Measurement of some Immunological parameters, Hematologic outcomes and Physiological features for Graves' disease in Babylon City, Iraq

Humam A. Hade

College of Science, Al-Qasim Green University, Iraq

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ABSTRACT Graves' disease (GD) is an autoimmune disease that infected some people and is described as a disorder in the thyroid gland that leads to a hyperactive thyroid gland leading to the overproduction of thyroid hormones resulting from an attached immune system for the thyroid gland.
The aims of this study were to Measurement of some immunological parameters (IL-2 & IgG), hematologic outcomes (routine CBC tests), and
physiological features of Graves' disease in Babylon City, Iraq.
Study subjects (GD and Control Groups) & Demographic data: Study conducted from January 2022 to March 2022, samples for immunologic (IL-2, IgG & hematologic tests (routine CBC) were blood samples taken from both (n=94) Hospital-admitted patients with Graves' disease symptoms from Imam Al-Sadiq Teaching Hospital\ Thyroid gland diseases Center- First Floor and compare them with (60) represent healthy control, in both sexes, of different age groups ranging from (>38-<52 years)., in Babylon city. The researchers follow the Ethics committee of health & academic directorates. Data analysis using SPSS system to determine significant differences between data, p-value less than (<0.05) ANOVA. All values are expressed as the mean standard deviation (SD) for sex, age groups, and test results in both GD & control groups.
Keywords : Graves' disease IgG IL-2 CBC test

INTRODUCTION

Graves' disease (GD) is named after Dr. Robert J. Graves, who first described a case of it in 1835. The thyroid gland is an endocrine gland that secretes thyroxine hormone. Thyroid gland autoimmune disturbances are caused by the immune system attacking healthy tissues by creating antibodies, known as thyroid-stimulating immunoglobulins (TSIs), which provoke the thyroid to grow and produce more thyroid hormones than necessary for normal body functions. GD is responsible for 60–80% of clinical cases of hyperthyroidism and stands as one of the most prevalent autoimmune diseases (Gallo et al., 2020). Like other autoimmune diseases, GD is usually triggered by environmental factors in genetically predisposed individuals. The diagnosis of GD is made in the presence of a diffusely enlarged thyroid gland, thyrotoxicosis, and the possible involvement of connective tissues around the orbits and in the skin. The disease is suspected based on symptoms and physical examinations, but confirmation requires further blood or imaging tests to differentiate it from other autoimmune thyroid diseases (Magnusson et al., 2020). In regions without iodine deficiency, GD constitutes about 80% of hyperthyroidism cases, while hyperfunctioning autonomous adenomas are more common in iodine-deficient areas. All body tissues require thyroid hormones for normal development, differentiation, metabolic balance, and physiological function. Consequently, hematological abnormalities are frequently reported in thyroid disorders; for instance, anemia is defined in 20–60% of patients with hypothyroidism (Raveinal et al., 2020). This study aims to evaluate these immunological, hematological, and physiological profiles within the patient population of Babylon City, Iraq

MATERIALS AND METHODS

Study Subjects & Demographic Data

We retrospectively selected $n=94$ patients diagnosed with Graves' disease presenting with hyperthyroidism, alongside $n=60$ healthy control samples. Samples were collected between January 2022 and March 2022 at the Imam Al-Sadiq Teaching Hospital, Thyroid Gland Diseases Center, Babylon City. All patients presented with a diffusely enlarged thyroid gland and elevated thyroid hormone levels according to standard diagnostic guidelines.

Ethical Issues

Approval was obtained from the Ethics Committee of the College of Science at Al-Qasim Green University, the Babylon Health Directorate, and the hospital's administration.

Blood Sampling and Processing

Blood samples were collected, centrifuged at 3000 rpm for 15 minutes to isolate serum, and stored at -80°C. Serum aliquots were held at -20°C until testing via Enzyme-Linked Immunosorbent Assay (ELISA) to measure IgG autoantibodies and IL-2 levels.

Statistical Analysis

Data analysis was conducted using SPSS version 26. Descriptive and correlation tests were used, with a significance threshold set at $p < 0.05$. One-way ANOVA was utilized to evaluate differences between groups and sexes, the Chi-square (χ^2) test was used for categorical data, and the independent Student's

t-test was implemented to compare means. Quantitative results are expressed as mean $\pm$ standard deviation (SD).

RESULTS AND DISCUSSION

General Analysis

Statistical variations within and between the groups showed significant differences, as detailed in Table 1.

Table 1: One-way ANOVA Results Between and Within Groups

One-way ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2307.293	5	461.459	26.118	0.000
Within Groups	3215.663	182	17.668		
Total	5522.957	187			

Age Distribution

The age group of 31–40 years demonstrated the most significant differences compared to younger and older cohorts (Table 2). These findings align with Koseva et al. (2021), who noted that while GD typically affects individuals between 30 and 50 years of age, clinical presentation and signs vary widely across different lifespans.

Table 2: Statistical Tests of Age Groups for GD Patients and Controls

Age Groups	Mean Difference	LSD	Sig.
21 - 30	6.692	3.346	0.000
31 - 40	7.431	3.348	0.000
41 - 50	6.643	3.321	0.000

Pathogenesis is multi-factorial, incorporating genetic predisposition and environmental triggers like smoking, high iodine intake, stress, and pregnancy. In pediatric populations, the disease peaks during adolescence and shows a higher prevalence in females (Shin et al., 2019). Furthermore, younger patients experience higher relapse rates, highlighting strong genetic susceptibilities supported by a 75% concordance rate observed in monozygotic twins.

Immunological Parameters (IL-2 & IgG)

The comparative data for IL-2 and IgG levels across sexes and study cohorts are provided in Tables 3 and 4.

Table 3: Immunological Parameters Evaluated by Disease Status and Sex

Parameter	Group	Male (M ± SD)	Female (M ± SD)	T-value
IL-2	Graves' Patients	0.6277 $\pm$ 0.43139	0.6483 $\pm$ 0.43558	-0.213
	Healthy Control	7.6995 $\pm$ 5.02144	7.2911 $\pm$ 8.80367	0.264
IgG	Graves' Patients	15.2831 $\pm$ 2.54698	15.3848 $\pm$ 1.81845	-0.194
	Healthy Control	7.1682 $\pm$ 3.42766	6.9978 $\pm$ 3.43961	0.190

Table 4: Statistical Evaluation of Sex Dynamics Across Cohorts

Parameter	Sex	Graves' Patients (M ± SD)	Healthy Control (M ± SD)	Table t-value
IL-2	Female	0.6125 $\pm$ 0.41690	7.6203 $\pm$ 5.03649	-11.093
	Male	0.6483 $\pm$ 0.43558	7.2911 $\pm$ 8.80367	-4.083
IgG	Female	15.2938 $\pm$ 2.56564	7.2647 $\pm$ 3.50879	15.229
	Male	15.3848 $\pm$ 1.81845	6.9978 $\pm$ 3.43961	10.940

Significantly higher levels of IL-2 were found in healthy controls compared to Graves' patients. Autoimmune thyroid diseases (AITD), encompassing Graves' disease and Hashimoto's thyroiditis, are characterized by lymphocytic infiltration of thyroid tissue by T and B cells (Chu et al., 2018). Key immune-related susceptibility genes include HLA, CTLA-4, CD40, and PTPN22 (Du et al., 2022). Due to the high polymorphism within the major histocompatibility complex (MHC), HLA genes act as dominant molecular backgrounds for GD expression (Stasiak and Lewiński, 2020). Recent next-generation sequencing (NGS) has validated these strong HLA haplotype links (Mehraji et al., 2017). Still, individual genetic predictors show relatively modest odds ratios (~ 2), highlighting the delicate balance between immune anergy and active autoimmunity (Zawadzka-Starczewska et al., 2022).

Regarding IgG, autoantibodies bind and stimulate the TSH receptor, escaping normal metabolic feedback loops (Weider et al., 2021). Serum autoantibodies (TG Ab, anti-TG Ab, TPO Ab, anti-TPO Ab) serve as definitive markers to assess disease severity and monitor clinical responses to antithyroid drug treatment (Nabi et al., 2022). These antibody levels are significantly elevated in hyperthyroid states compared to healthy controls (Paliwal et al., 2022).

Demographically, hyperthyroidism exhibits an estimated prevalence of 2% in females compared to 0.2% in males (Struja et al., 2017). This structural disparity highlights how biological sex influences immune system susceptibility, driven by differences in sex hormones, organ vulnerability, pregnancy state, and epigenetics (Jafarzadeh et al., 2010). GD is reported to have a global prevalence of 0.5% to 2%, with a female-to-male ratio ranging between 4:1 and 6:1 (Ali and Hamoud, 2022).

Hematologic Outcomes (CBC Tests)

The analysis of complete blood counts revealed key differences across multiple cell lines (Table 5).

Table 5: Complete Blood Count (CBC) Profiles for GD and Control Groups

CBC Test	Graves' Patients (M ± SD)	Healthy Control (M ± SD)	T-test	Significance
WBC	12.17 $\pm$ 4.94	6.40 $\pm$ 1.33	8.91	Significant (0.00)
LYM	3.76 $\pm$ 0.93	16.62 $\pm$ 39.44	-3.17	Significant (0.00)
MON	2.72 $\pm$ 1.00	0.48 $\pm$ 0.40	16.67	Significant (0.00)
GRA	6.29 $\pm$ 4.28	4.33 $\pm$ 1.18	3.50	Significant (0.00)
RBC	4.12 $\pm$ 1.67	4.03 $\pm$ 0.46	0.43	Random (0.67)
HGB	8.81 $\pm$ 2.03	14.87 $\pm$ 1.41	-20.39	Significant (0.00)
TLC	24.95 $\pm$ 5.46	3184.51 $\pm$ 9625.40	-3.19	Significant (0.00)
MCV	86.43 $\pm$ 8.34	9.25 $\pm$ 1.08	71.82	Significant (0.00)
MCH	30.04 $\pm$ 3.26	28.41 $\pm$ 2.46	3.34	Significant (0.00)
MCHC	35.72 $\pm$ 4.52	33.75 $\pm$ 0.82	3.37	Significant (0.00)
RDWS	35.75 $\pm$ 4.52	37.57 $\pm$ 5.06	-2.34	Significant (0.02)
RDWC	14.82 $\pm$ 5.90	13.22 $\pm$ 1.61	2.06	Significant (0.04)
PLT	284.56 $\pm$ 136.84	272.44 $\pm$ 113.98	0.57	Random (0.57)
PCT	14.11 $\pm$ 53.52	0.28 $\pm$ 0.25	2.02	Significant (0.05)
MPV	12.34 $\pm$ 17.88	8.48 $\pm$ 0.49	1.69	Random (0.09)
PDWS	10.31 $\pm$ 1.42	10.38 $\pm$ 1.44	-0.30	Random (0.76)
PDWC	36.65 $\pm$ 7.21	38.32 $\pm$ 1.38	-1.78	Random (0.08)

CBC Test	Graves' Patients (M ± SD)	Healthy Control (M ± SD)	T-test	Significance
P-LCC	59.83 $\pm$ 23.20	69.05 $\pm$ 22.01	-2.47	Significant (0.01)
P-LCR	26.86 $\pm$ 4.41	27.20 $\pm$ 5.07	-0.45	Random (0.66)

Our findings show that lymphocytes, monocytes, and overall white cell counts differ significantly in active disease states compared to controls, consistent with trends observed in other hyperthyroid groups (Mathew et al., 2021). Excess thyroid hormones accelerate erythropoiesis by altering immature erythroid progenitor proliferation and boosting erythropoietin (EPO) gene expressions (Ip et al., 2022). Conversely, concurrent increases in total plasma volume, abbreviated erythrocyte lifespan, iron turnover defects, or vitamin deficiencies often mask these mechanisms and culminate in anemia (Ortega et al., 2022). While severe outcomes like pancytopenia are rare, mild decreases in white blood cell counts and plateletes remain common complications of hyperthyroidism (Fishman et al., 2018).

In contrast to subacute thyroiditis where elevated WBC profiles are triggered by viral infections and cytokine-induced steroid responses (Yanai et al., 2019), Graves' disease presents a distinct profile. Functional TSH receptors located on myeloid and lymphoid cell surfaces directly mediate how thyroid imbalances shift circulating leukocyte pools (Çetin et al., 2021).

Our data showed mild anemia in approximately 40% of the evaluated Graves' disease patients, though red blood cell counts did not show drastic drops. This rate matches findings by Zahedi et al. (2017), who reported a 33% to 40% anemia rate in newly diagnosed thyrotoxic cases, which typically normalized within four months of antithyroid drug therapy. Variations in indices like MCV, MCH, and RDW (Shetty and Vijaya, 2019; Kim et al., 2020) further support the role of thyroid hormones in regulating human hematopoiesis, including B-cell production in the bone marrow. Increased RDW without iron deficiency indicates a clear need to check thyroid function along with folate and vitamin B12 levels (Turan, 2019).

Conclusions

Thyroid disorders significantly alter immunological parameters such as TG Ab and TPO Ab, making them reliable markers for clinical dysfunction. Furthermore, these conditions are associated with increased platelet activation, which may elevate the risk of atherothrombotic complications, alongside increases in inflammatory markers like CRP, PCT, and MPV (Artemniak-Wojtowicz et al., 2019).

Graves' disease is linked to clear hematological abnormalities that span all major blood cell lineages, indicating that bone marrow function may be affected. These hematological shifts are likely driven by a combination of autoimmunity, thyroid hormone excess, nutritional deficiencies, and genetic factors (Ahmed and Mohammed, 2020). Consequently, hematological parameters should be monitored periodically throughout the course of antithyroid treatment.

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