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RESEARCH ARTICLE

Investigating IL27 and IL1-Beta Levels in Graves' and Hashimoto's

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ABSTRACT

The prevalence of autoimmune thyroid diseases (AITD), one of the most prevalent organ-specific autoimmune disorders, varies by gender and affects 2-5% of the population (i.e., women are affected at a rate of 5-15% and men at 1-5%). AITD includes diseases like Hashimoto Thyroiditis (HT) and Graves' Disease (GD), among others. For hypothyroidism and hyperthyroidism, respectively, the main causes are HT and GD. We suggest that GD and HT patients will have higher levels of IL-27 and IL-1β than those without these illnesses, suggesting their role in the inflammation linked to autoimmune thyroid disorders. 150 participants in total 50 with Graves' Disease (GD), 50 with Hashimoto Thyroiditis (HT), and 50 control subjects all between the ages of 20 and 60 were progressively enrolled in this study at Al-Sader Teaching Hospital in Al-Najaf between August 2023 and March 2024. The research populations' serum concentrations of IL27 and IL1β were measured through the utilization of an ELISA kit obtained from MyBioSource (USA). Every subject also had their levels of T3, T4, TSH, TR.Ab, TPO.Ab, and TG.Ab measured. The Chi-square test was used to compare the frequencies of categorical variables in patients and controls. The F test (ANOVA) was used to compare the mean values of age, BMI, T3, T4, TSH, TR Ab, TPO, TG, IL27, and IL1β parameters across patients with Graves' disease (GD), Hashimoto's thyroiditis (HT), and the control group. According to the current study, patients with Graves' disease (GD) had higher levels of T3 and T4 than the control group, patients with Hashimoto's thyroiditis (HT), and both. On the other hand, there were statistically significant differences (P=0.001) in the TSH level between the HT and control groups and the GD group. Additionally, there were notable differences in the levels of autoantibodies directed against the thyroid gland, with GD patients having considerably greater levels of TR, TPO, and anti-TG than HT patients and the control group (P=0.001). Furthermore, GD and HT patients showed significantly higher levels of IL27 and IL1β than the control group (P=0.001). Comparing patients with Graves' Disease (GD) and Hashimoto's thyroiditis (HT) to healthy controls, our study found that these patients had significantly higher levels of two important cytokines, IL-27 and IL-1β. These results highlight the role that inflammatory processes and immunological dysregulation play in the etiology of autoimmune thyroid diseases.

INTRODUCTION

The thyroid gland is a target of autoimmunity, which resulting in autoimmune thyroid diseases (AITDs), which include Graves' disease (GD) and Hashimoto's thyroiditis (HT), these diseases are serious conditions characterized by aberrant immune responses. Thyroid tissue is damaged and dysfunctional because of complex interactions between immune system abnormalities, environmental factors, and genetic vulnerabilities (Casto et al., 2021; Jam et al., 2010). The IL27, a cytokine belonging to the IL6/IL12 family, influences both innate and adaptive immunity in immune cells through a variety of pleiotropic effects (Saleh *et al.,* 2024). It regulates T cell development, the production of cytokines, and immunological homeostasis via the IL27 receptor complex, which is made up of glycoprotein130 and IL-27Rα subunits (Sarin et al*.,* 2023). The importance of IL27 in immune-mediated diseases has been highlighted by its association with several autoimmune disorders, such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease (Clénet *et al.,* 2021). Nevertheless, little is known about its precise role in AITDs, especially GD and HT.

Analogously, pro-inflammatory cytokine IL1-Beta has strong impact on immune cells and inflammatory mechanisms (Galozzi et al*.,* 2021; Kanval et al., 2024). It serves a critical function in promoting both local and systemic inflammation and is primarily generated by activated macrophages (Dinarello, 2018). Several autoimmune diseases, including AITDs, have been linked to deregulated IL1-Beta signaling, which increases the production of autoantibodies and inflammation of thyroid tissue (Rashid et al., 2023; Ritvo & Klatzmann, 2019). Even though its role in the pathophysiology of GD and HT has been established, more research is needed to determine the precise pathways by which IL1-Beta plays this role.

This case-control study is investigating the levels of IL27 and IL1-Beta in patients with GD and HT in comparison to healthy controls. The current study assumes to establish more about the possible functions that these cytokines may play in the pathophysiology of various autoimmune thyroid disorders and investigate their potential use as biomarkers for prognosis and diagnosis. Comprehending the dysregulation of cytokines in GD and HT could provide new avenues for therapeutic targeting and approaches to controlling these difficult autoimmune diseases.

MATERIALS AND METHODS

Study population

A total of 150 participants, with age between 20 and 60 years, sequentially enrolled at Al-Sader Teaching Hospital in Al-Najaf between August 2023 and March 2024. The participants consisted of three groups: fifty cases diagnosed with Graves' Disease, fifty cases diagnosed with Hashimoto Thyroiditis, and fifty healthy control individuals. The sample was created carefully to represent the population in terms of ethnicity, educational attainment, and other demographic characteristics. To evaluate individuals from various groups, an extensive questionnaire was distributed, which covered a wide range of topics, such as lifestyle determinants and demographic information. The current study performed according to the rules issued by the research committee of the medical ethics unit under the Ministry of Health in Iraq, informed and written permission was obtained from each participant.

Sample collection

Participants' peripheral venous blood was aspirated under aseptic procedures. To separate the serum from the other blood components, samples were centrifuged for five minutes at 3000 rpm after the blood was allowed to clot for 30 minutes at room temperature. Using a micropipette, the serum was extracted for the ELISA hormone analysis test. It was then placed in two Eppendorf tube repeaters and frozen at -20°C.

Blood sample analysis

Study groups' serum levels of IL27 and IL1β were evaluated using an ELISA kit from MyBioSource (USA). Thyroid function blood tests, including thyroid stimulating hormone (TSH), free triiodothyronine hormone (fT3), and free thyroxine hormone (fT4), were performed utilizing the Cobas e411 instrument's full automated Chemiluminescence Immunoassay. The thyroid receptor antibody (TR. Ab), thyroperoxidase (TPO), and thyroglobulin (TG) serum concentrations for everyone were measured using the enzyme-linked immunosorbent assay (ELISA) by the Elabscience company (USA).

Statistical analysis

IBM's 2017 SPSS version 25 program for Windows was used to enter, arrange, and analyze participant data, including that of individuals with autoimmune thyroid illness and controls. Every variable was checked for mistakes or inconsistencies before being analyzed. The Chi-square test was used to determine the significance of variations in the frequencies of qualitative variables between patients and controls. While, F test (ANOVA) was used to compare the mean values of age, BMI, T3, T4, TSH, TR Ab, TPO, TG, IL27, and IL1β parameters between the patients with Graves' disease (GD), Hashimoto's thyroiditis (HT), and control group. The threshold for significance (P-value) was set at 0.05 or less. In addition, the Microsoft Word 2013 for Windows program was used to exhibit the outcomes and discoveries in tables.

RESULTS

Characteristics of the study population.

Table 1 displays the mean age ± SD of the GD, HT patient, and control groups. The results indicate significant differences between the matching groups at $p \leq 0.05$. Table (1) also displays the distribution of the age group into four categories. The results, which are presented as proportions and numbers for both groups under study, indicate that there are no significant differences between the control and patient groups at ($p = 0.543$). According to this study, patients between the ages of 41 and 50 were most likely to have both GD and HT, with age groups 51 to 60 years old and 31 to 40 years old following closely after.

According to the current study data, 38 (76%) of the GD patients and 41 (82%) of the HT patients have a negative family history, while 12 (24%), 9 (18%), and HT patients have positive family histories of illnesses. In respect of the percentage of female patients with GD (n=22/28) and HT (n=24/26) was the insignificantly (p=0.250) highest among the analyzed groups in the study that was given, based on group distribution according to sex.

According to the mean BMI, there were notable significant (p =0.001) variations between the patients and control participants, as indicated by the current data. The body mass index (BMI) ranged from normal to overweight and obese. A high percentage of GD patients in the current study were overweight (42%) and then normal (38%), while most HT patients were normal (44%) and overweight (32%), and the results indicated non-significant (p =0.585) variations when compared to the control group.

The results also showed there were significant (p =0.05) variances between patients GD, HT and control in residency as shown in Table (1). About 66% of GD and 54% of HT cases were urban and rural patients, respectively.

Table 1: Characteristics of study population

Characteristic	**GD** ***n*** **= 50**	**HT** ***n*****= 50**	**Control** ***n*** **= 50**	***p*-Value**
Age (years)				
Mean ±SD	42.9±10.5	41.8± 8.3	37.7 ± 10.12	**0.05 Sig.†**
< 30, *n* (%)	10 (20)	8 (16)	15 (30)	**0.543 NS¥**
31-40, *n* (%)	12 (24)	9 (18)	7 (14)	
41-50, *n* (%)	14 (28)	13 (26)	8 (16)	
51-60, *n* (%)	8 (16)	12 (24)	11 (22)	
≥60, *n* (%)	6 (12)	8 (16)	9 (18)	
Family history				
Positive, *n* (%)	12 (24)	9 (18)	0 (0.0)	**0.002 HS ¥**
Negative, *n* (%)	38 (76)	41(82)	50 (100)	
Sex				
Male, *n* (%)	22 (44)	24 (48)	30 (60)	**0.250 NS¥**
Female, *n* (%)	28 (56)	26 (52)	20 (40)	
Body mass index (BMI)				
Mean ±SD	28.33 ± 5.02	30.4 ± 5.9	25.13 ± 3.08	**0.001HS†**
Normal Weight	19 (38)	22 (44)	20 (40)	**0.585 NS¥**
Overweight	21 (42)	16 (32)	23 (46)	
Obesity	10 (20)	12 (24)	7 (14)	
Residence				
Urban, *n* (%)	33 (66)	23 (46)	19 (38)	**0.05 Sig.¥**
Rural, *n* (%)	17 (34)	27 (54)	31 (62)	

n: number of cases; **SD**: standard deviation; †: ANOVA test; ¥: Chi-square test; NS: not significant at $p > 0.05$.

Thyroid function tests

The current study's Table (2) showed that GD patients had significantly higher levels of T3 (p =0.001) and T4 (p =0.001) than HT and control groups, but their TSH levels were lower with statistically variations (p =0.001) in comparison to the HT and control groups. Conversely, HT patients exhibited

a significant reduction in T3 (P=0.001) and T4 (p =0.001) levels in comparison to the GD and control groups, meanwhile TSH showed a significant (p=0.001) high level.

Table 2: Serum thyroid function test among GD and HT in comparison with control

Parameter	GD *n*=50 mean± SD	HT *n*=50 mean± SD	Control *n*=50 mean± SD	Statistical test	P Value
Free T3 (nmol/l)	34.2 ± 12.5	8.2 ± 3.8	18.8 ± 3.14	Df:2, F= 141.6	0.001†
Free T4 (µg/dl)	55.9 ± 20.4	31.7 ± 9.7	47.7 ± 17.7	Df:2, F= 25.5	0.001†
TSH (µIU/ml)	0.55 ± 0.21	42.2 ± 18.5	2.8 ± 0.9	Df:2, F= 239.6	0.001†
GD: Graves' disease**; HT:** Hashimoto thyroiditis**; SD:** standard deviation**; †:**one way ANOVA test**; Df:** degree of freedom.					

Estimation the level of thyroid autoantibodies

In comparison to the HT and control groups, there were extremely significant differences in the levels of autoantibodies against the thyroid gland among the GD group, as demonstrated by Table (3). In addition, HT patients' levels of TR. Ab were found to be lower than those of the GD. Conversely, TPO and anti-TG levels among GD and HT patients were significantly (p =0.001) raised when compared to the control group.

Table 3: Serum level of thyroid autoantibodies among studied groups

Parameter	GD *n*=50 mean± SD	HT *n*=50 mean± SD	Control *n*=50 mean± SD	Statistical test	*p* value
TR. Ab (ng/ml)	6.8 ± 2.8	0.61 ± 0.12	0.48 ± 0.24	**Df:**2, **F= 231.5**	**0.001**†
TPO. Ab (IU/ml)	325.8 ± 150.8	379.3± 58.7	6.9± 3.4	**Df:**2, **F= 231.7**	**0.001**†
TG. Ab (ng/ml)	449.4± 109.5	424.5± 153.8	20.8 ± 9.07	**Df:**2, **F=** 128.2	**0.001**†
GD: Graves' disease**; HT:** Hashimoto thyroiditis**; SD:** standard deviation**; †:**one way ANOVA test**; Df:** degree of freedom**; TR. Ab:** thyroid receptor antibody**; TPO:** thyroperoxidase antibody**; TG. Ab:** Thyroglobulin antibodies.					

Measurement of the IL27 level in GD, HT and control.

Table (4) illustrated a highly significant increase in the levels of IL27 among patients with GD and HT in comparison with control groups (p = 0.001).

Table 4: level of IL27 among GD & HT patients in comparison with control.

Groups	Mean IL27 (pg/ml)	*SD*	*SE*	95% C. I		Compared groups		Sign.
				Lower	Upper			
GD	25.81	8.2	1.16	23.4	28.15	GD	HT	0.000*
							Con.	0.000*
HT	15.23	4.2	0.6	14.02	16.44	HT	GD	0.000*
							Con.	0.000*
Control	8.53	3.3	0.48	7.57	9.5	Con.	GD	0.000*
							HT	0.000*
GD: Graves' disease; HT: Hashimoto thyroiditis; SD: standard deviation; SE: standard error; C.I: confidence intervals; *: The mean difference is significant at the 0.05 level.								

Table 5: Level of IL-1β among GD & HT patients in comparison with control.

Groups	Mean IL1β (pg/ml)	SD	SE	95% C. I		Compared groups		Sign.
				Lower	Upper			
GD	37.06	8.6	1.2	34.5	39.5	GD	HT	0.000*
							Con.	0.000*
HT	49.4	4.9	0.69	48.00	50.8	HT	GD	0.000*
							Con.	0.000*
Control	15.7	3.8	0.54	14.65	16.84	Con.	GD	0.000*
							HT	0.000*
GD: Graves' disease; HT: Hashimoto thyroiditis; SD: standard deviation; SE: standard error; C.I: confidence intervals; *: The mean difference is significant at the 0.05 level.								

Measurement of the IL-1β level in GD, HT and control.

The mean IL-1β levels were significantly higher in individuals with GD and HT compared to healthy controls (P = 0.001) as shown in table (5).

DISCUSSION

The current study detects the insignificant role of age on incidence of GD and HT. Age has been found to be a key determinant in the incidence and prevalence of GD and HT in several investigations. Graves' disease is more common in early adulthood, especially between the ages of 30 and 50, according to Conrad *et al.* (2023), whereas HT typically affects those in their middle years and beyond. The incidences of both GD and HT increased with age, according to a meta-analysis by Effraimidis & Wiersinga (2014), with older age groups showing a larger occurrence.

Most cases of GD and HT in this study have family history, this finding is in line with other studies that show autoimmune thyroid disorders are inherited. The pathophysiology of GD and HT has been linked to genetic variables, such as mutations in genes related to thyroid function and immunological control (Razmara *et al.,* 2021; Vargas-Uricoechea, 2023). In some populations, for example,

polymorphisms in the thyroid stimulating hormone receptor (TSHR) gene have been associated with a higher risk of GD (Zaaber *et al.,* 2020).

Numerous studies have indicated that women are more likely than males to develop GD, with varied sex-ratios. The X chromosome, which affects the thyroid and immune system, and the hormone estrogen, which is often higher in females, are linked to the imbalance of sex hormones, which is why thyroid diseases are common globally, particularly among women (Kjaergaard *et al.,* 2021).

Many research works have indicated a possible correlation between obesity and a higher chance of acquiring GD and/or HT. Chronic low-grade inflammation and dysregulated adipokines are features of obesity that may exacerbate immune system malfunction and lead to the onset of autoimmune illnesses like GD (Tylor, 2021). Furthermore, a condition known as insulin resistance, which is defined by a decreased ability to respond to insulin, is frequently linked to obesity. Insulin resistance may have an impact on the metabolism of thyroid hormones and may be a factor in changes in thyroid function, such as the elevated production of thyroid hormones that is indicative of GD (Machado *et al.,*2022). Multiple epidemiological investigations have investigated at the association between thyroiditis caused by Hashimoto's and obesity. Some studies indicate a favorable correlation between obesity and the chance of developing autoimmune thyroid illnesses, such as Hashimoto's thyroiditis, however the results have been rather inconsistent. Song *et al.,* (2019) conducted a meta-analysis which revealed a correlation between obesity and a higher likelihood of autoimmune thyroid illnesses. However, the precise processes underlying this association are still to be determined.

The results of current study demonstrated the impact of environmental factors related to location on thyroid autoimmunity by revealing significant variations in the occurrence of GD and HT between urban and rural people. These factors include ultraviolet light, pollution, infectious agents, and iodine consumption (Ferrari *et al.,* 2017). Research has demonstrated regional differences in the frequency and occurrence of various illnesses, pointing to a possible environmental component in their etiology. Al-Khawari *et al.,* (2015) investigated the relationship between living in Kuwait's rural or urban regions and the likelihood of developing autoimmune thyroid disorders.

The current study defines elevated rates of T3 and T4 and decreased rates of TSH in GD, vice versa in HT. A study compared the thyroid hormone levels of patients with GD and HT to those of controls, the findings, which were consistent with hyperthyroidism, revealed that GD patients had considerably higher T3 and T4 levels than controls, whereas TSH levels were also considerably lower in GD patients than in controls, indicating decreased TSH secretion. (Zamwar & Muneshwar, 2023). Patients with autoimmune thyroid disorders, such as GD and HT, had their thyroid hormone levels compared in another investigation (Korevaar *et al.,* 2017). It was confirmed by the results that patients with GD had higher T3 and T4 levels and lower TSH levels, while patients with HT had higher TSH levels and lower T3 and T4 levels. One of the main signs of clinical hypothyroidism in HT is elevated TSH. These parameters facilitate a precise and quick diagnosis of GD and aid in the differential diagnosis of hyperthyroidism (Weng *et al.,* 2022).

The current study shows that autoantibodies TR. Ab, TG. Ab and TPO were elevated in GD and HT. The measurement of serum thyroid stimulating antibody (TSAb) and thyroid stimulating blocking antibody (TSBAb) is now the most important laboratory test (Cui *et al.,* 2019). For GD patients, TSAb is considered by many to be prominent. The occurrence of hypothyroidism is observed when TSBAb is the dominating antibody (Liu *et al.,* 2023). TG. Ab and TPO. Ab positivity is seen in about 70% of GD cases, but in HT patients, TR. Ab might also rise noticeably (Daramjav *et al.,* 2023). Our findings appear to be consistent with those of Al-Mofarji *et al.,* (2023), who discovered substantial variations in TPO antibody levels between individuals with hyperthyroidism and those in being healthy. The elevated TR-Ab levels observed in the present study are consistent with the findings of Fawzi *et al.,* (2018), who discovered elevated levels of TR-Ab in patients suffering from hyperthyroidism. Furthermore, when comparing the hypothyroid and hyperthyroid groups to the control group, a

Saudi Arabian study found a statistically significant rise in anti-TG and TPO levels (El Din *et al.,*2021). A different study conducted in India found that, in comparison to the control group, female hypothyroid patients had statistically significant increases in anti-TG and TPO levels (Thomas *et al.,* 2017).

Cytokines constitute a prerequisite for the development of autoimmune illnesses because they participate in the induction and effector phases of all inflammatory and immunological responses. The development of autoimmune inflammation is mostly influenced by increased, decreased, or inappropriate cytokine responses (Liu *et al.,* 2021).

The IL-12 family of cytokines has significant effects on inflammation and the immune system (Xin *et al.,*2021). The IL-12 family included two comparatively recent members, IL-27 and IL-35 (Gocher *et al.,* 2022). The aberrant activation of T cells is directly linked to GD, and the proinflammatory cytokine IL27 is crucial for the transcriptional activation and control of T cells (Xu *et al.,* 2024). One distinct member of the IL-12/IL-6 family of cytokines, IL27 is a heterodimeric cytokine that is primarily produced by Dendritic cells and monocytes/macrophages, among other antigen-presenting cells (Odoardi *et al.*, 2021). Because of its combined pro- and anti-inflammatory properties, IL-27 is presently being explored as a potential treatment for autoimmune disorders (Shahi *et al.,* 2020). In contrast to a study that discovered lower serum levels of IL-27 in Graves' disease patients (Saeed *et al.,* 2020), this study findings suggested that these cytokines may have an anti-inflammatory function (Saeed *et al.,* 2020). Th1 to Th2 ratio variations, which are non-dominant in Graves' illness, may be explained by the impact of IL-27 (Łukawska-Tatarczuk *et al.,* 2021).

Increased IL-1β levels have been linked to GD patients in numerous studies. GD patients had higher serum IL-1β levels than controls, indicating a potential role for this protein in the disease's development (Rayman, 2019). Furthermore, GD is associated with a hyperthyroid condition since IL-1β has been linked to the activation of thyroid follicular cells and the synthesis of thyroid hormones (Antonelli *et al.,* 2015).

Moreover, thyroid inflammation and autoimmune damage in HT are thought to be significantly influenced by IL-1β. Excessive expression of pro-inflammatory cytokines and chemokines has been demonstrated to be caused by IL-1β, which exacerbates tissue damage and thyroid inflammation in hypoxic thyroid disease (Wu *et al.,* 2018).

The T cells, B lymphocytes, and pro-inflammatory cytokines are immunological responses that are dysregulated in both GD and HT. Thyroxine synthesis and immune cell activation in the thyroid gland are regulated by IL-1β, a major inflammatory mediator (Barroso-Sousa *et al.,* 2018). Research indicates that IL-1β may enhance the development of T helper 17 (Th17) cells, a subtype of T cells linked to autoimmune disease, hence intensifying the inflammatory cascade in GD and HT conditions (Lechner *et al.,* 2023). High levels of IL-1β may be an effective biomarker for GD and HT patients in determining the course and severity of their diseases. Tracing the evolution of the disease and the response to treatment with IL-1β may be useful for clinicians (Abbate *et al.,* 2020).

CONCLUSION

The results of our investigation indicate that, in comparison to healthy controls, those with Graves' Disease (GD) and Hashimoto's thyroiditis (HT) had significantly higher levels of two important cytokines, IL-27 and IL-1β. These results highlight the role that inflammatory processes and immunological dysregulation play in the etiology of autoimmune thyroid diseases. In general, the discovery of increased amounts of IL-27 and IL-1β broadens scientists understanding of the immunological processes behind autoimmune thyroid conditions. To clarify the precise roles that these cytokines play in the pathophysiology of disease and to investigate if they could be used as therapeutic targets to treat GD and HT, more investigation is necessary.

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