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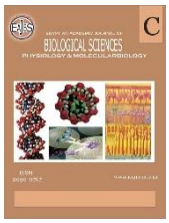

Evaluation of Some Auto-Antibody Levels in Patients with Autoimmune Thyroiditis

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ABSTRACT

The study aimed to evaluate the serum levels of the ATPO, ATG, and TRAB in patients with patients with autoimmune thyroiditis such as Hashimoto thyroiditis and Graves disease, and in control groups. The results of current study showed the concentrations of thyroid hormones T3, T4, TSH, FT3, FT4 were measured to determine the patients who have an underactive thyroid gland or have hyperthyroidism and compared them to a control group, where it was found that the patients who had an underactive thyroid had significantly decreased levels of T3, T4, FT3 and FT4 concentrations, while their TSH level increased significantly than the rest of the patients and the control group. Most of the patients' samples were patients who had hypothyroidism. After that, special tests were done to detect the presence of the specific antibodies associated with this disease (AITD), which are ATPO, ATG, and TRAb, in addition to the clinical diagnosis of patients by the physician using the ultrasound device. It was found that patients with Graves' disease had a significant increase in the level of TRAb concentration compared to Hashimoto's patients and healthy controls, while the concentration of ATG was significantly increased in patients with Hashimoto's disease compared to patients with Graves' disease and healthy controls, and the concentration of ATPO was significantly elevated in both patients with Graves' and Hashimoto's disease compared to healthy controls.

INTRODUCTION

Autoimmune thyroiditis is the most common organ-specific autoimmune disease (ADs), which affects 5% of the population with significant gender differences (i.e., women 5–15% and men 5%) ((Menconi *et al.*, 2008). Both Graves' disease (GD) and Hashimoto thyroiditis (HD) are part of autoimmune thyroiditis. The primary causes of hypothyroidism and hyperthyroidism, respectively, are HT and GD. Both (GD) and (HT) share the presence of a cellular and humoral immune responding to the thyroid gland antigens, the reactive infiltration of T and B cells, the formation of autoantibodies, and the subsequent emergence of clinical signs, which reflect the loss of immunological tolerance (Anaya *et al.*,2012). The thyroid gland's functionality is changed by lymphocytic infiltration, which also damages surrounding tissue. The thyroid cells are damaged when autoantibodies or sensitized T lymphocytes interact with them, triggering an inflammatory response and, occasionally, cell lysis. (Shin *et al.*,2009).

Graves' disease, Named after Robert Graves who was the first to identify the connection in 1835, (GD) is clinically distinguished by the presence of hyperthyroidism, widespread goiter Ophthalmopathy, and dermopathy (Menconi *et al*.,2008, Brent,2008). GD accounts for up to 80% of instances of hyperthyroidism, making it the most prevalent cause (Weetman, 2000). Ten times more women than men are impacted by it. There has been evidence of a high frequency between the ages of 40 and 60 (Brent,2008). A higher risk of GD and a younger onset age are linked to a family history of thyroid illness, particularly in maternal relatives (Cooper,2003). The presence of circulating TSH Receptor Ab (TRAb) causes GD by binding and activating the TSH receptor, causing follicular enlargement and hyperplasia, also an increase in thyroid hormone production and the T3 relative proportion to T4 in the blood (Davies,2005). Hashimoto Thyroiditis is the most related cause of hypothyroidism in the region where iodine is abundant. (Stathatos and Daniels,2012). Hakaru Hashimoto was the first to identify it in 1912 when he personified four ladies have a syndrome he named Struma Lymphomatosa (Rocchi *et al*.,2008).

MATERIALS AND METHODS

Study Design and Patients:

The samples were collected from patients with auto-immune thyroiditis Najaf Center for Diabetes and Endocrinology in Al-Sadr Medical City Hospital and specialized AL-Najaf Laboratory during the period from January 2022 to October 2022 after clinical diagnosis.

Serum Collection:

Five ml of blood were collected from healthy and infected patients. Blood samples were drawn in sterile plain tubes and left at room temperature for 30 min. Centrifugation was done at 3000 rpm for 5 min (Memmert, Germany**).** The serum was collected and kept in sterile tubes at deep freeze at -20 until use.

Serum Auto-Antibody Detection:

Three human auto-antibodies were used in this study: the ATPO, ATG, and TRAB. The ATPO and ATG biomarkers kits were provided by Aeskulisa Company, Germany, and TRAB biomarkers kits were provided by Eagle Biosciences company, Columbia and the level of biomarkers in serum was determined by using ELISA device (Human reader, Germany) according to the Manufacturer Company.

Statistical Analysis:

The well-known statistical program (Graph Pad Prism version 7) was employed, and the one-way anova analysis of variance test (by Tukey's multiple comparisons test) was performed to compare the measured parameters

RESULTS

ATPO Levels:

The results of subgroup comparisons were significant ($P<0.0001$) indicating there were significant differences in TPO antibody titer between patients and Control groups as shown in Figure (1). The mean of TPO in both Graves and Hashimoto disease was high (466.008 ± 12.467 IU/ml; 460.714 ± 11.167 IU/ml) respectively, while the mean of TPO in control was (22.612 ± 3.029 IU/ml).

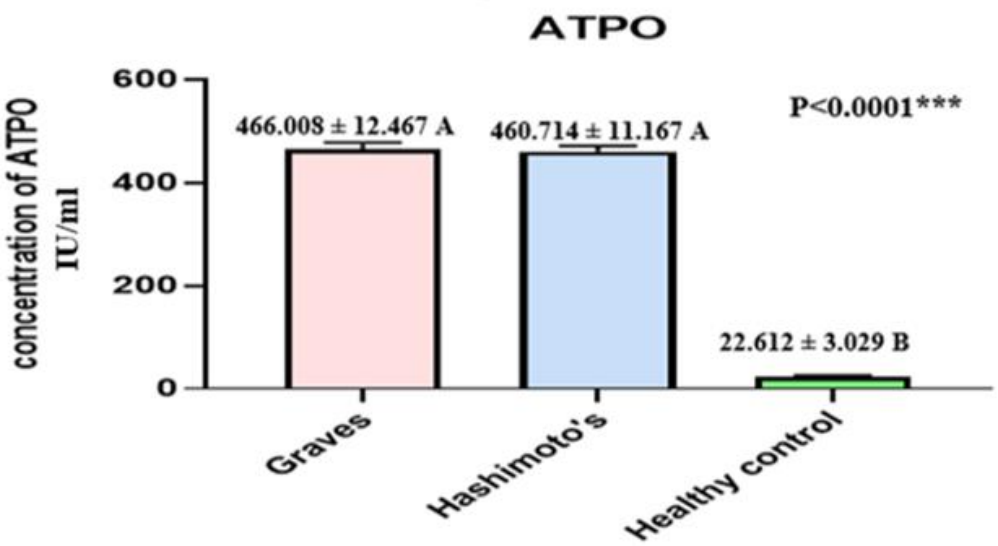

Fig. 1: Relation between thyroidal peroxidase antibody(ATPO) level among studied groups.

ATG Levels:

The results of subgroup comparisons were significant (P<0.0001) indicating there were significant differences in anti-Tg antibody titer among the subgroups as shown in Figure (2). The mean of ATG in Hashimoto disease was high(517.120 ± 26.795 IU/ml), while the mean of ATG in Graves disease and control was (96.458 ± 8.529 IU/ml; 12.424 ± 5.193 IU/ml) respectively.

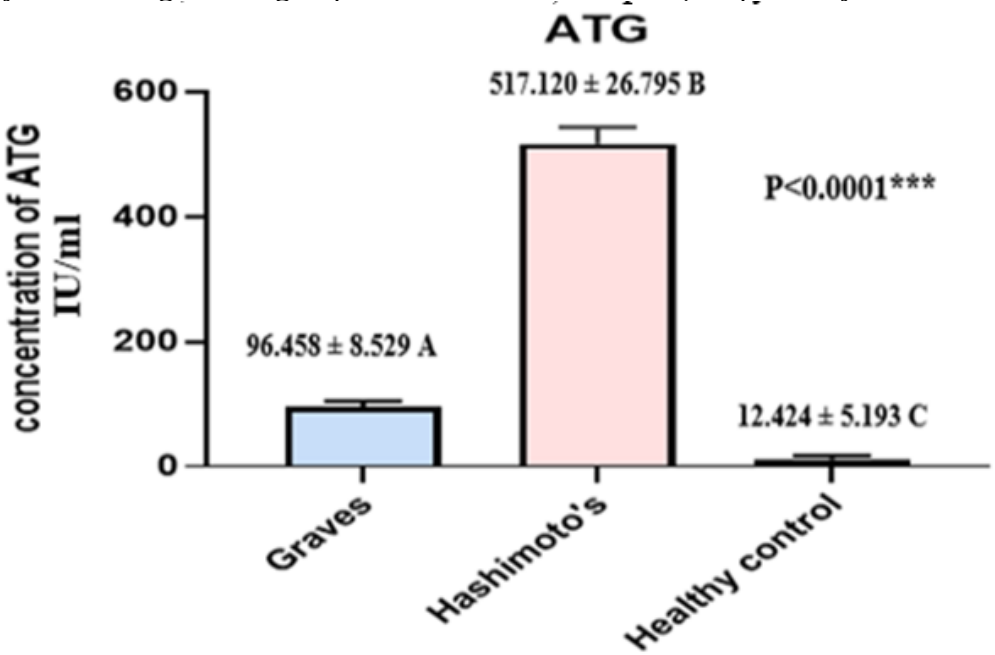

Fig. 2: Relation between Thyroglobulin antibodies (ATG) level among studied groups.

TRAB Levels:

The results of subgroup comparisons were significant (P<0.0001) indicating there were significant differences in TRAB titer among the subgroups as shown in Figure (3). The mean of TRAB in Graves disease was high (33.136 ± 1.591 IU/l), while the mean of TRAB in Hashimoto disease and control was (16.011 ± 1.469 IU/l; 1.771 ± 0.703 IU/l) respectively.

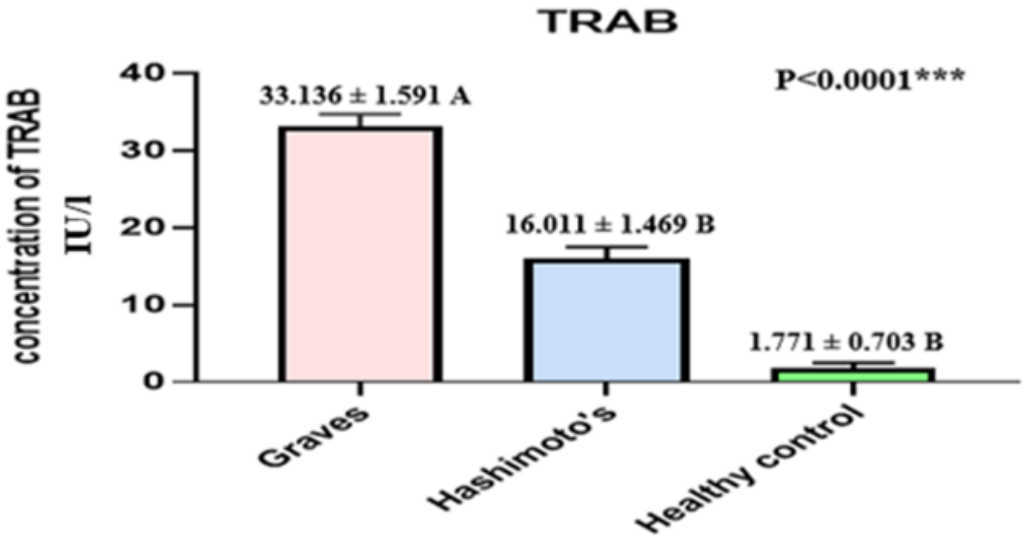

Fig. 3: Relation between Thyroid stimulating hormone receptor antibodies (TRAb) level among studied groups.

Comparisons Mean of T3, T4, TSH, FT3, and FT4 between the Studied Groups:

Hormones that include T3, T4, TSH, FT3, and FT4 levels were compared in Graves' disease, Hashimoto's disease, and control groups. The mean value of the T3 and T4 parameters in Graves disease was substantially greater than in Hashimoto thyroiditis and controls, which found high significant differences between these studied groups P<0.0001.The mean of T3 and T4 in Graves disease patients were (5.068 ± 0.131 nmol/L; 203.704 ± 7.913 nmol/L), while in Hashimoto thyroiditis were (0.183 ± 0.020 nmol/L; 24.003 ± 1.199 nmol/L), and in healthy control were (1.622 ± 0.052 nmol/L; 90.026 ± 1.321 nmol/L) respectively , as shown in Figures (4 &5).

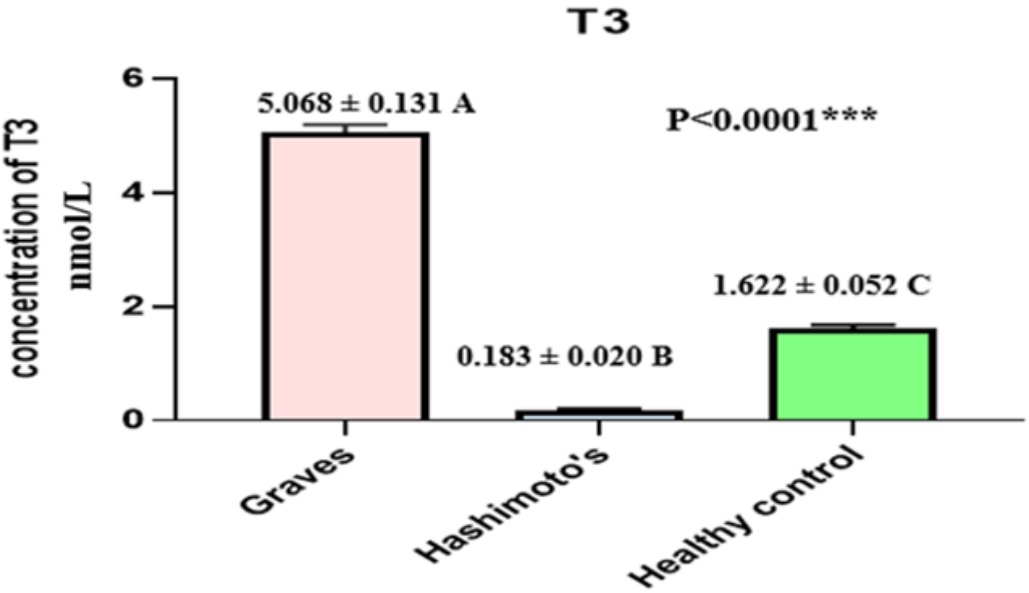

Fig. 4: Comparison between studied groups in a concentration of T3.

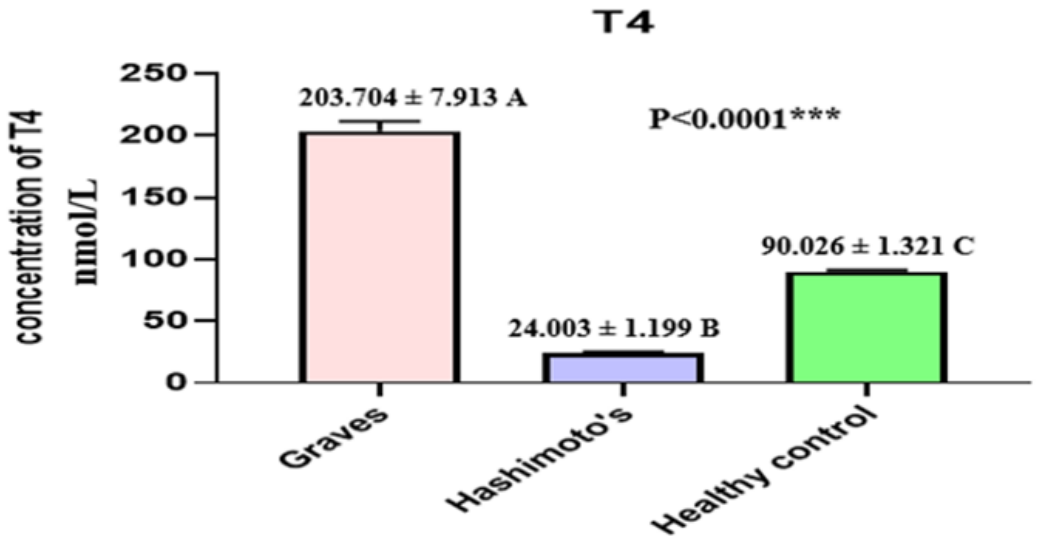

Fig. 5: Comparison between studied groups in a concentration of T4**.**

The mean of TSH in the GD patient group was (0.833 ± 0.117 µIU/ml), HT patient group was (21.986 ± 1.935 µIU/ml), whereas the mean of the Healthy control was (4.006 ± 0.116 µIU/ml), as shown in Figure (6). There was a significant difference between the analyzed groups P<0.0001.

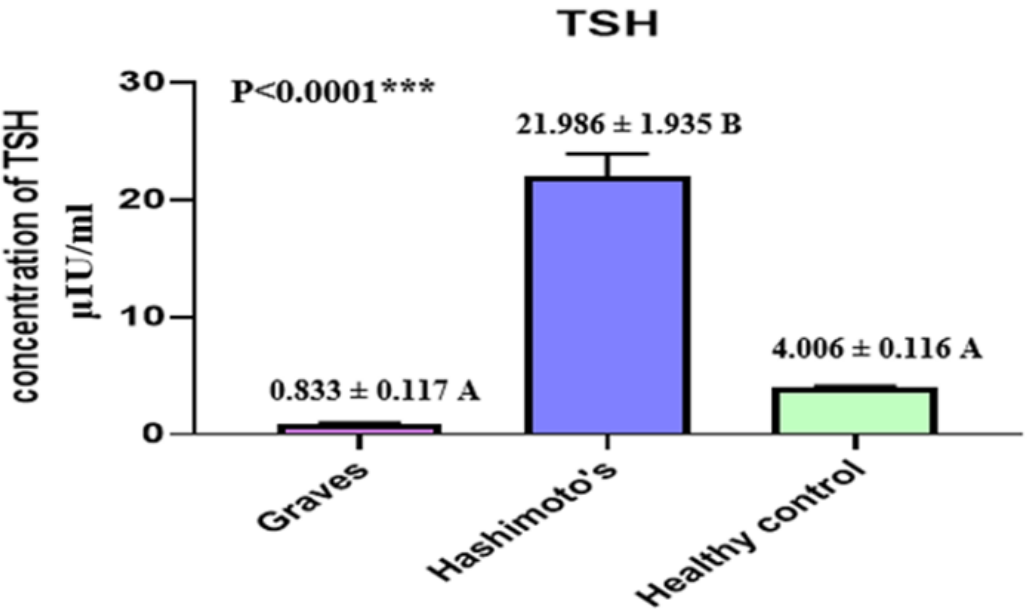

Fig. 6: Comparison between studied groups in a concentration of TSH**.**

Furthermore, the mean of FT3 and FT4 in Hashimoto thyroiditis were (1.151 ± 0.076 pmol/L; 0.194 ± 0.020 ng/dL), and in Healthy Control (4.812 ± 0.139 pmol/L; 1.267 ± 0.022 ng/dL), while in the Graves group were the highest (9.362 ± 0.239 pmol/L; 8.502 ± 0.328 ng/dL) respectively. The findings were extremely significant when compared to the studied group (P<0.0001), as shown in Figures (7 & 8).

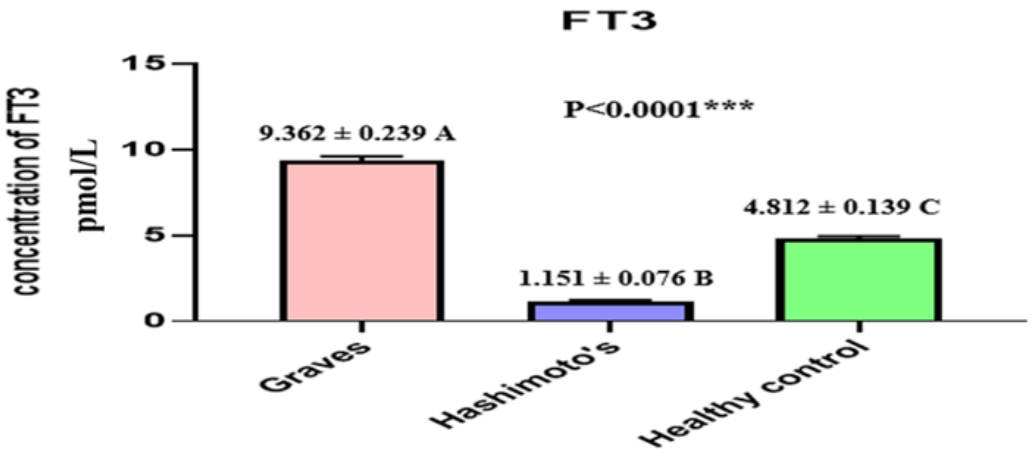

Fig. 7: Comparison between studied groups in a concentration of FT3**.**

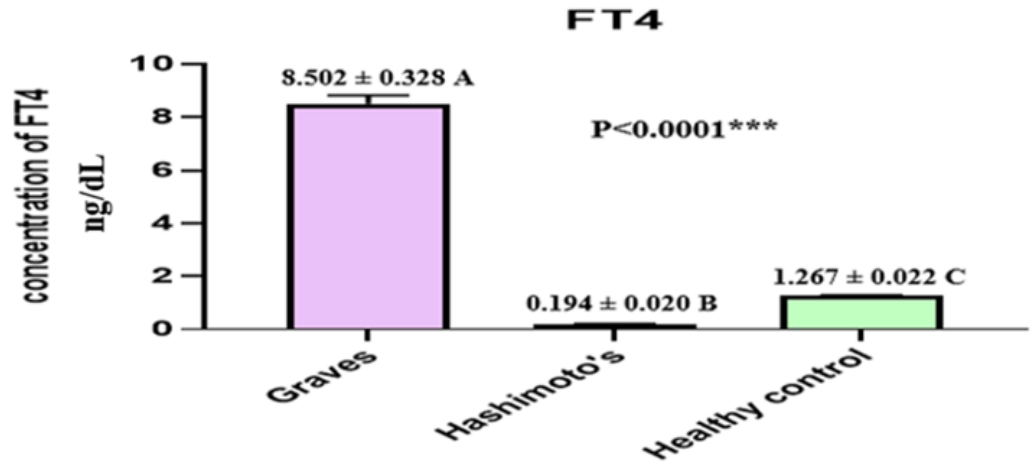

Fig. 8: Comparison between studied groups in a concentration of FT.

DISCUSSION

TPO antibodies are highly elevated in autoimmune thyroid diseases. Grave's disease and Hashimoto's thyroiditis are the two main autoimmune thyroid conditions (George,2023). TPO antibodies can be elevated in people who don't have any thyroid conditions. High TPO antibodies could make it more likely that you'll get a thyroid condition in the future (Anila *et al.*, 2019).Thyroid antibodies come in two different varieties: thyroglobulin antibodies and thyroid peroxidase antibodies. Thyroglobulin and thyroid peroxidase are required by the body to produce thyroid hormones. In autoimmune thyroid diseases, both types of thyroid antibodies are frequently elevated. According to Fröhlich and Wahl (2017), a higher proportion of persons with autoimmune thyroid disorders had elevated thyroid peroxidase antibodies and thyroglobulin which means these results agree with this study.

Diverse sources have reported on how Tg antibodies are distributed among the classes. One study found that IgG1 and IgG4 were the most significant classes in GD and HT patients, while other authors found that IgG1, IgG2, and IgG4 classes were distributed differently. IgG4 class predominated in GD patients and IgG2 class in HT patients, which is an interesting difference in distribution between the two groups of patients (Hattori *et al*.,2017). This disparate distribution can be a result of the many immunological responses the thyroid is experiencing (Eleonore and Richard, 2017).

TRAbs are IgG-type antibodies that fall into two categories: TSAbs (TSHR-stimulating antibodies), which stimulate the

TSH receptor, and TBAbs (TSHR-blocking antibodies), which block it. TSAbs are very common in GD patients and stimulate the TSHR. In between 10% and 90% of people with HT, TBAbs are detected and increase the risk of hypothyroidism. Additionally, TRAbs that can bind to the TSHR but have no effect on thyroid function have been discovered (Jungel *et al*., 2010; Yan *et al*., 2019; Coppedè, 2017). The basic mechanism of HT, according to the scientific consensus, is a cellular autoimmune response with a large inflammatory infiltrate that causes thyroid gland loss and consequent dysfunction. The presence of autoantibodies against the thyrotropin receptor (TRAb), which stimulates the growth and function of thyroid follicular cells (TFCs), has led some to believe that GD is primarily mediated by a humoral autoimmune response and is the primary cause of goiter and hyperthyroidism (Ana and Monica, 2016). In contrast, humoral and cellular immune mechanisms are closely related and cross-linked in AITD, as they are in other autoimmune disorders, and once they are activated, they go through subsequent feedback circuits that reciprocally amplify and perpetuate one of the responses while inhibiting the other, indicating the complex mechanisms involved in the pathogenesis of AITD (Rapoport and McLachlan, 2014; González and Marazuela, 2015).

Hypothyroidism (Hashimoto's thyroiditis) and hyperthyroidism (Graves disease) are the two clinical extremes of autoimmune thyroid disease (AITD), a spectrum of many disorders. Both conditions are characterized by a cellular and humoral autoimmune reaction, with an increase in the production and secretion of antibodies against different thyroid antigens, as well as a phenomenon of thyrocyte necrosis and apoptosis (in HT) and a persistent thyrotropin-receptor stimulation (in GD) (Skevaki and Wesemann, 2023; Bogusawska *et al*., 2022). The diagnosis of both entities is based on clinical, laboratory, and imaging data in a study by Hernando *et al* (2023), there are three main anti-thyroid antibodies that have been identified: thyroglobulin (TgAb), thyroid peroxidase (TPOAb), and the TSH receptor (TRAb). Each of these autoantibodies is crucial to the method used to diagnose autoimmune thyroid disease. TRAbs are a defining feature of GD because, among other things, they can predict how a patient will respond to treatment and if a decrease in these antibody levels, these indicate of effectiveness of treatment. TPOAb and TgAb, which are both positive when thyroid autoimmunity is present, enable the identification of people who have a higher risk of developing Hashimoto thyroiditis.

The results showed that both men and women with Graves' disease had extremely substantial increases in T3 and T4 levels as well as a highly significant decrease in TSH levels when compared to control subjects. Because T3 and T4 exert negative feedback on the pituitary and hypothalamic axis, the source of these antibodies is immune-competent plasma cells, and the antibodies bind with TSHR and block it leading to the start and increase in T3 and T4 synthesis and production regardless of a decrease in TSH (Abbas & Muttaleb, 2018).

According to Choksi *et al*. (2003), Graves' disease is the most frequent cause of hyperthyroidism (GD), which increases the effectiveness of thyroid gland tissue and causes hyperactivity in the production of one or both thyroid hormones (T3 and T4). In order to quickly identify Graves' disease, a total T3/T4 ratio and TSH value may be utilized (Yanagisawa *et al*., 2005; Carlé, 2013). Variations in T3 and T4 levels, as well as TSH, are goiter markers, according to Hüser *et al*. (2018). According to Diana *et al*. (2018), the (TSH) test is used to diagnose hyperthyroidism when it is suppressed, together with an increase in T4; and T3 to support the diagnosis.

Serum TSH concentrations were thought to be the most reliable indicator of thyroid function abnormalities among these hormones because the log/linear TSH/FT4 relationship dictates that an altered TSH will be the first abnormality to appear - as soon as the pituitary registers that FT4 has changed from its genetically-determined set point for

that particular individual (Pop *et al*., 2014). On the other hand, significant changes in serum TSH are triggered by even small changes in T4 concentration. To correctly detect moderate (subclinical) hypothyroidism or hyperthyroidism, the TSH reference range must be set up. According to research by Hussain *et al*. (2017), patients with more severe conditions (higher FT3 and FT4) had a far higher likelihood of not responding to medical treatment.

Imran *et al*., 2016 found that 20% of patients had hyperthyroidism, which was indicated by high blood levels of FT3, and FT4, and low serum levels of TSH, while 7.5% had hypothyroidism.

Conclusions:

Anti-TPO was high in both Graves and Hashimoto patients while anti-TG was high in Hashimoto patients, also anti-TSH receptor (TRAB) was high in Graves patients.T3 and T4 hormones were high and TSH hormone was low in patients with Graves disease in comparison with Hashimoto thyroiditis and healthy control, while T3 and T4 hormone were low and TSH hormone was high in patients with Hashimoto thyroiditis in compare with Graves disease and healthy control.

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