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Interleukin-34: A New Immunological Marker to Enhance the Initial Diagnosis of Thyroid Disorders

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Abstract

Background:*Thyroid disorders are divided into two types: hypothyroidism and hyperthyroidism, The prevalence of hypothyroidism is increased in India, Iraq followed by China, the Kingdom of Saudi Arabia, Europe, and America. As for, the prevalence is increasing in the north and Baghdad, while the prevalence of hyperthyroidism is increased in Sweden, the United States, and Europe. In Iraq, the prevalence of hyperthyroidism is increased in Basra, Nasiriyah, Baghdad, and Dohuk. The current study aimed to innovate a biochemical relationship between IL-34 and thyroid disorders and to evaluate the levels of (TSH, T4, T3, FT3, FT4, anti-TPO, anti-Tg) in sera samples of patients with thyroid disorders (hypothyroidisms and hyperthyroidisms) and healthy controls.* ***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 their ages ranged from 30 to 60 years. Sandwich-ELISA method was applied to determine the level of interleukin-34 in the serum samples of the study individuals. ***Results:*** *The results showed a significant elevation* ***(p=0.000)*** *of the TSH, T4, T3, FT4, FT3, anti-TPO, anti-Tg concentration in hypothyroidism patients group comparison to the those in the controls group, the same result was noted when the two patients groups compared together except anti-TG* ***(p=0.07)****, while no noted significant elevation of the TSH* ***(p=0.706)****, anti-TPO* ***(p=0.282)*** *when comparing between hyperthyroidism group and healthy control group. The results showed a significant elevation* ***(p=0.000)*** *of the interleukin-34 level in hypothyroidism patients group comparison to the those in the controls group, the same result was reported when the two sickness groups compared together and when compared between hyperthyroidismgroup and controls group.*

Key Words: Interleukin-34, Thyroid Disorders, TSH, anti-TPO, anti-TG,

Introduction

Hypothyroidism is a common problem worldwide, National Health and Nutrition Examination SurveyIII (NHANESIII) study found the prevalence of overt hypothyroidism among adults in the United States (12 years of age and older) to be 0.3% and subclinical hypothyroidism 4.3% [1]. Many studies reporting the prevalence of hypothyroidism worldwide with a range of 0.2%–5.3% in Europe,whilein adult Chines was 13.95% [2] and in Iran it composed 0.2% [3]. A study conducted in Libya found that the prevalence of hypothyroidism to be 6.18% and in certain areas of Saudi Arabia Kingdom the prevalence was reported to be 47%. In India, it was noted a significant variability in the rate of hypothyroidism depending on geographical location, with

coastal regions having lower rates compared to inland areas, while in Africa remains unknown due to the limited number of studies as well as the underreporting of cases [4] increasing age were associated with higher TSH and the prevalence of antithyroid antibodies. Hypothyroidism is more prevalent in women with small stature at birth and low BMI during childhood [1].

Iraq is a country with low iodine supplyand endemic goiter is prevalent. A greater prevalence of endemic goiter was found in northern area of Iraq and Baghdad [5]. Prevalence of hyperthyroidism in worldwide ranges from 0.2-1.3%, with sufficient iodine intake[6]. In the United States, is approximately 1.2% (0.5% overt and 0.7% subclinical) [7]. In Australia, the prevalence of subclinical and overt hyperthyroidism is 0.3% [8], 0.8% in European [9]. The prevalence of hyperthyroidism was estimated to be 2% in women and 0.2% in men, with an annual incidence of 20/1000000 in the general population, and a male: female ratio of 1: 5 – 7 in the general population [10]. In Sweden have reported an incidence between 25.8 and 43/100000 inhabitants per year [11].

For Graves' disease, the onset is most common between the ages of 20 and 50, and more often in women [10]. The incidence of Graves' disease is higher in African Americans than in non-Hispanic Caucasians [12]. In Iraq, prevalence of hyperthyroidism in Basra is about 67.7%in women, as for subclinical hyperthyroidism, it reaches 8.4% [13]. In Nasiriya City the prevalence of hyperthyroidism is about 64% in females and about 36% in males [14]. While its prevalence in Duhok about 0.31%, asfor as subclinical hyperthyroidism it reaches 2.2% [15]. In Baghdad about 18.3% in males and the highest relevance rate was 81.7% in females [16].

Materials and Methods

Patients' selection and exclusion criteria: the current study required exclusion the following cases: All participators (patients with thyroid disorders 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, and pregnant women.

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 diagnosis parameters (TSH, T4, T3, FT3, FT4, anti-TPO, anti-TG) in the study groups: ECLIA method was applied to determine the level of (TSH, T4, T3, FT3, FT4, anti-TPO, anti-TG) in the serum samples of the study individuals.

Assessment of interleukin-34 in the study groups: Sandwich-ELISA method was applied to determine the level of interleukin-34 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

Levels of serum TSH were measured in the three studied groups; hypothyroidism and hyperthyroidism as well as healthy individuals. Results of table 1 have shown significant statistical difference (**p=0.000**) in TSH levels when comparing hypothyroidism and hyperthyroidism groups together. Similarly, a significant difference was recorded between hypothyroidism group and control group, while no significant statistical difference **(p=0.706)** was reported between hyperthyroidism and healthy controls group. **Table 1** has shown significant difference in the levels of (T4, T3, FT4, FT3, Anti-TPO) whencomparing hypothyroidism and hyperthyroidism groups together, the same results noticed when comparing hypothyroidism group with control healthy group. No significant difference **(p=0.070)** in anti-TG levels when comparing between hypothyroidism and hyperthyroidism groups together as shown in **Table 1.**

Levels of serum Interleukin-34 were measured in the three studied groups; hypothyroidism and hyperthyroidism as well as healthy individuals. **Table 2** shows that there is significant statistical difference (**p=0.000**) in interleukin-34 levels when comparing hypothyroidism and hyperthyroidism groups. Same results **(p=0.000)** were gained when comparing the group of thyroid disorders patients (hypothyroidism and hyperthyroidism) to the healthy group.

When comparing subgroups (based on the gender of the group members), statistical analysis showed that there were significant differences in the concentration of interleukin-34 when comparing males with hypothyroidism with those with hyperthyroidism (**p=0.022**), as well as with healthy males (**0.000**), in addition, similar results appeared when comparing hyperthyroidic male patients and healthy males (**p=0.013**). The results of the current work were more clear when comparing females subgroups together. The study showed a significant increase in the concentration of interleukin-34 in the group of hypothyroidic female patients compared to those in the hyperthyroidic subgroup (**p=0.000**), as well as those in the healthy females subgroup (**p=0.000**). Remarkably, the results of the present study came to confirm the presence of

significant differences in the levels of interleukin-34 in hyperthyroidism female patients compared to their counterparts in the control group (**p=0.000**). Moreover, the results of the current work demonstrated that there are no differences in the level of interleukin-34 when comparing females to males in one group, as shown in **Table 3.**

Cytokines are proteins with low molecular weights that are produced by a group of cells, and they are involved in inflammation and immunity [17].There are many factors that affect the level of cytokines, including age, the treatment used, as well as demographic and clinical variables [18]. Cytokines contribute to cancer, infection, inflammation, and autoimmunity [19]. It is well known that interleukin-34 is a cytokine mainly produced by macrophage, monocyte, and microglia. Interleukin-34 is involved in inflammation as an inflammatory cytokine. Interleukin-34 is derived from osteoblasts, a co-receptor linked to the macrophage colony-stimulating factor receptor (CSF-1R) [20], and has the same properties as colony-stimulating factor in cell regulation, differentiation, and proliferation [20]. As for autoimmune diseases, it was noted that interleukin-34 involved in the development of many diseases, as its high level was observed in many autoimmune diseases such as SLE, rheumatoid arthritis, systemic sclerosis (SSc) and inflammatory bowel diseases. The level of interleukin-34 in patients' serum depends on the severity, duration, and progression of the disease. By future, it could be a new diagnostic and prognostic biomarker for the disease, and it could be an important and powerful target for therapeutic intervention, also it has been suggested that it could be a biomarker for autoimmune diseases [21, 22].

The results of the present study came is in agreement with a previous study conducted in 2018, in which it was observed that the level of interleukin-34 decreased in Hashimoto's patients, and it was also able to resist programmed cell death in Hashimoto's patients. This study hypothesized that interleukin-34 could be a predictor for assessing thyroid damage in Hashimoto's patients. Interleukin-34 may play an important role in the inhibiting inflammatory and chronic diseases [23].

Interleukin-34 is a recently discovered pro-inflammatory cytokine and is a vital regulator in different tumor types. Serum levels of interleukin-34 are significantly increased in patients with rheumatoid arthritis (RA) and correlated with disease severity. In addition, interleukin-34 expression is elevated in the serum and intestine of patients with inflammatory bowel disease (IBD). Several studies have also demonstrated the contribution of interleukin-34 in cancer and demonstrated that interleukin-34 plays a pro-tumorigenic role in the tumor microenvironment. Increased expression of interleukin-34 has been found in patients with different kinds of carcinomas, including breast, lung, ovarian, and blood cancer, and it has been reported that its expression is correlated with the progression of tumor metastasis [24].

Interleukin-34 has been recognized as an anti-virus cytokine during infection with influenza A virus (IAV), hepatitis B Viruses (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV). The high serum level of interleukin-34 was observed in many autoimmune diseases. Many correlation studies assessed the serum level of interleukin-34 and suggested interleukin-34 is a positive biochemical for autoimmune response [21]. Interleukin-34 may promote the production of proinflammatory cytokines, such as TNF-α and interleukin-6, which can damage thyroid cells. Interleukin-1B and interleukin-6 induced expression of interleukin-34 [25]. The evidence suggests that interleukin-34 may be a biochemical marker for prognosis, diagnosing, and response to treatment in inflammatory disorders, infection, cancer, autoimmune disease [21].

It is important to note that the relationship between interleukin-34 and Graves' disease is still partially understood. More researches are needed to fully understand the role that interleukin-34 which plays a key role in many diseases and how it can be used to develop new treatments for Graves' disease. Programmed cell death is regarded as one of the factors responsible for the development of Hashimoto disease, as an increased level of programmed cell death which has been observed in the thyroid tissue of

Hashimoto's patient. Interleukin-34 can reduce apoptosis in the thyroid gland through the interleukin-34/CSF-1R/STAT3 pathway, as a low level of interleukin-34 was observed in Hashimoto's patients, and an inverse relationship was observed between it and the levels of thyroid antibodies [23].

Efficiency of interleukin-34 in the diagnosis of thyroid disorders: In order to illustrates the diagnostic ability of interleukin-34 for thyroid disorders, ROC-AUC curve was tested. **Table 4** illustrated that the sensitivity of interleukin-34 was 93.33% when 28 of hypothyroidism cases were illustrated interleukin-34 levels higher than mean of these criteria recorded in the healthy controls, while, the specificity of interleukin-34 was 90% when 27 of hypothyroidism cases had been registered inerleukin-34 levels higher than the cutoff value. **Table 5** illustrated that the sensitivity of interleukin-34 was 67% when 20 of hyperthyroidism cases were illustrated interleukin-34 levels higher than mean of these criteria recorded in the healthy controls, while the specificity of inerleukin-34 was 90% in hyperthyroidism group.

Table 1: Levels of Thyroid Diagnostic Parameters in the Samples of Thyroid Disorders Patients and Control Groups

Parameter	Subjects (N) Mean ± S.E. Minimum-Maximum			*p-value*
	***Hypothyroidism* 30**	***Hyperthyroidism* 30**	***Controls* 30**	
TSH (µIU/mL)	53.167±6.654 8.320-100.000	0.040±0.010 0.005-0.160	2.094±0.131 0.900-3.300	**0.000 For G1 *vs* G2 0.000 For G1 *vs* C 0.706 For G2 *vs* C**
T4 (µg/dL)	3.295±0.244 0.960-4.930	25.493±1.543 16.710-51.610	10.121±0.233 7.140-12.870	**0.000 For G1 *vs* G2 0.000 For G1 *vs* C 0.000 For G2 *vs* C**
T3 (ng/mL)	0.628±0.031 0.280-1.100	4.881±0.361 2.810-11.800	1.270±0.498 0.900-1.840	**0.000 For G1 *vs* G2 0.035 For G1 *vs* C 0.000 For G2 *vs* C**
FT4 (pmol/L)	7.310±0.645 1.580-11.390	37.058±2.137 25.140-73.470	16.634±0. 334 12.520-20.000	**0.000 For G1 *vs* G2 0.000 For G1 *vs* C 0.000 For G2 *vs* C**
FT3 (pmol/L)	2.273±0. 185 0.700-5.500	9.211±0.361 7.150-15.860	4.218±0.174 2.170-6.130	**0.000 For G1 *vs* G2 0.000 For G1 *vs* C 0.000 For G2 *vs* C**
Anti-TPO(IU/mL)	775.640±155.742 5.000-2000.000	150.903±39.124 5.000-845.000	9.006±0.842 5.000-20.910	**0.000 For G1 *vs* G2 0.000 For G1 *vs* C 0.282 For G2*vsC***
Anti-Tg (IU/mL)	282.803±46.129 10.500-725.000	476.145±120.558 12.040-2000.000	22.208±2.786 10.000-62.430	**0.070 For G1 *vs* G2 0.015 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.

Table 2: Interleukin-34 Levels in the Sera Samples of Patients with Thyroid Disorders and Controls Groups

Parameter	**Subjects (N) Mean ± S.E. Minimum-Maximum**			***p-value***
	Hypothyroidism 30	***Hyperthyroidism 30***	***Controls 30***	
Interleukin-34 (ng/L)	8.207±0.180 6.395-11.142	7.143±0.148 5.849-8.864	6.104±0.114 4.599-7.614	**0.000 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.

Table 3: Levels of Interleukin-34 (ng/L) in the Sample of the Study Individuals

Subjects (n)	***Sex (n)***	***IL-34 (ng/L) Mean ± S.E.***	***Minimum-Maximum***	***p-value***
Hypothyroidism (30)	**Male 10**	8.026±0.260	6.847-9.206	0.401 **For 1 *vs* 2**
	Female 20	8.298±0.239	6.395-11.142	0.930 **For 3 *vs* 4**
Hyperthyroidism (30)	**Male 10**	7.162±0.282	5.958-8.398	0.510 **For 5 *vs* 6**
	Female 20	7.133±0.178	5.849-8.864	**0.022 For 1 *vs* 3**
Controls (30)	**Male 11**	6.236±0.220	4.599-7.614	**0.000 For 1 *vs* 5**
	Female 19	6.027±0.129	4.989-7.241	**0.013 For 3 *vs* 5**
				0.000 For 2 *vs* 4
				0.000 For 2 *vs* 6
				0.000 For 4 *vs* 6

1: Males with 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.

Table 4: Receiver Operating Characteristic Analysis of the interleukin-34 as Diagnostic Markers for Hypothyroidism

	AUC	*SE*	*p-value*	*Cutoff value*	*Sensitivity%*	*Specificity%*	*CI (95%)*
Interleukin-34	0.975	0.016	0.000	6.804	93.33	90	0.944-1.000

AUC: Area Under Curve, SE: Standard Error

Table 5: Receiver Operating Characteristic Analysis of Interleukin-34, as Diagnostic Markers for Hyperthyroidism

	AUC	*SE*	*p-value*	*Cutoff value*	*Sensitivity%*	*Specificity%*	*CI (95%)*
Interleukin-34	0.847	0.049	0.000	6.780	67	90	0.751-0.944

AUC: Area Under Curve, SE: Standard Error

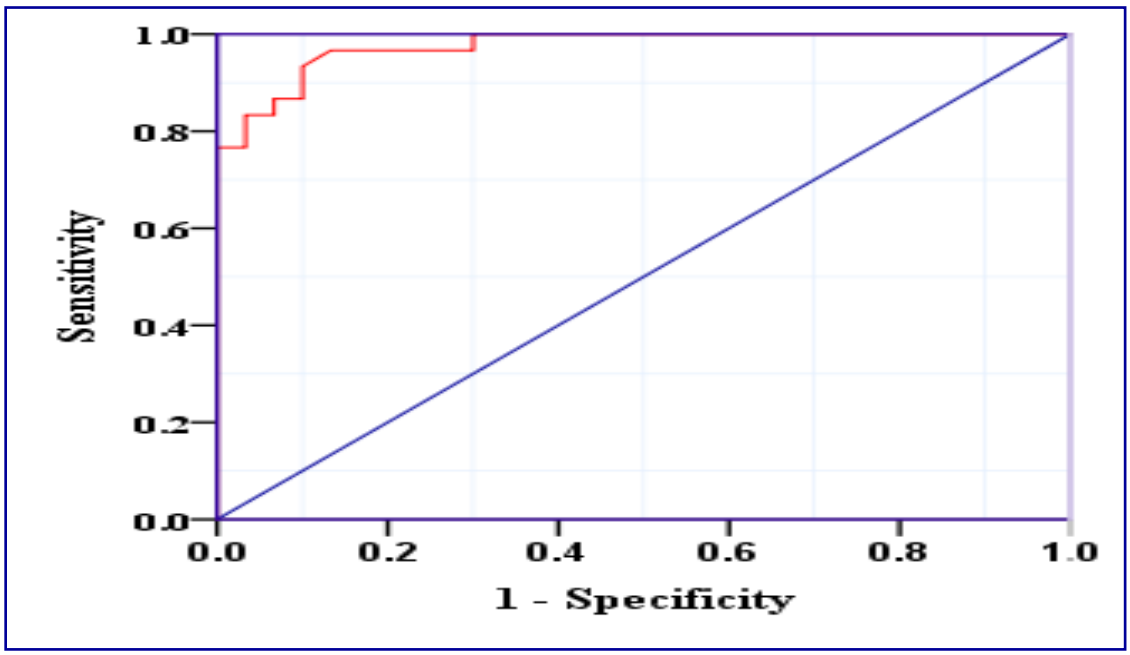

Figure 1: Receiver Operating Characteristic Curve of Interleukin-34 in Hypothyroidism

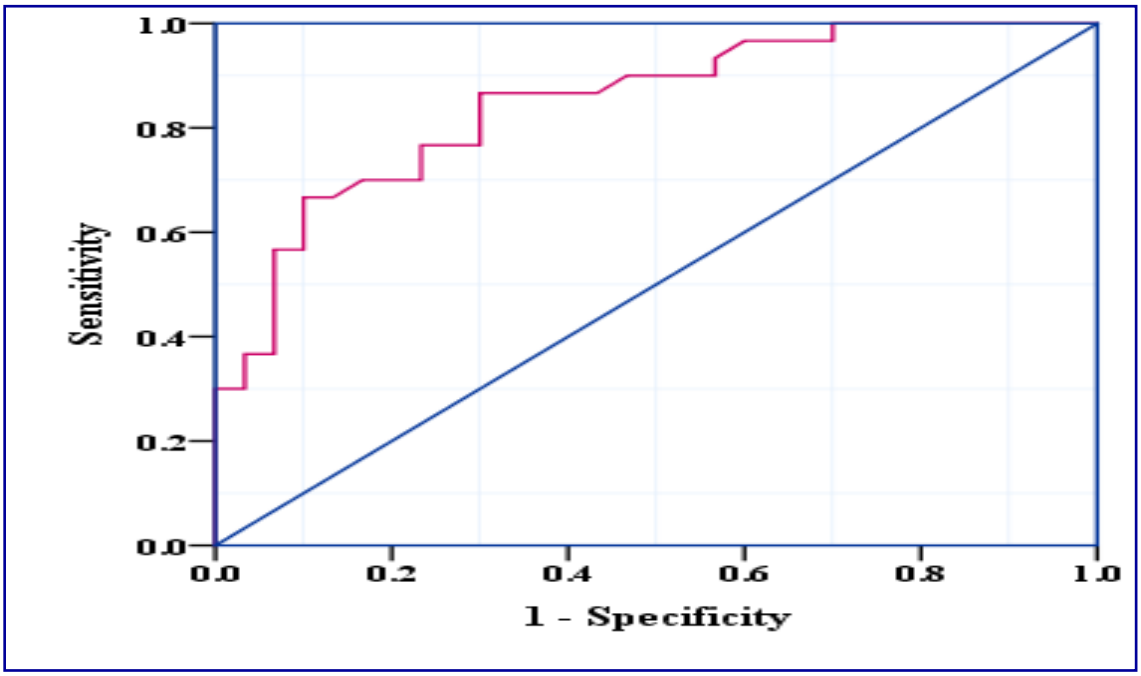

Figure 2: Receiver Operating Characteristic Curve of Interleukin-34 in the Hyperthyroidism

Conclusions

Interleukin-34 is excellent tool for distinguishing thyroid disorders from healthy individuals, as well as distinguishing between hypothyroidism and hyperthyroidism. The change in the levels of interleukin-34 correlates with the change in biochemical criteria (TSH, T4, T3, FT4, FT3, anti-TPO, anti-Tg) used in routine diagnostic examinations.

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