Characterizing Thyrotropin Receptor Antibodies and Hormonal Dynamics in Graves' Disease: A Comparative Analysis of Treated and Untreated Patients

Zahraa Hasan Shnawa[1], Lina A. Hassan[1]

[1]*Faculty of Science, University of Kufa, Najaf, Iraq*

[*]*Corresponding author: Linaa.manghi@uokufa.edu.iq*

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ABSTRAC: Graves' disease (GD) constitutes a significant proportion, ranging from 60% to 80%, of diagnosed cases of hyperthyroidism, emerging as a prevalent autoimmune disorder primarily affecting women aged between 30 and 50 years. Notably, GD stands as the foremost etiology of hyperthyroidism within populations endowed with adequate iodine levels. This investigation endeavors to quantify Thyrotropin receptor antibodies (TRAb) in cohorts comprising both untreated individuals and those undergoing pharmacotherapy with anti-thyroid agents, while concurrently evaluating hormonal indices encompassing Thyroid Stimulating Hormone (TSH), Free Thyroxine (FT4), and Free Triiodothyronine (FT3) levels. The study adopts a case-control design encompassing a sample size of 70 participants, inclusive of 40 subjects subjected to anti-thyroid medication and 30 untreated counterparts. Measurement of TRAb levels was facilitated through the utilization of commercially available Enzyme-Linked Immunosorbent Assays (ELISA, whereas assessment of T4, T3, and TSH) levels was executed via immunoenzymatic assays. Before starting carbimazole (CMZ) therapy, people with GD showed significant symptoms.elevated concentrations of FT4, FT3, and TRAb, juxtaposed with diminished TSH levels relative to both treated individuals and healthy controls, achieving statistical significance at $p<0.001$. Furthermore, a predominance of female participants was observed across all study groups, constituting proportions ranging from 70% to 83.3%. Among the various age strata under scrutiny, the cohort aged over 44 years exhibited the highest mean percentage, with negligible disparity discernible between the second (25-34 years) 11 third (35-44 years) age brackets. The results of this study highlight the unique hormonal profiles observed in patients with GD, characterized by increased levels of FT4, FT3, and TRAb, along with lower TSH levels. The study also shows that GD is more common in females and becomes more prevalent with advancing age.

Keywords: Graves' disease, TRAb, carbimazole, FT4, FT3, Case-control study.

1. Introduction

Graves' disease is an autoimmune disorder that mainly impacts the thyroid gland and is frequently observed in individuals with a genetic predisposition. It is the leading cause of hyperthyroidism, predominantly occurring in females. A hallmark of Graves' disease is the presence of anti-TSH receptor antibodies [1]. These TSH receptor antibodies (TRAbs) stimulate thyroid cells, resulting in excessive

production of thyroid hormones. The presence of these antibodies is a distinguishing feature of Graves' disease, being present in nearly all diagnosed patients. Additionally, the concentration of circulating TRAbs correlates with the severity of the disease and serves as a prognostic indicator[2]. Graves' disease is one of the most prevalent causes of hyperthyroidism worldwide, with an estimated annual happening of 20 cases per 100,000 individuals. The condition predominantly affects women, particularly those aged 30 to 60 years[3]. It is significantly more common in females, affecting 2% of women compared to 0.2% of men[4].

2. Materials and methods

In this study, a case-control design was utilized to investigate 70 patients, aged between 15 and 60 years, who attended the Diabetes and Endocrinology Center of Dhi-Qar province in Iraq from July to December 2023. There were two groups of patients: 30 patients with untreated GD and 40 patients with GD who were being treated with CMZ. Additionally, 20 individuals who appear to be in good health were included as a control group, matched to the GD patients by sex and age. From all participants, the blood samples were collected from the venous, processed to obtain serum, and stored at -20°C. The levels of Thyrotropin receptor antibodies (TRAb) were measured using commercially available ELISA assays. Additionally, the levels of Thyroxine (T4), Triiodothyronine (T3), and Thyroid Stimulating Hormone (TSH) were measured using immunoenzymatic assay kits. Exclusion criteria for participants included a history of thyroid surgery, alternative treatment therapies, presence of thyroid malignancy, and pregnancy. The study received ethical approval from both the Ethics Committee of the Faculty of Science, Kufa University, Al-Najaf Province, Iraq, and the Medical Ethics Committee of the Ministry of Health in Iraq.

2.1. Statistical Analysis

The ongoing investigation's data were evaluated statistically by using SPSS program (version 26). Least significant difference – Independent sample t test, One-way ANOVA was used to compare between means, Chi-square test was used to compare between percentages. The result was stated as Mean ± S.D and Person correlation, at *P. value* < 0.05 was considered significant.

3. Results and Discussion

The results revealed a non-significant difference between males and females within the same groups, except for a notable rise in Thyrotropin Receptor Antibody (TRAb) levels among females ($p < 0.05$). TRAb values were notably elevated in both treated and untreated

female patients, while remaining relatively stable in male patients within their respective groups. Across different groups, females generally exhibited higher mean TRAb levels compared to males, although these differences did not reach statistical significance in the treated ($p = 0.111$) and untreated ($p = 0172$) patient groups. In contrast, the control group showed significantly lower TRAb levels overall, with a notable disparity between females and males ($p < 0.001$).

Post-hoc inspection revealed noteworthy variations in TRAb levels between the control group and both treated and untreated patient groups across sex ($p < 0.001$). Additionally, significant variation was noted between female and male participants within each group ($p < 0.001$).

Table (1): Level of TRAb according to sex.

	Groups	Female	Male	p. value
		Mean ± S.D		
TRAb	Treated	33.2 ± 9.53[a]	29.3 ± 6.78[a]	0.111
	Untreated	39.5 ± 10.2[a]	32.1 ± 11.3[a]	0.172
	Control	7.60 ± 1.40[c]	5.22 ± 0.80[b]	< 0.001
p. value		< 0.001	< 0.001	
p. value of LSD		< 0.001[1,2], < 0.001[1,3] 0.01[2,3]	< 0.001[1,3], < 0.001[2,3]	

* Different small letters in the same column indicate a significant difference between means. While the groups indicated by numbers (treated, untreated and control). Data are presented as mean ± standard deviation (S.D).

3.1. Level of TSH in Study Groups According to Sex

The outcomes of the present investigation indicate variation alterations in Thyroid Stimulating Hormone (TSH) levels across different groups, with noteworthy differences observed between untreated and treated patients, as well as between patient groups and controls. Specifically, TSH levels exhibited a significant decrease in both female and male untreated patient groups, followed by increases in treated patients. Moreover, no major variations in disparity were observed between male and female participants within

the same group at a significance level of $p < 0.05$, as evidenced by the findings presented in Table (2). In Table (2), the mean TSH levels stratified by sex across study groups are delineated. Among treated patients, both females and males displayed comparable mean TSH quantities of 1.40 ± 0.42 and 1.34 ± 0.36, correspondingly, with no statistically significant difference observed between the sexes ($p = 0.720$). Similarly, in the untreated patient group, females exhibited a mean TSH level of 0.140 ± 0.078, while males had a slightly lower mean level of 0.098 ± 0.030; however, these variations are not up to statistical significance ($p = 0.251$). Conversely, the control group exhibited higher mean TSH levels overall, with females and males registering mean levels of 1.87 ± 0.61 and 2.13 ± 0.26, respectively, although again, no significant difference was noted between sexes ($p = 0.195$). Additionally, post-hoc analysis showed significant differences in TSH levels across all groups ($p <0.001$). However, there were no significant differences observed between male and female participants within each group, highlighting the consistency of TSH alterations irrespective of gender.

Table (2): Level of TSH in study groups according to sex.

Parameters	Groups	Female	Male	P. value
		Mean ± S. D		
TSH	Treated	1.40 ± 0.42[b]	1.34 ± 0.36[b]	0.720
	Untreated	0.140 ± 0.078[c]	0.098 ± 0.030[c]	0.251
	Control	1.87 ± 0.61[a]	2.13 ± 0.26[a]	0.195
P. value		< 0.001	< 0.001	
P. value of LSD		For all	For all	

* Different small letters in the same column indicate a significant difference between means. Data are presented as mean ± standard deviation (S.D).

3.2. Level of FT4 in Studies Groups According to Sex

The study's results revealed notable variations in Free Thyroxine (FT4) levels among untreated and treated patients compared to controls. Specifically, FT4 levels showed a

significant increase in both untreated male and female patients, followed by treated patients, whereas a noteworthy decrease was observed in the healthy group. Moreover, there was no statistically significant difference between male and female participants within the same group at a significance level of $p < 0.05$, as depicted in Table (3). Table (3) presents the mean FT4 levels stratified by sex across study groups. Among treated patients, both females and males displayed similar mean± **SD** FT4 levels of 3.23 ± 147 and 2.55 ± 102, correspondingly, with no noteworthy difference observed between the sexes ($p = 0.194$). Conversely, in the untreated patient group, females exhibited a mean±**SD** FT4 level of 5.96 ≤ 1.58, while males showed a slightly higher mean level of 718 ± 182; however, this difference did not reach statistical significance ($p = 0.238$). In contrast, the control group exhibited lower means of FT4 levels, overall, females and males registering mean levels of 1.22 ± 0.44 and 1.38 ± 0.41, respectively, with no significant difference between the sexes ($p = 0.459$). Moreover, analysis using post-hoc tests revealed significant differences in FT4 levels across all groups ($p < 0.001$). However, no noteworthy differences were observed between male and female participants within each group, underscoring the consistency of FT4 alterations regardless of gender

Table (3): Level of FT4 in study groups according to sex.

Parameters	Groups	Female	Male	P. value
		Mean ± S.D		
FT4	Treated	3.23 ± 1.47[b]	2.55 ± 1.02[b]	0.194
	Untreated	5.96 ± 1.58[a]	7.18 ± 1.82[a]	0.238
	Control	1.22 ± 0.44[c]	1.38 ± 0.41[c]	0.459
P. value		< 0.001	< 0.001	
P. value of LSD		For all	For all	

* different small letters in the same column indicate a significant difference between means. Data are presented as mean ± standard deviation (S.D).

3.3. Level of FT3 According to Sex

The study's findings revealed significant fluctuations in Free Triiodothyronine (FT3) levels across untreated and treated patients compared to controls. Specifically, FT3 levels exhibited a significant increase in both untreated male and female patients, followed by

treated patients, whereas a significant decrease was noticed in the control group. Additionally, there was no statistically significant difference between male and female participants within the same group at a significance level of $p < 0.05$, as depicted in Table (4). Table (4) presents the mean ± SD FT3 levels stratified by sex across study groups. Among treated patients, both females and males displayed comparable mean FT3 levels of 3.55 ± 0.79 and 3.65 ± 0.59, respectively, with no significant difference observed between the sexes ($p = 0.687$). Conversely, in the untreated patient group, females exhibited a mean FT3 level of 6.21 ± 1.73, while males demonstrated a slightly lower mean level of 5.73 ± 2.41; however, this difference did not reach statistical significance ($p = 0.603$). In contrast, the control group exhibited lower mean of FT3 levels, overall, females and males registering mean levels of 2.19 ± 0.65 and 2.11 ± 0.43, respectively, with no significant difference between the sexes ($p = 0.776$). Further analysis utilizing post-hoc tests revealed significant differences in FT3 levels across all groups ($p < 0.001$). However, no significant differences were observed between male and female participants within each group, indicating consistent FT3: 4 nations irrespective of gender.

Table (4): Level of FT3 in study groups according to sex.

G7	Groups	Female	Male	P. value
		Mean ± S. D		
FT3	**Treated**	3.55 ± 0.79^{b}	3.65 ± 0.59^{b}	0.687
	Untreated	6.21 ± 1.73^{a}	5.73 ± 2.41^{a}	0.603
	Control	2.19 ± 0.65^{c}	2.11 ± 0.43^{c}	0.776
p. value		< 0.001	0.001	
p. value of LSD		For all	$0.006^{1,2}$, $0.027^{1,3}$. $< 0.001^{2,3}$	

* different small letters in the same column indicate a significant difference between means. while the number is indicated on the groups (treated, untreated and control). Data are presented as mean ± standard deviation (S.D).

Graves' disease (GD) pathogenesis is related to autoimmune production for antibodies of TSH receptor (TRAbs). The existence of an increased TRAb titer is a key

component when making a diagnosis of GD [5]. However, TRAbs are diverse and might have either an inhibitory effect (TSH-receptors blocking antibody TBAb) or a stimulating effect (TSH-receptor stimulating antibodyTSAb), which is the most frequent case in GD, and finally a neutral effect on the TSH-receptors and that's one is rare [6].

The study by Al-Jabery *et al.,* (2023) in Iraq, showed that TSHR-Ab was responsible for Grave's disease[7]. On the other hand, the finding by Abdulrazaq, (2023) in Basra, Iraq,demonstrated that patients had positive TRAb results, where hyperthyroidism was the dominant disease[8]. In a study conducted by Abbas *et al*., (2018), it was revealed that the Thyroid Receptor Antibody (TRAb) assay could serve as a valuable tool for distinguishing Graves' disease from other autoimmune thyroid conditions such as toxic nodular and toxic nodules[9]. Link between Anti-TSH Antibodies and Hyperthyroidism, Helial and Mohammed (2022) presented findings from a study conducted in Al-Nasiriya City, revealing that a significant percentage of patients in the hyperthyroidism group, specifically 85%, were found to carry anti-Thyroid Stimulating Hormone (TSH) antibodies[10]. This observation suggests a heightened susceptibility to Graves' disease development.

Indeed, studies conducted by Villagelin *et al*. (2020) indicated that TRAb levels usually tend to be reduced during ATD treatment. Nevertheless, the decline may manifest in two ways: either entering into a negative state or showing a volatile trend. However, it should be pointed out that in 10% of patients, TRAb may stay positive even after a five-year course of anti-thyroid drugs[11].

According to the study conducted by Kościuszko *et al.* (2021) the level of TSHR-Ab was significantly lower after treatment compared to the pretreatment level[12]. Likewise, Zhang *et al.* (2023) identified a high level of TRAb remission and negative conversion in newly diagnosed GD patients treated with ATD[13]. These observations suggest that such rates may be used as prognostic indices for TRAb remission or negative conversion, thus helping to forecast prognosis for patients suffering from GD.

Similarly, Ibrahim *et al*. (2022) highlighted TSH, T4, and T3 as being significant physiologic indicators of thyroid hormone activity. Fluctuations in these hormones can lead to various health complications. Notably, TSH stands out as the most common chemical marker used in thyroid disorders; low TSH signifies hyperthyroidism while high TSH indicates hypothyroidism. This explains the diagnostic and prognostic value of

TSH in the identification of thyroid dysfunction and establishes it as an important tool in clinical assessment and management. When hyperthyroidism patients are treated with ATD, TSH should be normalized together with the normalization of thyroid hormone. However, some patients undergo the suppression of TSH in the enduring sense even when thyroid hormone is normalized[14].

High levels of T3 and T4 in people with Graves' Disease can be explained by the increased synthesis of these hormones and reduced levels of TSH. This is thought to be due to the existence of autoantibodies. These autoantibodies are generated through immune competent plasma cells and react with the TSH receptors (TSHR). These antibodies attach to TSHR and increase the production and release of T3 and T4 even when the level of TSH is low. This can be clarifiedthrough the mechanisms of negative feedback by T3 and T4 on the pituitary and hypothalamic axis.

a recent study assessing the levels of T3, fT, TRAb, and TSH before and after anti-thyroid drug (ATD) treatment in patients with Graves' Disease, it was observed that post-treatment there was an increase in TSH levels alongside a decrease in fT3, fT4, and TRAb. A study by Gokce *et al.* (2022) also demonstrated significant differences in T3, f4, and TRAb levels before and after ATD treatment, all of which decreased post-treatment. In contrast, TSH levels were noticeded to increase both before and after ATD treatment[15].

Our study illustrated that females were more than males in all studied groups. In this regard, Ali and Hamoud, (2022) observed the percentage of GD patients group was (33.3%) of males and (66.7%) of females and the age of the patients and healthy group in the study ranged from 14 to 73 years[16]. So, the gender has effects onautoimmune disease risk via unique immune responses, organ vulnerability, sex hormones, reproductive capability, parental inheritance, and epigenetics. The emergence and progression of autoimmune diseases are influenced by gender, making it an essential aspect to consider [17].

Numerous studies, congruent with our investigation, delineate a pronounced female predominance in thyroid disorders, as evidenced by research carried out in Iraq's Diyala Governorate, wherein the incidence of thyroid diseases among females was notably higher (86.57%) than among males (13.43%) [18]. Given the pervasive nature of thyroid disorders, particularly among females, elucidating the underlying reasons for this gender disparity remains imperative. One plausible explanation implicates the influence of female sex hormones[19].

Based on this premise, the preceding study by Hade and Al-Shukri (2023) delineated the multifaceted impact of gender on autoimmune disease susceptibility, encompassing immune response modulation, organ vulnerability, sex hormone dynamics, reproductive capacity - including pregnancy-related factors, parental inheritance patterns, and epigenetic mechanisms [20]. Consequently, gender assumes a pivotal role as a determinant in delineating the intricate processes governing the onset and progression of autoimmune diseases, thereby accentuating its significance in the broader context of disease pathogenesis and clinical management.

Conclusion

This study's findings emphasize the distinct hormonal profiles found in GD patients, which include elevated levels of FT4, FT3, and TRAb, as well as reduced TSH levels. The study also found that GD is more frequent in women and increases with age.

Generative AI:

The authors employed [QuillBot] in the process of preparing this work to rephrase and clarify a few portions in the introduction. After utilizing this tool, we went over and made any necessary edits to the content, and we are fully accountable for the publication's content.

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