University of Thi-Qar Journal of Science (UTJsci)
E-ISSN: 2709-0256, P-ISSN: 1991-8690, Vol. 11, No. 2, Dec. 2024

Evaluating the patient's serum for thyroid stimulating immunoglobulin and Cytotoxic -T-lymphocyte associated protein 4 in Graves hyperthyroidism

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Received: 2024-05-28, Revised: 2024-07-05, Accepted: 2024-07-17, Published: 2024-12-05

***Abstract*— Graves' disease (GD) is a common autoimmune disorder that causes an excess of thyroid hormone production. It is mainly due to the production of IgG antibodies that activate the thyrotropin receptor. The aim of this study is to measure the levels of Cytotoxic -T-lymphocyte associated protein 4 and thyroid stimulating immunoglobulin in individuals with GD. It also seeks to establish the connection between thyroid stimulating immunoglobulin and thyroid stimulating hormone (TSH), as well as to assess the predictive value of CTLA4 for Graves' disease. The study was a case-control investigation conducted at the Alrusafa Center for Diabetes and Endocrinology. It involved a total of 45 people diagnosed with Graves' illness. The healthy group consisted of 45 individuals who had no previous medical history or clinical indications of hyperthyroidism or any other chronic condition. Blood samples from 90 individuals diagnosed with Graves' illness and a control group were analyzed to measure the levels of CTLA4, TSI by ELISA. The findings indicated a robust and statistically significant association (P.value<0.01) serum levels of CTLA4 in the patients for Graves' disease comparing to the control group. The patient samples included 13 men (28.89%) and 32 women (71.11%). There was no significant relationship found between TSI and TSH.**

***Keywords*— Graves disease, Cytotoxic -T-lymphocyte associated protein 4, thyroid stimulating immunoglobulin, thyroid stimulating hormone.**

I. INTRODUCTION

One prevalent endocrine system issue is hyperthyroidism. The condition known as Graves' disease is an immune system problem. Affecting the thyroid gland, which is observed in people who are predisposed genetically. It is the primary cause of the majority of hyperthyroidism cases, and the primary cause of hyperthyroidism that occurs most often. [1] The condition is a result of the presence of autoantibodies that target the TSH receptor. Its primary characteristic is the presence of goiter. [2]. The occurrence of (GD) is estimated to be around 1% to 1.5% in the general population.[3] Furthermore, its prevalence has experienced a substantial rise in recent years. [4] Prevalent among roughly 2% of females and 0.2% of males [5]. The highest occurrence of GD typically happens in individuals aged between 30 and 50 years. [6,7]. Thyroid-stimulatory immunoglobins (TSI), specifically TRAbs, are responsible for causing Graves' disease (GD) in people who are suspected of having hyperthyroidism. These antibodies function to be a diagnostic tool for GD [8,9]. TSI, also known as thyroid-stimulating immunoglobin, imitate the action of TSH by promoting the synthesis of two primary hormones of the thyroid, triiodothyronine (T3) and thyroxine (T4) [10]. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a T-cell co-receptor, also known as T-lymphocyte-associated protein 4, is a receptor that suppresses immunological responses. CD28 belongs to the immunoglobulin subfamily known as CD28 and is mostly expressed on T-cells. The ligands CD80 and CD86 are typically situated on the surface of antigen-presenting cells. They have the ability to bind either CD28 or CTLA-4, leading to a costimulatory or co-inhibitory response, respectively. CTLA-4 plays a vital role in regulating T-cell balance and the body's ability to tolerate itself due to its ability to reduce activity[11]. The inhibitory function of CTLA-4 can be classified into two categories: cell-intrinsic, which affects the T-cell expressing CTLA-4, and cell-extrinsic, which impacts secondary cells. Studies conducted in the past ten years demonstrated that CTLA-4 primarily functions outside of cells by competing with CD28, facilitating the internalization of CD80 and CD86 through CTLA-4, and directly inducing tolerance in the interacting cell. However, intrinsic CTLA-4 signaling has been linked to T-cell movement and the control of CTLA-4's subcellular location, among other functions. CTLA-4 is widely acknowledged as a crucial immune checkpoint and has become a prominent target for therapy in the areas of autoimmunity and cancer [12].

II. OBJECTIVE:

The aim of this study is to measure the levels of Cytotoxic -T-lymphocyte associated protein 4 in individuals with GD. It also seeks to establish the connection between thyroid stimulating immunoglobulin and thyroid stimulating hormone (TSH), as well as to assess the predictive value of CTLA4 for Graves' disease.

III THEORY

The study was a case-control investigation conducted at the Alrusafa Center for Diabetes and Endocrinology. It involved a total of 45 people diagnosed with Graves' illness. This study encompassed the time frame spanning from

https://doi.org/10.32792/utq/utjsci/v11i2.1259

September 2023 to January 2024. Thyroid ultrasonography and TSI test were employed to detect all people with Graves'. The healthy group consisted of 45 individuals who had no previous medical history or clinical indications of hyperthyroidism or any other chronic condition.

A. Inclusion criteria

To confirm Graves' disease (GD), we needed serum TSH levels below the normal range (<0.3 µIU/mL), positive serum TSI levels, and relevant clinical findings like hyperthyroidism symptoms, a diffuse goiter (as shown by Ultrasounds), or thyroid orbitopathy

B. Exclusion criteria :

Individuals diagnosed with non-Graves hyperthyroidism and those who had undergone thyroid surgery were eliminated from the study. Additionally, patients with missing data were also excluded.

C. Ethical Issue :

The research gets approval from the Ethics Committee from the College of Medicine at Al-iraqia University and the Al-Rusafa Center for Diabetes and Endocrinology.

D. Blood Sample Processing:

Blood samples from 90 individuals diagnosed with Graves' illness and a control group were analyzed to measure the levels of CTLA4 and TSI. The serum was obtained using an Eppendorf's tube and stored at a temperature of -20°C for the purpose of conducting an ELISA test. Human CLTA4 ELISA kit and Human TSI ELISA kit were from company My Bio Source and Country of origin U.S.A

E. Statistical Analysis:

The SAS (2018) program was employed to identify the influence of parameters for distinct groups (patients and control) on the study parameters. We used t-test to compare the means for statistical significance. The test of chi-square was employed to determine the statistical significance of the comparison of percent at probabilities of 0.05 and 0.01. Calculation of the correlation coefficient between variables (Pearson's correlation) in this study. The Receiver Operating Characteristic (ROC) curve was employed to assess the accuracy of markers in predicting the presence of the disease. The markers were evaluated based on the area under the curve. The analysis was conducted using MedCalc Software. A p-value less than 0.05 is regarded as statistically significant.

IV RESULTS

All the patients were examined by thyroid ultrasound, a TSI test, and evaluations of thyroid function (TSH and TT4). The patient group comprised 13 males, accounting for 28.89% of the total, and 32 females, represented 71.11%. as in table1. The study included participants varied in age from 8 to 80 years. CTLA4 levels were examined in both the Graves' disease group and the control group. The average value of CTLA4 in the patients was 24.72 ±10.47, while the mean value in the controls was 434.26 ±20.38. The findings exhibited remarkable significance in comparison to the research groups. (P < 0.005), as shown in Table (2) and Figure (1)

Table 1: Distribution of sample study according to Sex in patients and control

Factor		Patients (No=45)	Control (No= 45)	P-value
Sex: No (%)	Male	13 (28.89%)	13 (28.89%)	1.00 NS
	Female	32 (71.11%)	32 (71.11%)	1.00 NS
	P-value	0.0026 **	0.0026 **	---
(P≤0.01), NS: Non-Significant.				

Table2: Mean comparison of immunological parameter CTLA4 among study's group (n =90)

Immunological parameter (mean+SE)	Patients N=45	Control N=45	Significance
CTLA4 (ng/ml)	24.72 ±10.47	434.26 ±20.38	P- 0.0001 T-test= 45.529

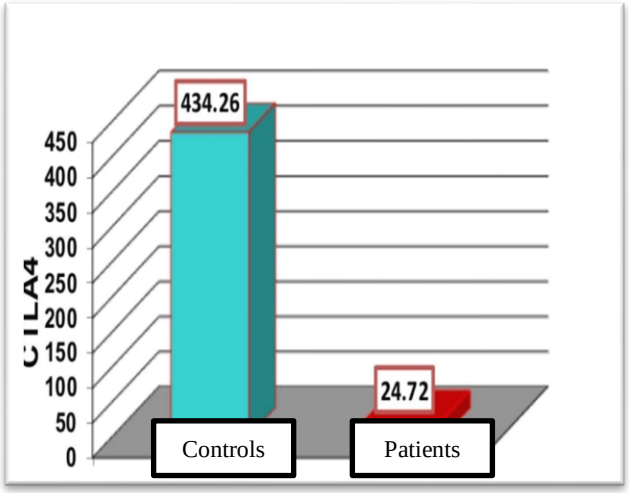

Figure1:Comparsionbetween patients and control group in CTLA4 among studies group n=90

CTLA4 as predictive diagnostic test for Graves' disease

Furthermore, the cutoff point value of CTLA4 for detect patients with Graves disease was >377.68 with sensitivity of 100%, specificity of 71.11%, positive predictive value (PPV) of 77.59%, negative predictive value (NPV) of 100%, and excellent area under the ROC curve (AUC) of 0.86 (*P*=< 0.0001)(Table 3) (Figure 2).

Table 3 Predictive value of CTLA4 for diagnosis of Graves' disease among study's sample (n=90)

Parameter	Validity of model						
	Sensitivity (sn)	**Specificity** (sp)	**Positive predictive value** (ppv)	**Negative predictive value (NPV)**	**Accuracy**	**Area Under the curve (AUC)**	**Significancy (*P*-value)**
CTLA4	100	71.11	77.59	100	85.56	0.86	<0.0001

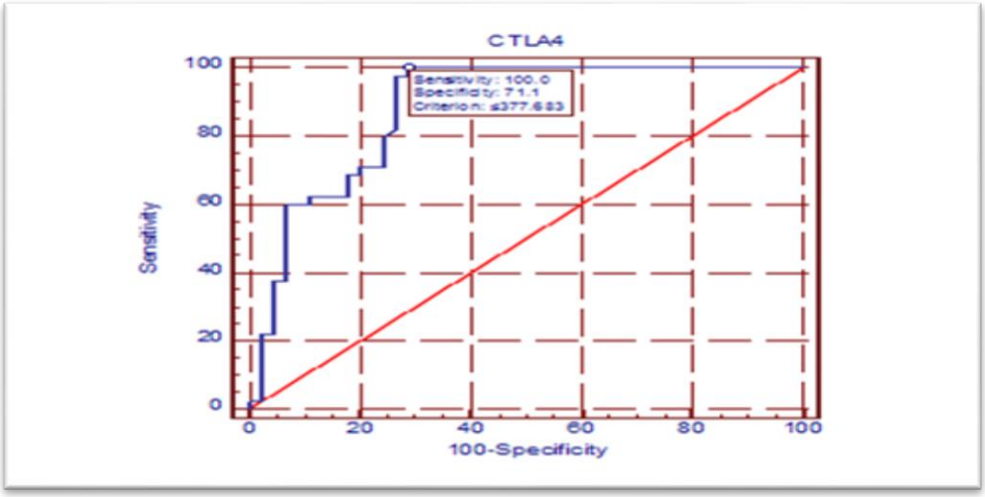

Figure 2:ROC Curve of graves diseases predicted by immunological parameter of CTLA4 among study's sample (n=90)

Regarding the relationship between TSI values and thyroid stimulating hormone (TSH) levels in a group of patients, it was determined that there is no statistically significant link between TSI values and TSH (*P*= 0.923).as shown in table4

Table 4: Estimate of correlation coefficient between TSI with TSH

Parameters	**Correlation coefficient-r**	**P-value**
TSI & TSH	-0.02	0.923

V. DISCUSSION

The current study has demonstrated a significant decrease in sCTLA4 levels in patients with GD compared to the control group (p=0.0001). Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a receptor that inhibits immune responses. It is a member of the CD28 immunoglobulin subfamily and mostly found on T-cells.[13]. T regulatory cells (Tregs) are a specific group of CD4+ T lymphocytes that play a role in suppressing inflammation. They have an impact on different disorders such as autoimmune, infections, cancer, antimicrobial resistance, allergies, and transplantation. Tregs play a crucial role in this process since they function as inhibitory regulators of inflammation in different circumstances and maintain self-tolerance[14]. Distinct subgroups of CD8+ Tregs have been recognized by their distinctive expression of certain markers, such as CTLA-4. Alterations in the function or frequency of Treg cells are anticipated to influence the onset and progression of GD[15]. Alterations in the function or frequency of Treg cells and may contribute to alteration in the CTLA4 concentration. There was no statistically significant association between TSH and TSI readings in the current investigation. Serum TSI levels reflect the degree of GD. Their rises throughout methimazole therapy serves as an indicator of continued disease activity.[16] Autoantibodies known as thyroid stimulating hormone (TSH) receptor autoantibodies (TRAbs) are the etiology of Graves' disease (GD). Thyroid hormone production, secretion, and thyroid cell development are all stimulated when TRAbs attach to and activate TSH receptors [17].

VI. CONCLUSION

Patients with Graves' disease had lower serum levels of CTLA4 than the control group . This implies that CTLA4 may be a helpful diagnostic tool for determining whether Graves' disease is present or not. The TSH and TSI levels are not statistically significantly correlated.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest

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