assay. All blood samples were collected at morning after overnight fasting (≥ 10 hours), to control for circadian rhythm variation in melatonin secretion. Fasting status was confirmed verbally and documented for all participants.

- **Immunological Study**

  Serum levels of melatonin and TNF-α were measured using a sandwich ELISA assay, following the manufacturer's instructions (Elabscience, China).

- **Statistical analysis**

  Computerized statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), version 23 (IBM Corp., USA). Statistical analyses were performed using one-way ANOVA, Chi-square (χ^2) tests, and independent sample t-tests. Significance was determined by using the probability value ($p \leq 0.05$). Other statistical tests included the 95% confidence interval (95% CI) and the ROC curve analysis, area under the curve (AUC) statistic.

RESULTS

Characteristic of the study participants

Within this study, 90 serum samples obtained from individuals visiting Merjan Medical City were analyzed for the levels of melatonin and TNF-α. Regarding gender distribution, the complete cohort consisted 38 males (42.2%) and 52 females (57.8%). Among hyperthyroid patients, 10 (33.3%) were male and 20 (66.7%) were female. The hypothyroidism patients comprised 18 males (60.0%) and 12 females (40.0%). The control group included 10 males (33.3%) and 20 females (66.7%). Statistical analysis revealed no significant difference in gender distribution across the study groups (P = 0.054) as in table 1.

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Table (1): Comparison between patients and control groups in gender.

Study groups		Gender		Total	p-value
		Male	Female		
Groups	Hyperthyroidism	10 (33.3%)	20 (66.7%)	30	0.054 ¥NS
	Hypothyroidism	18 (60.0%)	12 (40.0%)	30	
	Control	10 (33.3%)	20 (66.7%)	30	
Total		38 (42.2%)	52 (57.8%)	90	

¥: Chi-square test; **NS**: not significant at $P \leq 0.05$

Serum melatonin level in participants

Mean melatonin concentrations were 5.71 ± 0.84 ng/mL in the hyperthyroidism group, 5.57 ± 1.05 ng/mL in the hypothyroidism group, and 3.21 ± 0.31 ng/mL in the control group. Both patient groups exhibited significantly higher melatonin levels compared to the healthy controls (P < 0.001). However, no significant difference was observed between the hyperthyroidism and hypothyroidism groups (Table 2).

Table (2): Melatonin levels in patients and healthy controls.

Groups		Melatonin levels
Hyperthyroidism	Mean ± SE	5.71 ± 0.84^{A}
	Range	1.23-23.30
Hypothyroidism	Mean ± SE	5.57 ± 1.05^{A}
	Range	1.28-34.00
Control	Mean ± SE	3.21 ± 0.31^{B}
	Range	0.84-6.50
p-value		**0.042**** †

SD: standard deviation; †: one-way ANOVA; **: significant at $P \leq 0.05$

ROC curve analysis for melatonin level among participants

Table 3 presents the diagnostic performance of serum melatonin levels in distinguishing patients with hyperthyroidism and hypothyroidism from healthy controls using ROC curve analysis. For hyperthyroidism, a melatonin cutoff value larger than 3.98 ng/mL resulted in an AUC of 0.761, demonstrating adequate discriminative capacity with 76.7% sensitivity and 80.0% specificity. For hypothyroidism, a cutoff larger than 3.76 ng/mL resulted in an AUC of 0.718, indicating moderate diagnostic precision, with a sensitivity of 73.3% and specificity of 70%. That can indicated melatonin level is a relatively accurate biomarker for discriminate between patients with thyroid dysfunctions and control group. Additionally, an optimal melatonin cut-off value of greater than 3.76 ng/mL was identified to differentiate hypopatients with thyroid dysfunction from healthy controls, with a sensitivity of 73.3%, specificity of 70.0%, and AUC of 0.718. These data indicate that melatonin levels may exhibits as diagnostic performance biomarker for identifying hypopatients with thyroid dysfunction from control individuals.

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Table (3): Roc curve of Melatonin levels

Characteristic	Hyperthyroidism / control	Hypothyroidism / control
Cutoff value	> 3.98	> 3.76
P value	0.001	0.001
Sensitivity %	76.7 %	73.3 %
Specificity %	80.0 %	70.0 %
PPV %	79.3 %	71.0 %
NPV %	77.4 %	72.4 %
AUC (95% CI)	0.761 (0.636- 0.887)	0.718 (0.587- 0.849)

CI: Confidence interval, AUC: Area under curve.

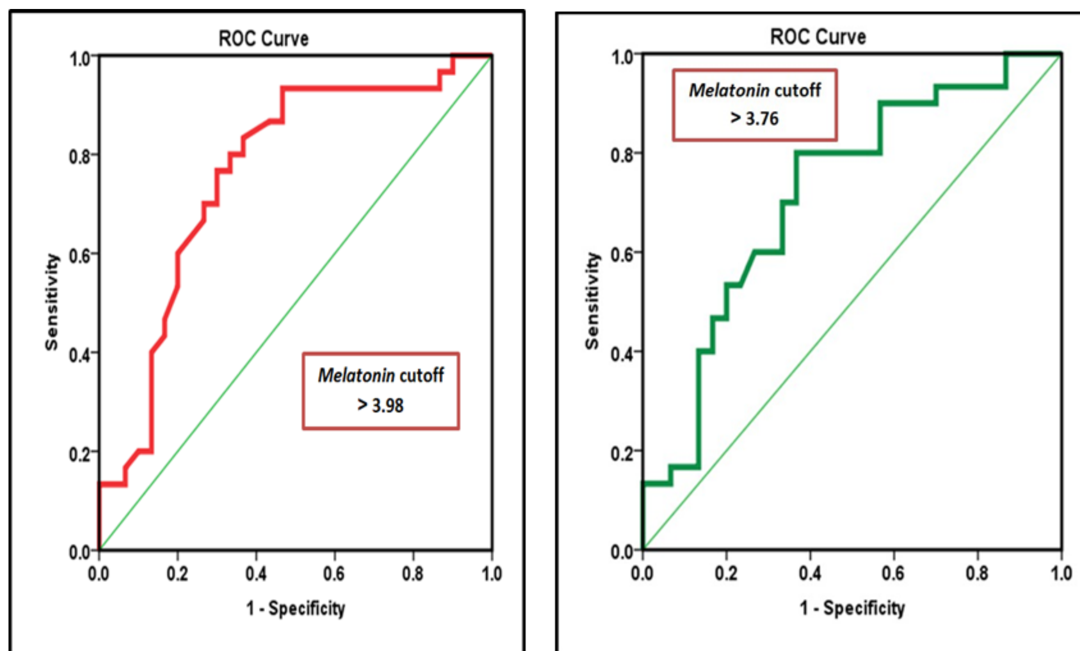

Figure 2: ROC curves for serum melatonin levels. Red curve represents melatonin's diagnostic performance for hyperpatients with thyroid dysfunction, while green curve refers to hypopatients with thyroid dysfunction versus controls levels.

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Serum TNF-α levels in participants

Table 4 presents, serum TNF-α levels were elevated in both thyroid dysfunction groups relative to controls group, with the highest level detected in hyperthyroid patients (23.56 ± 11.77 pg/mL), followed by hypothyroid patients (19.72 ± 12.05 pg/mL). The control group showed the lowest levels (15.15 ± 9.18 pg/mL). Although this pattern indicates increased inflammatory activity in thyroid disease, the differences between groups were not statistically significant ($P = 0.101$), demonstrating that, within the current sample size, the variation in TNF-α levels may represent random fluctuation in contrast to a consistent pathological elevation attributed to thyroid dysfunction.

Table-4: TNF-α levels in patients and control groups.

Groups		TNF-α levels
Hyperthyroidism	Mean ± SE	23.5616±11.774
Hypothyroidism	Mean ± SE	19.720 ± 12.05126
Control	Mean ± SE	15.1507 ± 9.17732
p-value		P=0.101

Figure 3 demonstrates the comparative analysis of TNF-α levels among hyperthyroid patients, hypothyroid patients, and control group. The bar graph exhibits that TNF-α levels are increased in patients with thyroid dysfunction highest in the hyperthyroid followed by the hypothyroid patients' groups, while the control group has the lowest level. Although this pattern indicates a potential association between thyroid dysfunction and Systemic inflammatory response.

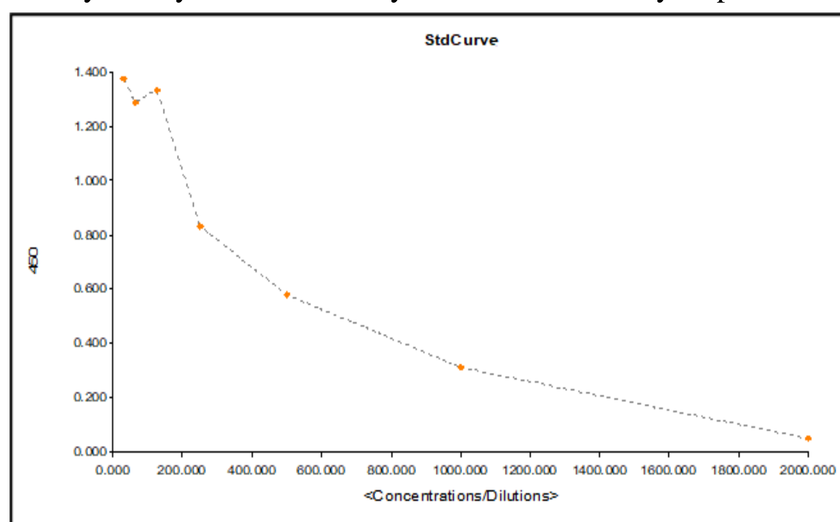

Figure 3: TNFα level between patients and control

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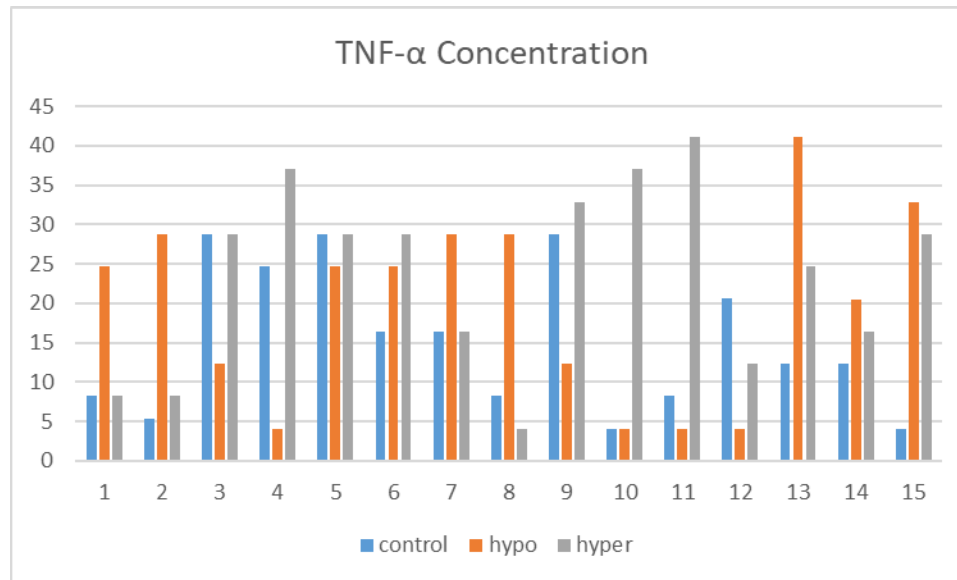
Figure 4 presents a comparative analysis of TNF-α levels between the combined patient and the control groups, that shows that patients with thyroid dysfunction show higher levels of TNF-α compared to controls, indicating a potential increase in systemic inflammatory response among patients with thyroid dysfunction. Although, this observed elevation, the difference does not reach statistical significance ($p = 0.101$), suggesting that while there may be a trend toward increased inflammation in thyroid disease, it is not conclusively supported by the current results.

Figure 4: comparative between the patient and control group



DISCUSSION

Thyroid dysfunction is often considered a silent condition due to its subdued symptoms, demanding attentive observation. Currently, thyroid enlargement is one of the most prevalent clinical presentation, with a prevalence of 4–7% in the study population. The primary cause of these disorders is dysregulation of thyroid hormone production, which is critical to metabolism, mental health, and muscle strength [17].

Sex based differences in thyroid dysfunction have been documented distinct from patterns observed in metabolic abnormalities. Various thyroid conditions, including Hashimoto's thyroiditis, occur conditions in females, with rising incidence with age in both sexes [16]. Previous studies reported significantly higher rates of hypothyroidism and hyperthyroidism in females compared to males [18,19]. However, Najeb and Mohammed reported no significant difference in sex distribution among hypothyroidism, hyperthyroidism, and control groups [20].

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One of the most prevalent endocrine disorders, according to worldwide surveys on illness prevalence, is thyroid abnormalities, which are far more common in women (25%) than in men (0.6%) [21]. Several studies have repeatedly demonstrated that thyroid conditions are more common in women, especially among middle age group (27–29 years). The results of Habash study indicated that female patients had more thyroid dysfunctions, than male patients which is consistent with our findings. Sex hormones including progesterone and estrogen are thought to affect thyroid function and autoimmunity, and their modulatory actions may be the cause of this gender variation gap [17].

In our study in table 2 demonstrated significantly increased in patient than controls; the elevated serum melatonin levels observed in the current study in patients with thyroid disorders can be explained by several interconnected mechanisms. Because melatonin is a highly effective free radical scavenger capable of stimulating antioxidative enzymes while inhibiting prooxidative ones, its elevation may reflect a compensatory protective response by the pineal gland. While according to Kader et al. [22] in comparison to the control group, melatonin hormone levels were significantly lower in the groups with hyperthyroidism and hypothyroidism. Moreover, the hypothyroidism group's melatonin levels were significantly reduced. Since zinc is a necessary trace metal for the catalytic activity of numerous enzymes involved in the metabolism of hormones, including thyroid hormones [23], impaired melatonin levels may be the consequence of decreased zinc levels in hypothyroidism [24]. Zinc also increases the production of melatonin from serotonin by binding to the arylalkylamine N-acetyltransferase (AANA) enzyme, activating it and raising serotonin's affinity for binding to AANAT [25].

Melatonin has strong protective effects on normal cells, according to other findings. By promoting DNA damage responses that lower the likelihood of genomic instability, you should mention what is MLT then write abbreviation (MLT)boosts normal cells' tolerance to the harmful effects of ionizing radiation. Additionally, under normal circumstances, melatonin suppresses autoimmune reactions and lessens the impact of the immune system [11]. Melatonin has been shown in some studies to have an antiapoptotic effect on normal cells, but there is evidence that it either promotes or triggers apoptosis in cancer cells [26, 27]. Although in the current study mean TNF-α concentrations were higher in patients with thyroid dysfunction compared to controls, the differences did not reach statistical significance ($P = 0.101$). Previous studies have demonstrated elevated TNF-α in autoimmune thyroid diseases such as Graves' disease and Hashimoto's thyroiditis, reflecting the pro-inflammatory immune response characteristic of these conditions [1]. The lack of significance variation in our findings may be due to inter-individual variability, disease stage, or insufficient statistical power. Also, Choudhury et al. [28] reported that patients with thyroid dysfunction exhibited significantly higher serum TNF-α compared to healthy controls ($p < 0.05$).

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CONCLUSIONS

Our results demonstrated that serum melatonin levels were significantly increased in both hyper and hypothyroid patients compared to control groups, conforming its function as a potential supplementary biomarker for thyroid dysfunction. Conversely, TNF-α exhibited only a non-significant increase, suggesting a less robust association with thyroid condition in this study sample.

Acknowledgments:

The authors would like to express their sincere gratitude to the Imam Al-Sadiq Hospital in Babylon for their assistance, as well as providing nominated sources to the laboratories of the University of Babylon to take detailed drawings.

Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

تشمل اضطرابات الغدة الدرقية، بما في ذلك فرط نشاط الغدة الدرقية وقصورها، تغيرات أيضية وهرمونية ومناعية. قد تساهم السيتوكينات الالتهابية، مثل عامل نخر الورم ألفا (TNF−α)، والهرمونات المنظمة، مثل الميلاتونين، في الفيزيولوجيا المرضية للمرض. على الرغم من ارتباط أمراض الغدة الدرقية المناعية الذاتية بارتفاع مؤشرات الالتهاب، إلا أن فائدتها التشخيصية في حالات خلل وظائف الغدة الدرقية الأوسع نطاقًا لا تزال غير مؤكدة. تهدف هذه الدراسة إلى تقييم ومقارنة تركيزات الميلاتونين وعامل نخر الورم ألفا (TNF−α) في مصل الدم لدى مرضى الغدة الدرقية ومجموعة ضابطة، وتقييم إمكانية استخدامها كمؤشرات حيوية تشخيصية. شملت هذه الدراسة المقطعية 90 مشاركًا، تم توزيعهم بالتساوي على ثلاث مجموعات: فرط نشاط الغدة الدرقية (ن = 30)، قصور الغدة الدرقية (ن = 30)، ومجموعة ضابطة من الأصحاء (ن = 30). تم قياس تركيزات الميلاتونين وعامل نخر الورم ألفا (TNF−α) في مصل الدم باستخدام مقايسة الامتصاص المناعي المرتبط بالإنزيم (ELISA). ارتفعت مستويات الميلاتونين في مصل الدم بشكل ملحوظ لدى مجموعتي المرضى المصابين بفرط نشاط الغدة الدرقية (5.71 ± 0.84 نانوغرام/مل) وقصور الغدة الدرقية (5.57 ± 1.05 نانوغرام/مل) مقارنةً بالمجموعة الضابطة (3.21 ± 0.31 نانوغرام/مل؛ P < 0.001)، دون وجود فرق ذي دلالة إحصائية بين المجموعتين. وأظهر تحليل منحنى خصائص التشغيل (ROC) أداءً تشخيصيًا مقبولًا في التمييز بين خلل وظائف الغدة الدرقية والمجموعة الضابطة: فرط نشاط الغدة الدرقية، AUC = 0.761، الحساسية = 76.7%، النوعية = 80.0%؛ قصور الغدة الدرقية، AUC = 0.718، الحساسية = 73.3%، النوعية = 70.0%. ارتفع متوسط تركيز عامل نخر الورم ألفا (TNF−α) في مصل الدم لدى مرضى فرط نشاط الغدة الدرقية (23.56 ± 11.77 بيكوغرام/مل) ومرضى قصور الغدة الدرقية (19.72 ± 12.05 بيكوغرام/مل) مقارنةً بالمجموعة الضابطة (15.15 ± 9.18 بيكوغرام/مل)، إلا أن هذه الفروقات لم تكن ذات دلالة إحصائية (P = 0.101). ان مستوى الميلاتونين أعلى بشكل ملحوظ لدى مرضى فرط نشاط الغدة الدرقية وقصورها، مما يشير إلى إمكانية استخدامه كمؤشر حيوي مساعد لاضطرابات الغدة الدرقية. أظهر عامل نخر الورم ألفا (TNF−α) اتجاهًا غير دال إحصائيًا نحو الارتفاع، مما يدل على احتمال وجود دور التهابي يستدعي مزيدًا من الدراسات للتحقق من ذلك.

الكلمات المفتاحية: الغدة الدرقية، T3، TSH، T4، الميلاتونين.