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Measurement Of Thyroid Peroxide (Tpo) And Thyroglobulin Antibodies (Tg) Levels and Its Association with Thyroid Dysfunction in Graves' Disease Patients

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Abstract: *The prevalent autoimmune disease causes most cases of hyperthyroidism in the world—the disease Graves (GD). Thyroid peroxide (TPO) and thyroglobulin (TG) antibodies levels were assessed using an enzyme-linked immunosorbent assay (ELISA), while TSH, T3 and T4 hormone levels were evaluated using a Cobas e 411 analyzer. Auto immun thyroid Graves' disorder constitutes one of the most prevalent auto immunes diseases (AID). The blood samples from patients with the condition and controls were taken to determine how TPO Ab and TG Ab relate to thyroid dysfunction in Graves' disease patients. The results indicated that there were more females (89%) than males (11%) and that their mean age is (36.86) which is higher than other ages. Those findings demonstrate why the TSH, T3, and T4 levels were highly significant. Additionally, patients had significantly higher levels of TPO Ab and TG Ab compared to the control group.*

Keywords*: *GD, TPO Ab, TG Ab, TSH, T3, T4 Hormones.

1.Introduction

An autoimmune thyroid gland illness called Graves' disease only affects those predisposed to genetics. It is the most common cause of hyperthyroidism, and women are more likely to experience it. Antibodies against the TSH receptor are what define it [1]. Symptoms of GD include extensive goiter and thyrotoxicosis.[2,3].

Autoantibodies against the thyroid stimulate hormone receptor antibodies (TSHR Ab), function as antagonists, and stimulates excess thyroid hormone release, freeing the thyroid gland from pituitary regulation and causing Graves' hyperthyroidism. Pretibial myxedema and Graves' orbitopathy (GO) are TSHR autoantibody-related conditions. [4,5].

GD is not typical in youngsters, affecting women between 5 and 10 times more frequently than men. Grave's disease is the leading cause of hyperthyroidism. It is a complicated illness in which endogenous, genetic, and environmental factors contribute [6]. The disorder can develop at any age, although it is most prevalent in people between the ages of thirty and fifty. Women are more likely than men to be affected. [7]. The primary autoantigen in Graves' disease (GD) is the Thyroid stimulating hormone receptor (TSHR),

expressed primarily in the thyroid gland yet also in adipocytes, fibroblasts, bone cells, and numerous other locations, including the heart.[8], This ultimately concerns the IgG antibody production boosting the TRAb (thyrotropin receptor antibody). TRAb activates the thyroid hormone receptor (TSHR) and imitates the activity of Thyroid stimulating hormone (TSH), causing goiter and hyperthyroidism (an overactive thyroid gland) [9].

2. Methodology

Patients Selection Criteria:

Patients diagnosed with Graves' disease were chosen for the study based on their doctors' initial assessments of them, as well as the preliminary results of hormone and serological examinations that initially indicated hyperthyroidism, a TSH rate under normal and T3 with T4 levels up normal were found to be false positives. Additionally, specific patient indications and symptoms call for submission to the lab for confirmation.

Exclusion criteria:

Pregnant women and all patients receiving therapy were removed from the study since these factors impacted the findings, assessment, and disease-related criteria.

Sample collection:

Because of the necessary hormone - ally and immunological tests associated with Graves' disease in the present research in Najaf City, 100 patients with Graves' disease and 32 healthy subjects served as a control group. Patients' ages ranged from 20 to 59 years old. Case-control investigation.

Using a disposable disposable syringe, 3 ml of blood was drawn after thoroughly sanitizing the region with 70 % ethyl alcohol. blood is then put into a gel tube free of anticoagulants, where 3 ml of blood is inserted. The gel tube is left at room temperature for 30 to 45 minutes to allow the blood to clot. The serum is then isolated by centrifuging the gel tube for 10 minutes at 4000 rpm. The serum is then put into Eppendorf tubes and stored at -20 degrees Celsius until it is needed for the study's requisite immunological testing [10].

Immunological and hormonals tests among patents with Graves' disease.

Hormonal testing were completed using the German-made Cobase 411 Analyzer, which measured the levels of TSH, T3, and T4 hormones.[11]. The Cobase 411 immunoassay, an automated analyzer that uses the electroch - emiluminescence immunoassay (ECLIA) technology to assess thyroid problems, was used to test the patients for thyroid hormone assays.

The enzyme-linked immunosorbent test (ELISA), a sandwich technique developed by the Sunlong firm in China, was also used to assess immunological assays. An antigen is particular to Ab has been precoated on the Microelisa strip plate included with the kit. The proper Microelisa strip plate wells are filled with standards or samples, then mixed with the designated antigen. Each Microelisa stripplate well is then full with an antigen specific for Ab conjugated to Horseradish Peroxidase (HRP). During laundering, loose pieces are removed. The TMB substrate solution is applied to each well. Only the holes containing Ab and HRP-linked Ab-Antigen will turn blue when a stop solution is added. [11]The essential enzyme in manufacturing thyroid hormones is thyroid peroxidase (TPO). [12]. T3 and T4 are precursors to TG, the thyroid hormones. [13].

Biometry Statistics the Statistics Package for Social Sciences system (SPSS) version 24 were used for the data analysis. Results shown as mean, standard deviation, and p-value [14].

3. Results and discussion:

Demographic study: In terms of demography, the age with years for patients did not appear to be significantly difference compared to the controls, where P-Value > 0.05) and as shown

in Figure (1): According to the statistical analysis, according to the ages of the patients, age categories (30-39) years showed a higher percentage of the incidence of Graves' disease. Its peak is between the ages (30-50) [14].

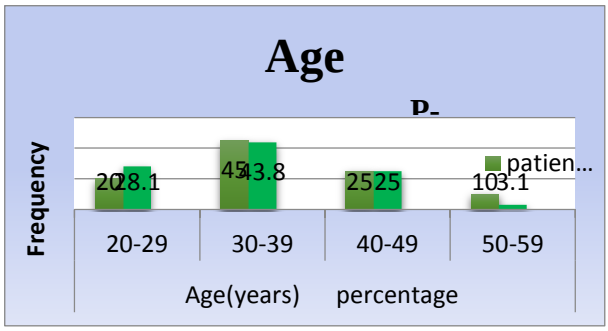

Figure (1): differences in age data between patients and the control group.

Hormonal and immunological determination :

The findings of thyroid hormone hormonal testing were perfect. They provided unmistakable evidence of hyperthyroidism but also the greatest frequent reason.[14]. TSH levels in the patient samples were below normal while T3 and T4 hormone concentrations were higher than normal (high levels). As a result, this is regarded as the initial step in diagnosing Graves' illness. [15].

Utilizing the statistical package SPSS version 24 and Excel programs, the mean and the standard deviation of thyroid-stimulating hormone (TSH) concentrations with a P-value were calculated for comparison between both study groups (patients and the control group), as shown in Figure (2). The mean TSH was 0.08 to 0.10 for patients compared to 2.64 to 1.02 for control groups.

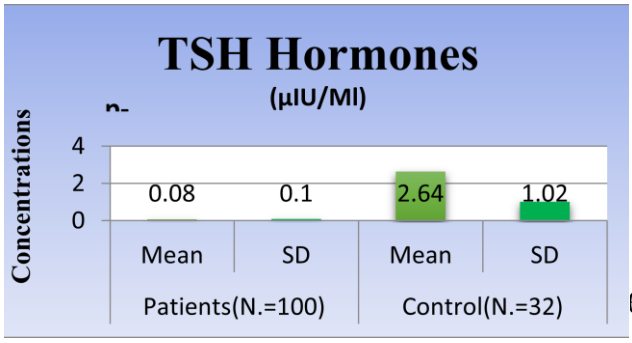

Figure (2): Thyroid stimulating hormones (TSH), the mean and SD for patients and categories:

A T-test results with a P value of (0.001) indicate elevated significance (HS) in Thyroid stimulating hormones (TSH) concentration when compared with control groups. The results also demonstrate the measurement and variance in TSH concentration between patient and control groups. The arithmetic means as well as the standard deviation of the patients were (0.08 ±0.10) in comparison to the control group, which were (2.64 ±1.02), indicating a highly significant drop (P=0.000) with the level of TSH. (P value 0.05)[16]. Additionally, Bahn noted that TSH should always be utilized as a preliminary test due to its extremely high specificity and sensitivity for assessing and testing thyroid gland function. [17]. It agrees with Mohamed's account of the patients' low TSH levels. [8]. Additionally, Abdalaziz stated that the low TSH in GD is due to the surge in thyroid hormones. [18]. As shown in figure (3), the triiodothyronine (T3) hormone's mean and standard deviation for the patient and control groups were 4.65 ±1.36 and 2.15 ±0.57, respectively.

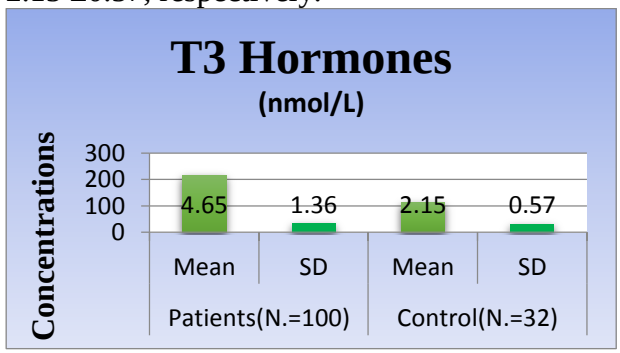

Figure (3): Triiodothyronine hormone (T3), the mean and SD for patients and control groups:

The T-test, with a p-value of (0.001), revealed the high significant (HS) difference in T3 hormone when comparing with the control groups, as shown in Figure 3.

When these hormones are elevated in addition to the low levels of TSH, the T3 and T4 tests are crucial as a preliminary step in diagnosing Graves' disorder.19]. It is proven, and it shows that there is a significant difference between the patients and the control group. According to Mohammed's work, other research has demonstrated the importance of the T3 hormone in Graves patients [8]. In the current study, the mean values for the T4 hormone were (216.37±36.07) for patients and (114.73±31.58) for control groups.

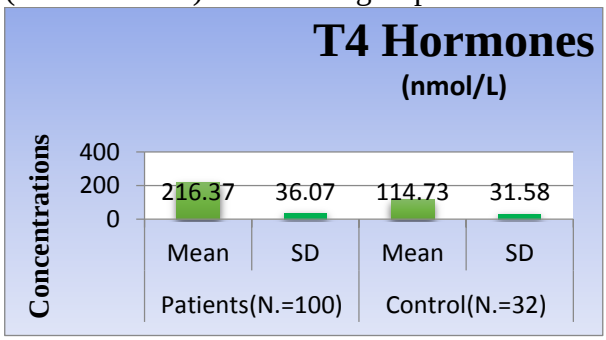

Figure (4): thyroxine hormone (T4), the mean and SD for patients and control groups: **p-value=(0.000)**

The T4 hormone showed significant differences in the T test with a P-Value =(0.001) since its concentrations were obviously higher than the patients' normal levels. The current research's TSH, T3, and T4 results were likewise in line with Al-Ghazali's observation that individuals with Graves' illness had significantly higher levels of these hormones than those in the control group.[19]. **The immunological tests** TPO Ab and TG Ab represented the current study. When patients were compared to the control group, there was a substantial difference in TPO antibodies. The patients' average and standard deviation were (449.04187.43), and the controls were (252.1245.40), respectively, as shown in Table (1).

Table (1): statistical of concentrations (mean and SD) o TPO Ab in the patients and control

Marker	`Patients (No. = 100)		Control (No. = 32)		T test	P value (Sig.)
	Mean	SD	Mean	SD		
TPO Ab. (pg/ml)	449.04	187.43	252.12	45.40	9.66	0.000 (HS)
TG Ab. (pg/ml)	365.62	199.49	202.49	58.65	7.26	0.000 (HS)

Table (1 shows that a highly significant (HS) difference between patients and the control group developed, dependent on the findings of statistical analysis using the t-test , with p-value equals (0.001), which agrees with several research, including[20]. These results were in line with much other research, including those of Elmugadam, who noted that TPO was highly significant in patients compared to the healthy control, with a p-value of 0.000. [20,21].

Although TG Abs are one of the characteristics of AITD, healthy people also experience them rather frequently. When combined with aberrant thyroid function tests, TG Abs, which are not specific to AITD but often have greater levels than in the general population, can be used to determine the presence of the condition. [22]. The average and standard deviation of the results are as follows, as shown in Table 1: (365.62199.49) for the patient group and (202.4958.65) for the control group. Statistical analysis using the t-test produced results with an elevated significance (HS) on patients compared with the control categories, as the p-value was (0.001), which is compatible with the study. [23]. Anti-TG and anti-TPO antibody levels are primarily elevated in autoimmune thyroid disorders, but low levels are also present in healthy persons. [9, 24,25]. Also, Figura and Saeed mentioned separately that anti-TG levels of the cases were also significant differences from controls ($p < 0.001$) [,26,27].

Conclusions

Compared with the control group, the results demonstrated a statistically significant drop in TSH. Regarding TPO and TG

Antibodies, which were present in high quantities in patients and registered a highly significant rise in disease compared to healthy persons, their P-value was (0.05). T3 and T4 hormones showed a clear and statistically significant increase compared with the control.

Ethical Approval:

All patients and healthy controls in this investigation agreed to participate in the trial and complete the questionnaire that obtained the necessary consent from the formal health institutes and Al-Qadisiya University.

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