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Changes in Interleukin-35 (IL-35), Anti-TSHR antibody and Human Insulin- like Growth Factor 1 Receptor (IGF1R) in Graves' Disease Patients Treatedwith Radioactive Iodine

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ABSTRACT

Objective: The objective of the current study was to examine the changes in the serum level of some immunological markers in Graves' disease (GD) patients in comparison to healthy controls. In order to evaluate its utility as clinical biomarker of autoimmune disorder as well as to establish the changes of some immunological markers and clinical outcome that is associated with radioactive iodine treatment.

Methodology: Serum levels of interleukin-35 (IL-35), anti-TSHR antibody and human insulin- like growth factor 1 (IGF-1) were measured using ELISA assay. The blood samples were collected from a total of 70 Iraqi GD patients who were enrolled in this study during the period between February and April 2022. They were divided into two groups: The first group involved 35 patients with GD without treatment and the second group involved 35 patients who received radioactive iodine therapy (RAI). In addition, 30 people apparently healthy worked as control group.

Results: The results showed a significant decrease in serum levels of IL-35 and anti-TSHR in untreated and treated GD patients as compared to healthy control. The data also revealed that serum level of IGF-1 was higher in untreated GD patients compared to the control group, but it was decreased in treated GD patients. Also, there were a difference at serum levels of IL-35 and TSH-R when compared between the untreated and treated GD patients.

Conclusions: The results of IL-35 and anti-TSHR, and IGF-1 levels in the serum of the studied groups suggests the potential anti-inflammatory function of these biomarkers in GD.

Keywords: Graves' disease, IL-35, Anti-TSHR, IGF-1, Radioactive iodine therapy.

INTRODUCTION

Graves' disease (GD) is the most common cause of hyperthyroidism. The reported prevalence is 0.8% in the USA and 1.3% in Europe [(1)]. Graves' disease is a type of autoimmune disease affect thyroid resulting in an overactive thyroid gland [(2)]. Grave's disease affects elderly females who previously had normal thyroid function [(3)].However, the thyroid gland plays a critical role in overall body metabolism, including hematopoiesis [(4)]. Thyroid-stimulating hormone (TSH) is used often to test the thyroid gland disorders. A small substance called thyrotropin-releasing hormone (TRH) releases from hypothalamus. which stimulate release of thyroid-stimulating hormone (TSH) which later induce the thyroid gland to produce FT3 and FT4 hormones that control the metabolism of the body [(5)].

Graves' disease mainly seems as a result of absence of immune tolerance to organ-specific self-antigens, such as TSH receptors . Determination of the level of TSH can be applied as a useful marker at different stages of GD diagnosis as well as before and after treatment [(6)]. Insulin-like growth factor receptor type1 (IGF-1) is a large transmembrane tyrosine kinase. It has been reported that IGF-1 can play important role on promoter of TSH signaling. In addition the concentrations of IGF-1 serum were negatively connected with FT4 levels at different stages of GD diagnosis and treatment [(7)].

Cytokines are a little proteins have a major role in regulating the activity and development the immunological and inflammatory responses of immune system. Interleukin-35 belong to the IL- 12 family with a unique IL-12R2:gp130 heterodimeric inhibitory cytokine composed of the p35 subunit [(8)] .However, the information about the functions of IL-35 is still under investigation but it has been reported that IL-35 involved in suppression of Th17 proliferation as well as work as anti-inflammatory role [(9)]. Interleukins play an important role in the development of autoimmune disorders[(10)].

Radioactive Iodine (RAI), Because of its ease, low cost, and low rate of serious complications, has been recommended as the best treatment option for patients with Graves' disease. It is based on the thyroid follicular cells' unique ability to trap and organize iodine. However, the role of radioactive iodine therapy on regulation of autoimmune disease is still unclear.

The aim of this study was to examine the potential role of IL- 35 in GD patients in comparison with healthy controls. In order to evaluate its utility as clinical biomarkers of autoimmune disorder ,as well as to establish the changes and assess outcomes of GD patients treated with RAI.

MATERIALS AND METHODS

The serum levels of interleukin-35 (IL-35), anti-TSHR antibody and human insulin-like growth factor 1

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receptor (IGF-1), were measured using commercially ELISA assay (Sun Long Biotech, China), adhering to the manufacturer's instructions. The blood samples were collected from a total of 70 Iraqi GD patients (age ranged from 25 to 55 years). who attended to the Hormonal Unite at Specialized Center for Endocrinology and Baghdad Center for Radiotherapy and Nuclear Medicine, German hospital for Nuclear Medicine, Baghdad, Iraq during the period between February and April, 2022.

They were divided into two groups: The first group involved 35 patients with GD without treatment and the second group involved 35 patients who received radioactive iodine (RAI) therapy. In addition, 30 people apparently healthy worked as control group. The automated quantitative hematology analyzer was used for hematological parameters. The D-Cell 60 analyzer directly measures the white blood cells, red blood cells, hemoglobin, hematocrit, platelets, granulocytes, monocytes and lymphocytes.

Ethical clearance

The Ethical Committee, Department of Biology, College of Science, University of Bagdad's approved this work in Baghdad Iraq. The authorization is referenced as CSEC/0122/0035.

Statistical Analysis

The Statistical Analysis was done using SPSS program (version 23). To compare means, the least significant difference -LSD test (Analysis of Variation-ANOVA). was used. The Chi- square test was used to compare percentages that were not equal. The result was stated as Mean ± SEM and $p \leq 0.05$ was considered significant.

RESULTS

The hematological parameters were examined in the blood samples of the studied groups. The findings revealed that hemoglobin level (Hb) were higher in GD patients with and without treatment was (13.55 ± 0.24 and 13.89 ± 0.84 g/dL respectively) when compared with Hb level in healthy subjects (12.71 ± 0.27 g/dL) but there was no significant differences ($p = 0.306$) as shown in table (1). Similar results were obtained with red blood cells (RBC) data as shown in table (1). However, there were a significant difference in WBC levels among studied groups ($p = 0.0493$) with a significant difference between GD patients with and without treatment (8.06 ± 0.37 and 9.11 ± 0.38 $\times 10^9$/L respectively). The results showed that the platelets level (PLT) in GD patients with and without treatment (237.43 ± 11.28 and 263.84 ± 15.34 ×10*9/L respectively) were higher when compared with PLT level in healthy subjects (229.40 ± 9.34 ×10*9/L) but There were no significant differences ($p = 0.131$) in PLT levels in patients compared to the healthy group as shown in table (1).

Table (1): Hematological parameters of grave patients in relation to treatment and control

Groups	Mean ± SE				
	Lymphocyte	**WBC**	**RBC**	**PLT**	**Hb**
GD Patients	28.77 ± 0.83	8.06 ± 0.37 [b]	4.78 ± 0.07	237.43 ± 11.28	13.55 ± 0.24
GD with RAI Treatment	31.39 ± 1.63	9.11 ± 0.38 [a]	4.89 ± 0.12	263.84 ± 15.34	13.89 ± 0.84
Healthy Control	30.53 ± 0.92	8.01 ± 0.26 [b]	4.67 ± 0.08	229.40 ± 9.34	12.71 ± 0.27
LSD value	3.384 NS	0.995 *	13.606 NS	35.057 NS	1.521 NS
P-value	**0.278**	**0.0493**	**0.384**	**0.131**	**0.306**

Means having with the different letters in same column differed significantly, * ($P \leq 0.05$).

The concentrations of IL-35 were examined in the serum samples of the studied groups. The results showed that the level of IL-35 in GD patients with treatment was 397.70 ± 6.47 mg/dl and in GD patients without treatment was 320.31 ± 10.65 mg/dl while IL-35 level in healthy subjects was 676.67 ± 26.19 mg/dl. There was a significant differences between studied groups as well as between GD patients and healthy control (p=0.0001) (Table 2).

Thyroid Stimulating Hormone Receptor (TSH-R) results showed that the level of TSH-R in GD patients with treatment was 1.141 ± 0.02 mg/dl and in GD patients without treatment was 1.590 ± 0.07mg/dl while in healthy subjects was 2.147 ± 0.06 mg/dl.

There was a significant differences between studied groups as well as between GD patients and healthy control (p=0.0001) as shown in table (2). Also, the results showed that IGF-1 levels in GD, patients with treatment was 5.09 ± 0.13mg/dl and in GD patients without treatment was 3.38 ± 0.06 mg/dl while in healthy subjects was 4.01 ± 0.08 mg/dl. However, there was a significant differences between GD patients and healthy control (p=0.0001), (Table 2).

Table (2): Comparison between IL-35, TSH-R and 1GF1R

Groups	Mean ± SE (mg/dl)		
	IL-35	**TSH-R**	**IGF-1**
GD Patients	397.70 ± 6.47 [a]	1.141 ± 0.02[a]	5.09 ±0.13
GD with RAI Treatment	320.31 ± 10.65 [b]	1.590 ± 0.07 [b]	3.38 ± 0.06
Healthy Control	676.67 ± 26.19 [c]	2.147 ±0.06 [c]	4.01 ± 0.08
LSD value	43.989 **	0.163 **	0.288 **
P-value	**0.0001**	**0.0001**	**0.0001**
Means having with the different letters in same column differed significantly, ** (P≤0.01).			

In addition, the results, of correlation between age, weight, BMI and biomarkers studies were demonstrated in tables (3), (4) and (5). In general, there were no significant differences between them in exception of the correction between BMI and IL-35. as shown in table 3.

Table (3): Correlation coefficients-r between Age, Weight, BMI and IL-35 levels

Groups	**IL-35**		
	Age	**Weight**	**BMI**
GD Patients	0.10 NS	0.12 NS	0.09 NS
GD with RAI Treatment	0.06 NS	0.13 NS	0.37 *
Healthy Control	0.03 NS	0.02 NS	0.14 NS
* (P≤0.05), NS: Non-Significant			

Table (4): Correlation coefficients-r between Age, Weight, BMI and TSHR level

Groups	**TSHR**		
	Age	**Weight**	**BMI**
GD Patients	0.02 NS	0.07 NS	0.07 NS
GD with RAI Treatment	0.02 NS	0.09 NS	0.02 NS
Healthy Control	0.05 NS	0.04 NS	0.14 NS
NS: Non-Significant			

Table (5): Correlation coefficients-r between Age, Weight, and BMI and IGF-1 levels

Groups	IGF-1		
	Age	**Weight**	**BMI**
GD Patients	0.08 NS	0.11 NS	0.02 NS
GD with RAI Treatment	0.13 NS	-0.04 NS	0.04 NS
Healthy Control	0.08 NS	-0.07 NS	0.08 NS
NS: Non-Significant			

DISCUSSION

The current study indicated that there was no statistically significant differences between the hematological parameters including RBC, platelets, Hb and lymphocytes levels, while the results of WBC had a significant differences. In a previous study, the role of GD was indicated in the case of hematological parameters data. However, these results indicated that the state of the thyroid gland has an effect on the hematopoietic system, and the changes caused by thyrotoxicosis can have an effect on all three lineages of hematopoietic cells and anemia is the most common manifestation. Few cases of pancytopenia have been documented in the literature, most of which have been associated with GD, but they are often not fatal According to **Hamid *et al* .**[11].Another previous study suggested a possible role for GD in hemoglobin status. However, it suggests that hepcidin (a protein produced in the liver that controls iron absorption and level) may be the possible link between anemia and hypothyroidism. Hepcidin is the main regulator of iron homeostasis. During the treatment of GD, the concentration of hepcidin decreases However, GD treatment results in the restoration of cell number in most cases of pancytopenia or leukopenia associated with GD. Recovery can take anywhere from a few weeks to over a year [11].Another study indicated the role of radioiodine iodine treatment in improving complete blood count levels. The most frequent haematologic abnormalities identified at GD diagnosis according to a prior study return to normal following the start of radioiodine treatment and the distribution of white blood cells may differ in GD in contrast to other infectious disorders, including leukopenia [12].

Our results showed that TSH-R levels were significantly decreased in serum of GD patients with and without treatment in comparison with healthy controls. However, TSH-R autoantibodies play an important role in the pathogenesis of GD. Therefore, TSH-R when clinical features are inconclusive in the diagnosis of GD, the assay can be used as a gold standard diagnostic marker. The level of TSH-R increased significantly immediately after therapy and followed by a gradual decline. In contrast, TSH-R decreased in patients over the first year as they became euthyroid due to medication or surgery. After one year, patients in all therapy groups continued to enter remission of TSH receptor autoimmunity According to **Mumtaz *et al.*** [13].Graves' disease is characterized by agonistic thyroid stimulating antibodies (TSAbs) binding to and stimulating the TSHR in the same way that TSH does, preventing TSH from binding to and stimulating the receptor. Hyperthyroidism results in an excess of FT4 and FT3 hormone production and release. When the hypothalamus detects an increase in FT4 and FT3 levels in the blood, it reduces thyroid releasing hormone secretion and, as a result, the pituitary gland reduces TSH secretion. However, because TSAbs stimulate the thyroid gland rather than TSH, there is no change in thyroid hormone synthesis and release, resulting in an excess of T4 and T3 in the body. According to **Brent *et al.*** [14]**.** In patients with Graves' disease, however, the link between TSH and receptor antibodies and increased thyroid hormone synthesis shows that B cells are activated and humoral immune responses are dominant According to **Brand *et al.*** [15].. Autoantibodies attach to the receptor and activate thyroid cells by mimicking TSH action[15].

The results of present study revealed that levels of IL-35 decreased significantly in untreated DG patients and following RAI treatment in comparison with healthy controls. Interleukin-35 (IL-35) is a member of the IL-12 family that have an inhibitory effect through suppressing T cell proliferation and regulating T cells to limit inflammatory responses According to **Choi *et al.*** [16]. Many studies have revealed that IL-35 plays a significant role in controlling autoimmune diseases. Many observations indicate that IL-35 plays more critical role in the development of many autoimmune diseases and may serve as a biomarker for disease progression The information about the role of IL-35 in GD is very limited but [16]. found that serum levels of IL-35 were significantly lower among Juvenile Idiopathic Arthritis children patients. IL-35 plays an impartment role in balancing between Th-17 and T-reg cells. Also, IL-35 inhibits the function of Th-17, which is critical in development of IL-35 that can induce differentiation of naive T cells to trigger T-reg cells, which in turn can contribute to the pathogenesis of several autoimmune diseases.. According to **Akin *et al.*** [17]. To the best of our knowledge, this is the first study to investigate the role of IL-35 in GD patients before and following treatment with RAI. Our findings support the idea that serum IL-35 could be used as a marker of GD activity and that a decrease in IL-35 levels could be related to achieving euthyroidism and the immunomodulatory effects of RAI treatment.

Moreover, serum IGF-1 levels in GD without treatment were significantly higher than in the other two study groups. These results agree with many researches, which showed that IGF-1 is specific for GD According to **Pritchard *et al.*** [18]. .Graves' disease express higher IGF-IR levels than do those found in healthy control Furthermore, in vitro studies have revealed that the insulin-like growth factor-1 receptor (IGF-1R) is a key player as associated autoantigen in

the pathogenesis of GD According to **Wang *et al.*** [19]. A population study by [7] ..discovered that high levels of serum IGF-1 were connected with decreased serum TSH levels in females.

Another study revealed that hypothyroidism patients had lower serum IGF-1 levels in comparison with controls Also, people with subclinical hypothyroidism had lower IGF-1 levels but the subclinical hyperthyroidism had similar IGF-1 levels like healthy control group [19]. It has reported that protein and mRNA levels of IGF-1 and IGF-1R were significantly higher in tissues of patients with different thyroid disorders when compared to controls According to **Liu *et al.*** [20].

CONCLUSION

In conclusion, the elevation of serum biomarkers included IL-35, TSHR and IGF-1 levels in Graves' disease particularly in patients with radioactive iodine treatment. This explains the importance of these parameters in progression of Graves' disease.

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