Interleukin-37 and Galectin-9: Suggested Biochemical Tools for Diagnosis of Untreated Thyroidopathies

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Abstract

Background: Thyroid disorders are amongst the most dominant of medical conditions after diabetes disease, especially in women, it affects about 200 million people worldwide. Thyroid disorders are commonly separated into two major types, hyperthyroidism and hypothyroidism, depending on whether serum thyroid hormone levels (T4 and T3) are increased or decreased, respectively. Thyroid disease generally may be sub-classified based on etiologic factors, physiologic abnormalities, and also involved goiter and thyroid cancer.

Materials and Methods: 90 persons were participated in the current study, they were classified into two group, patients and healthy individuals. Patients group included 30 patients with hypothyroidisms (10 males: 20 females), while the second group included 30 patients with hyperthyroidisms (10 males:20 females). The controls group included 30 individuals (11 males: 19 females) who appeared healthy and whose ages ranged from 30 to 60 years. Sandwich-ELISA method was applied to determine the level of Interleukin-37 and galectin-9 in the serum samples of the study individuals.

Results: The current study aimed to evaluate the levels of interleukin-37 and galectin-9 in sera samples of patients with thyroid disorders (hypothyroidisms and hyperthyroidisms) and healthy controls. The results showed a significant elevation (p=0.000) of the interleukin-37 concentration in both of hypothyroidism and hyperthyroidism patients group comparison to the those in the controls group, while no such variations were noted when the two sickness groups compared together. Outcomes show significant increase (p=0.000) in the interleukin-37 of male patient's subgroup (hypo- and hyperthyroidism) comparison to those of healthy males, same results were recorded when the female subgroups(patients and healthy) compared together. It was noted that galectin-9 concentration increases in the patients'groups compassion to healthy individuals, moreover; the highly elevations of this parameter was shown in the hyperthyroidism group. Statistically, highly significant differences were illustrated when the three study's groups when compared together (p=0.000, p=0.046), in addition; the results did not record statistical variations between both sexes within study groups (either patientsor controls) on the other side, the outcomes have been shown a significant increase (p=0.000) of galectin-9 levels in the males' as well as females' patients subgroups when compared together or when compared to their peers in the control subgroups. The sensitivity of interleukin-37 was 97% in the hypothyroidism cases, while, the specificity of interleukin-37 was 100%. The sensitivity of galectin-9 in the hyperthyroidism was 100% and the specificity of galectin-9 was 90%. The combined specificity of interleukin-37 and galectin-9 reached to 100% when they were studied together in both hypo- and hyperthyroidism.

Conclusion: Interleukin-37 and galectin-9 can be used as clinical tools to diagnose thyroid disorders before receiving treatments and can also replace routine examinations in monitoring response to treatment.

Key words: Interleukin-37, Galectin-9, Hypothyroidism, Hyperthyroidism.

Introduction

Thyroid gland is the largest endocrine gland in the human body [1], is located in the lower front part of the neck [2]. It weighs about 25g [3], Its size increasing during pregnancy and menstruation [4]. Responsible for regulating body temperature, as well as the metabolic rate, in addition to the development of the child's neurocognition [5,6]. Thyroid disorders are the most common type of endocrine disease after diabetes [7]. It affects about 200 million people worldwide [8]. There are about 1.6 billion people at risk of developing thyroid disorders distributed over 110 countries [9]. Common causes of thyroid disorders include iodine deficiency, autoimmune diseases, geographical factors and race [10]. Risk factors include age, gender, race, geographic factors, and radiation exposure, as the level of thyroid

disorders increases with age, and women are more likely to develop thyroid disorders than men [10]. Symptoms of thyroid disorders include sleep disturbances, weight gain, weight loss, hand tremors, constipation, diarrhea, dry skin, bradycardia, irregular menstruation, intolerance to cold and intolerance to hot [11].

Interleukin-37 is a protein with a molecular weight of 17-25 kDa, with five isoforms in humans [12]. It belongs to the interleukin-1family of cytokines and it is considered the seventh member of it [13, 14]. It was discovered in the year 2000 [15], using silico methods in human cells [14], previously; it was known as interleukin-1F7 [16]. It has anti-inflammatory functions that control key immune pathways and adaptive and innate infections, as well as signaling through pattern recognition receptors such as Toll-like receptors (TLRs), tumor necrosis factor (TNF), interleukin-1, in addition to the adaptive type I, II, and III immune response, because of its powerful functions, it has been called the peacemaker [12]. Interleukin-37 is found in the uterus, skin, placenta, testis, kidneys, thymus gland, lymph nodes, heart, bone marrow and lung. It can reduce the immune response by both intracellular and extracellular inhibition by reducing anti-inflammatory secretion, however, it's role in autophagy is currently unknown [17]. Interleukin-37 is located on chromosome 2q12-13. It has an important role in pathophysiology, including enteritis and lupus erythematosus also have a role in the development of cancer [18]. It also has a distinctive role in suppressing of breast, lung, cervical and skin cancers [19].

Galectin-9 is a galectin bound to a β-galactoside with a 34-36 KD a molecular weight [20].

Contains two CRDS [21]. Galctin-9 is encoded by the *LGALS9* gene located at 17q11.2, as well as two *LGALS9*-like genes (*LGALS9B* and *LGALS9C*) located at (17p11.2) [22]. It is found in the lung, liver, kidneys, bladder, digestive system, endocrine tissues, gallbladder, female tissues [23]. The small intestine and the thymus gland [24], also in the nucleus, plasma membrane, cytosol [25], brain and retinal cells [24]. It has an important role in apoptosis and has multiple biological functions [26], cell proliferation and inhibition of cancer cell proliferation [27].

Materials and Methods

The Study Individuals: throughout five months, from the beginning of March 2023 to the beginning of August 2023, 90 persons were participated in the current study. These participants were classified into two group, depending on their health status (patients and healthy individuals) and the type of disorder suffered by the study patients. The first (patients) group included 60 patients with hypothyroidisms (10 males: 20 females), their ages ranged between 30 and 60 years, while the second group included 30 patients with hyperthyroidisms (10 males:20 females), whose ages ranged between 30-60 years. 17 patients in the group of patients with hypothyroidism were diagnosed with Hashimoto's disease (13 females and 4 males), while the group of patients with hyperthyroidism included 12 patients with Creve's disease (9 females and 3 males). The controls group included 30 individuals (11 males: 19 females) who appeared healthy and whose ages ranged from 30 to 60 years.

Limitations of the Study: the current study required exclusion the following cases: All participators (patients with breast tumors or healthy controls) who had suffered chronic diseases, *i.e.*; liver, renal, cardiovascular diseases, diabetes, hypertension and morbid obesity from participating in the current study, smoker, cases who underwent surgical intervasive within 5 years, patients taking treatment for thyroid disorders, patients who have had their thyroid gland completely or partially removed, Persons who take protein supplements, pregnant.

Samples Collection: 5 milliliters of venous blood samples were collected from the study subjects (patients and healthy ones) using gel tubes. After separating the serum from the study samples using a centrifuge at 5000 xg for 5 minutes. Serum samples were preserved using Eppendorf tubes at -20°C and stored until use.

Assessment of interleukin-37 and galectin-9 in the study groups: Sandwich Enzyme Linked Immune Sorbent Assay (Sandwich-ELISA) method was applied to determine the level of Interleukin-37 and galectin-9 in the serum samples of the study individuals.

Statistical analysis of the data: outcomes of the present study were analyzed through the statistical package for the social sciences (SPSS) version 26 software application statistical analysis system and excel (statistical package). The variables were illustrated by Mean±SE, minimum, maximum, frequencies,

percentages and cumulative percentages. Rock curve was applied to present the sensitivity of the evaluated parameters. Inferential data analysis included: One way analysis of variance (ANOVA) test was applied for examining the probable variations among the evaluated biochemicals. The probability of deflection than controls are considered statistically significant if *p*-value is below 0.05. Sensitivity and specificity percentage were calculated according to biomedical statistical.

Results and Discussion

Interleukin-37 levels in the study groups: Interleukin-37 levels were measured in the sera samples of the study groups. The results showed a significant elevation (p=0.000) of the interleukin-37 concentration in both of hypothyroidism and hyperthyroidism patients group comparison to the those in the controls group, while no such variations were noted when the two sickness groups compared together, as shown in **Table 1**.

Table 1: Levels of Interleukin-37 (ng/L) in the Sera Samples of Patients with Thyroid Disorders and Controls

Parameter	Subjects (N) Mean ± S.E. Minimum-Maximum			*p-value*
	***Hypothyroidism* 30**	***Hyperthyroidism* 30**	***Controls* 30**	
Interleukin-37 (ng/L)	25.988±1.160 0.684-36.619	23.753±1.843 1.315-39.621	1.223±0. 056 0.937-2.374	**0.212 For G1 *vs* G2** **0.000 For G1 *vs* C** **0.000 For G2 *vs* C**

G1: Group of Hypothyroidism, G2: Group of Hyperthyroidism, C: Group of Healthy Controls, The difference is considered significant at p<0.05.

Results of the current work showed there was no significant differences in the interleukin-37 levels between male and female when the comparison was done in the same group (weather patients or controls). **Table 2** show significant increase (p=0.000) in the interleukin-37 of male patient's subgroup (hypo- and hyperthyroidism) comparison to those of healthy males, same results were recorded when the female subgroups (patients and healthy) compared together. The highest levels of interleukin-37 (39.621 ng/L) were recorded in the hyperthyroidism female patients subgroup, and it was for a woman in the sixth decade, while the lowest value was recorded (0.684 ng/L) for a hypothyroidism female patient at 49 years old.

Table 2: Levels of the Serum Interleukin-37 in the Two Study Sexes

***Subjects* (n)**	***Sex* (n)**	***Interleukin-37 (ng/L)* Mean ± S.E.**	***Minimum-Maximum***	***p-value***
***Hypothyroidism* (30)**	**Male 10**	**26.095±1.116**	**22.108-31.470**	0.953 **For 1 *vs* 2** 0.387 **For 3 *vs* 4** 0.946 **For 5 *vs* 6** 0.214 **For 1 *vs* 3** **0.000 For 1 *vs* 5** **0.000 For 3 *vs* 5** 0.528 **For 2 *vs* 4** **0.000 For 2 *vs* 6** **0.000 For 4 *vs* 6**
	Female 20	**25.934±1.669**	**0.684-36.619**	
***Hyperthyroidism* (30)**	**Male 10**	**22.187±3.838**	**1.574-35.829**	
	Female 20	**24.536±2.047**	**1.315-39.621**	
	Male	**1.336±0.122**	**0.979-2.374**	

Controls (30)	**11**			
	Female 19	**1.158±0.052**	**0.937-1.892**	

1: Males wit Hypothyroidism, 2: Females with Hypothyroidism, 3: Males with Hyperthyroidism, 4: Females with Hyperthyroidism,5: Healthy Males, 6: Healthy Female. The difference is Considered Significant at p<0.05.

Cytokines are produced by immune and non-immune cells [28, 29] to reduce inflammatory reactions during innate and adaptive immune responses through intracellular and extracellular inhibition by reducing the secretion of pro-inflammatory chemokines [30, 31]. Interleukin-37 is a natural inhibitor of the innate immune response. It also has a role in stimulating inflammation. It is also able to enhance anti-inflammatory pathways. There is a close relationship between thyroid function and inflammation in the elderly [32]. Interleukin-37 has been implicated in the pathogenesis of autoimmune diseases including Graves disease, it is regulated and increased with the severity of the disease [33]. Interleukin-37 has an important role in influencing the response of cells, including Th1 and Th17. It has a role in the development of autoimmune diseases, including Hashimoto's disease [34]. Oxidative stress is one of the causes of autoimmune diseases, interleukin-37 is expected to be secreted in response to oxidative stress, in Hashimoto's disease, interleukin-37 has a protective role by counteracting oxidative stress and inflammation [34]. Interleukin-37 can reduce the activity of proinflammatory signals through interaction with receptors (interleukin-1R8 and interleukin-18 Rα) on the cell surface [28]. One study indicated the negative effect of treatment on the level of interleukin-37, where it was noted that its levels decreased in patients with Graves who were treated with RAI and CMZ [28]. It has the ability to reduce excessive inflammation, as it is expressed differently in patients with autoimmunity, cancer, and infections. Interleukin-37 helps improve excessive autoimmune responses in Hashimoto's patients [35]. Interleukin-37 has been shown to play a role in the development and progression of thyroid disorders, such as Graves disease and Hashimoto's thyroiditis. In Graves disease, interleukin-37 levels are elevated in the blood and thyroid tissue, due to the overproduction of thyroid hormones, which can lead to inflammation. Interleukin-37 may help to reduce inflammation and protect the thyroid gland from damage [36]. In Hashimoto's thyroiditis, interleukin-37 levels are decreased in the blood and thyroid tissue due to the destruction of thyroid cells by the immune system. The decrease in interleukin-37 levels may contribute to the inflammation and damage that occurs in Hashimoto's thyroiditis [37]. Recent evidence suggests that there is cross-talk between interleukin-37 and thyroid hormones in modulating chronic inflammation. For example, interleukin-37 has been shown to increase the expression of thyroid hormone receptors in macrophages. Thyroid hormones, in turn, can upregulate the expression of interleukin-37 [31]. The mutual relationship between interleukin-37 and thyroid hormones may be important in protecting target organs from damage in age-related metabolic and vascular conditions, for example, studies have shown that interleukin-37 can protect against cardiovascular disease, diabetic nephropathy, and Alzheimer's disease. Thyroid hormones have also been shown to protect against cardiovascular disease and Alzheimer's disease [38, 39]. Expression of interleukin-37 is induced in response to pro-inflammatory stimuli to control inflammation and limit excessive tissue damage [40]. It is known that there is an imbalance between antioxidants and free radicals in Hashimoto's patients, and this means an increased level of oxidative stress, which indicates that interleukin-37 production increases in response to oxidative stress [41].

Evaluation of galectin-9 levels in the sera samples of thyroid disorders cases and healthy individuals: galectin-9 levels were appraised in thyroid disorderspatients and healthy subjects. It was noted that galectin-9 concentration increases in the patients'groups compassion to healthy individuals, moreover; the highly elevations of this parameter was shown in the hyperthyroidism group. Statistically, highly significant differences were illustrated when the three study's groups when compared together (p=0.000, p=0.046), as shown in **Table 3**.

Table 3: Levels of Galectin-9 (ng/L) in the Sera Samples of the Study Groups

Parameter	Subjects (N) Mean ± S.E. Minimum-Maximum			*p-value*
	***Hypothyroidism* 30**	***Hyperthyroidism* 30**	***Controls* 30**	
Galectin-9 (ng/L)	15.738±0.599 12.243-31.089	47.441±3.908 20.890-108.144	9.161±0.453 4.638-13.830	**0.000 For G1 *vs* G2** **0.046 For G1 *vs* C** **0.000 For G2 *vs* C**

G1: Group of Hypothyroidism, G2: Group of Hyperthyroidism, C: Group of Healthy Controls, the difference is considered significant at p<0.05

Rresults of the present study did not record statistical variations between both sexeswithin study groups (either patientsor controls). On theother side, the outcomes have been shown a significant increase (p=0.000) of galectin-9 levels in the males' as well as females' patients subgroups when compared together or when compared to their peers in the control subgroups, as clarified in **Table 4**. The focus observation indicated that the highest concentration of galectin-9 (108.144 ng/L) was recorded in thehyperthyroidism femalepatients, while the lowest concentration of this protein (4.638 ng/L) was noted in the sample of healthy female.

Table 4: Levels of Galectin-9 (ng/L) in the Sample of the Study Individuals

Subjects (n)	*Sex (n)*	*Galectin-9 (ng/L) Mean ± S.E.*	*Minimum-Maximum*	*p-value*
***Hypothyroidism* (30)**	**Male 10**	**15.076±0.461**	**13.481-17.435**	0.842 **For 1 *vs* 2** 0.676 **For 3 *vs* 4** 0.755 **For 5 *vs* 6** **0.000 For 1 *vs* 3** 0.378 **For 1 *vs* 5** **0.000 For 3 *vs* 5** **0.000 For 2 *vs* 4** 0.072 **For 2 *vs* 6** **0.000 For 4 *vs* 6**
	Female 20	**16.069±0.868**	**12.243-31.089**	
***Hyperthyroidism* (30)**	**Male 10**	**46.059±6.371**	**22.444-77.895**	
	Female 20	**48.132±5.028**	**20.890-108.144**	
***Controls* (30)**	**Male 11**	**10.121±0.561**	**7.287-13.830**	
	Female 19	**8.605±0.612**	**4.638-13.597**	

1: Males wit Hypothyroidism, 2: Females with Hypothyroidism, 3: Males with Hyperthyroidism, 4: Females with Hyperthyroidism,5: Healthy Males, 6: Healthy Female. The difference is Considered Significant at p<0.05.

These results were consistent with Leskela's study using qRT-PCR that showed thyroid tissue increased galectin-9 levels in patients with Hashimoto's disease and Graves disease, galectin-9 has a suppressive effect on the release of TH1, TH2, and TH17 cytokines. Through its ability, it can be said that it is likely to be involved in the pathogenesis of autoimmune thyroid diseases [42]. Galectins, are responsible for regulating the immune response and regulating the cell cycle and inflammation, so, galectin-9 can regulate immunosuppressive immune

cell by promoting differentiation and regulatory expansion of T cells by a cluster of differentiation 44 (CD44), galectin-9 has an important role in inflammatory diseases [43], helps stimulate programmed cell death [44]. Galectin-9 also prevents autoimmune conditions by facilitating timely shutdown of the adaptive immunity. The physiological changes caused by galectin-9 are associated with changes in its concentration inside and outside cell, skewing signals by neighboring cells, and the cell surface receptors available for binding, galectin-9 assists in ROS production and neutrophils degranulation through a Tim-3 dependent pathway and impairs the cytotoxicity and cytokine production efficiency of NK cells *via* Tim-3 independent pathway, galectin-9 actively participates in the adaptive immune response by regulating T-cell development and homeostasis. Galectin-9 plays critical role [45]. Research has shown that galectin-9 levels may be altered in AITDs, for example, one study found that patients with Graves disease had lower levels of galectin-9 in their peripheral blood dendritic cells than healthy controls. Another study found that galectin-9 levels were increased in the thyroid tissue of patients with Hashimoto's thyroiditis [46].

Relationship of interleukin-37 to galectin-9: results of the current work show an increase in the levels of interleukin-37 Galectin-9 in thyroid disorder and healthy individuals groups as illustrated in **Table 5**.

Table 5: Relationship of Interleukin-37 to Galectin-9

Parameter	Subjects					
	Hypothyroidism		*Hyperthyroidism*		*Controls*	
Galectin-9	*r*	*p*	*r*	*p*	*r*	*p*
	0.634**	**0.000**	**0.859****	**0.000**	**0.775****	**0.000**

***** Correlation is significant at the 0.01 level***

The investigation in previous researches did not find any evidence to evaluate the relationship between interleukin-37 with galectin-9 in patients with thyroid disorders.

Efficiency of interleukin-37 and galectin-9 in the diagnosis of thyroid disorders: In order to illustrates the diagnostic ability of interleukin-37 and galectin-9 for thyroid disorders, ROC-AUC curve was tested. **Table 6** illustrated that the sensitivity of interleukin-37 was 97% when 30 of hypothyroidism cases were illustrated interleukin-37 levels higher than mean of these criteria recorded in the healthy controls, while, the specificity of interleukin-37 was 100% when 30 of hypothyroidism cases had been registered interleukin-37 levels higher than the cutoff value. The sensitivity of galectin-9 was 100% when 30 of hyperthyroidism cases were illustrated galectin-9 levels higher than mean of these criteria recorded in the healthy controls. The specificity of galectin-9 was 90% when 30 of hyperthyroidism cases had been registered galectin-9 levels higher than the cutoff value. **Table 8** shows the individual sensitivity of the evaluated criteria in the current study for distinguishing hyperthyroidism, the highest sensitivity and specificity (100%) was recorded in galectin-9. The combined specificity of interleukin-37 and galectin-9 reached to 100% when they were studied together., as **Table 7** and **Table 9** showed.

Table 6: Receiver Operating Characteristic Analysis of the Interleukin-37, Galectin-9, as Diagnostic Markers for Hypothyroidism

Parameters	*AUC*	*SE*	*p-value*	*Cutoff value*	*Sensitivity%*	*Specificity%*	*CI (95%)*
Interleukin-37	**0.967**	**0.033**	**0.000**	**9.953**	**97**	**100**	**0.902-1.000**
Galectin-9	**0.987**	**0.010**	**0.000**	**12.107**	**100**	**90**	**0.967-1.000**

AUC: Area Under Curve, SE: Standard Error

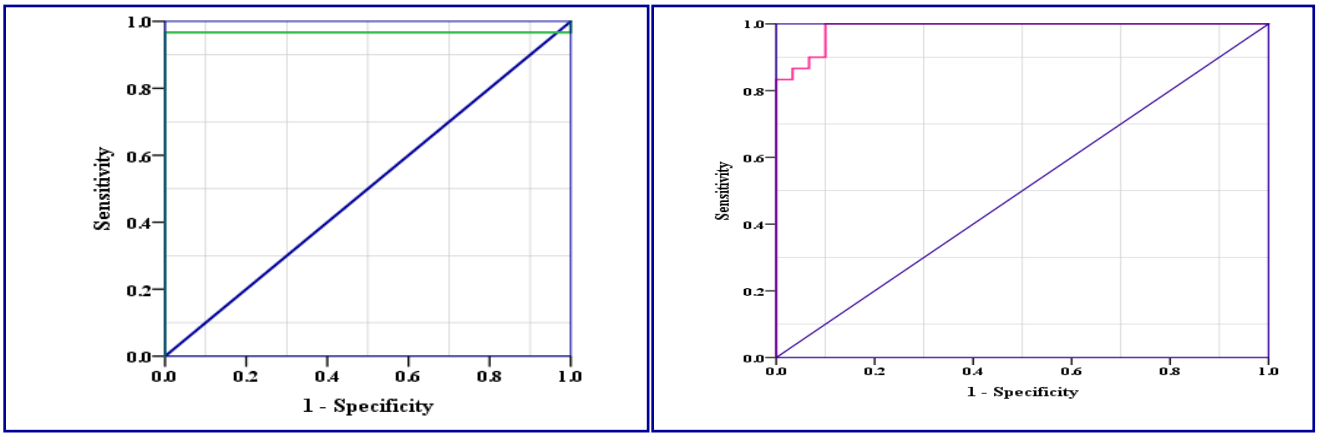

Figure 1: Receiver Operating Characteristic Curve of (A) Interleukin-37 (B) Galectin-9 in the Hypothyroidism

Table 7: The Individual and Combined Sensitivity of the Evaluated Parameters in Group of Hypothyroidisms

Parameters	*Interleukin-37*	*Galectin-9*
Interleukin-37	97	100
Galectin-9	100	100

Table 8: Receiver Operating Characteristic Analysis of the Interleukin-37, Galectin-9, as Diagnostic Markers for Hyperthyroidism

Parameters	*AUC*	*SE*	*p-value*	*Cutoff value*	*Sensitivity%*	*Specificity%*	*CI (95%)*
Interleukin-37	0.988	0.010	0.000	5.675	90	100	0.968-1.000
Galectin-9	1.000	0.000	0.000	17.36	100	100	1.000-1.000

AUC: Area Under Curve, SE: Standard Error

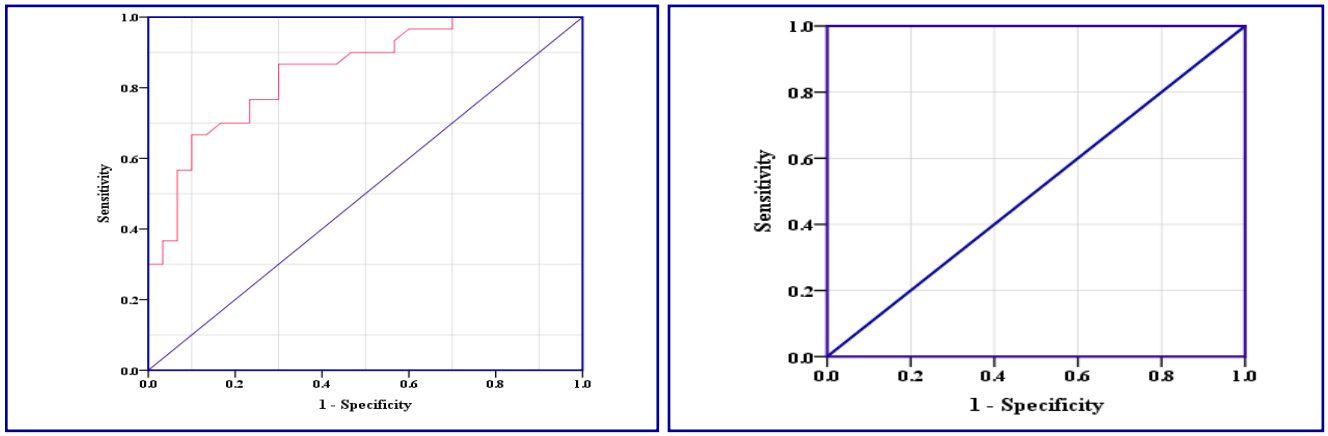

Figure 2: Receiver Operating Characteristic Curve of (A) Interleukin-37 (B) Galectin-9 in the Hyperthyroidism

Table 9: The Individual and Combined Specificity of the Evaluated Parameters in Group of Hyperthyroidisms

Parameters	*Interleukin-37*	*Galectin-9*
Interleukin-37	100	100
Galectin-9	100	90

Conclusions

Interleukin-37 and galectin-9 can be used as clinical tools to diagnose thyroid disorders before receiving treatments and can also replace routine examinations in monitoring response to treatment.

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