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Effect of Radioactive Iodine Therapy on Interleukin-2 (IL-2), IL-17 and Physiological Parameters in Iraqi Patients with Graves' Disease

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ABSTRACT

Objective: The causes behind graves' disease are still unclear but it is suggested to be the results of a combination of risk factors include genetic and environmental factors as well as immune factors. So, it may be useful to investigate the potential association between some serum biomarkers of greaves disease patients before and after treatment with Radioactive Iodine (RAI). **Subjects and methods:** Serum levels of Interleukin-2 (IL-2), IL-17 were measured using ELISA assay. The blood samples were collected from a total of 70 Iraqi GD patients were enrolled in this study. They were divided into two groups: The first group involved 35 untreated GD patients 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 hormonal results indicated that before radiation, most of GD patients had greater free triiodothyronine (FT3) and free thyroxine (FT4) levels, and lower TSH levels than healthy controls. Also, there were a significant difference in the lipid levels of TRG and HDL in the GD patients before and after treatment with RAI. The results showed a significant increase in serum levels of IL-2 in untreated and treated GD patients as compared to healthy control. The data also revealed that the serum level of IL-17 was lower in untreated GD patients compared to the control group. There were no significant differences before and after treatment with IL-17 and there were no significant differences observed among studies groups.

Conclusions: The results of IL-2 and IL-17 levels in the serum of the studied groups suggests the potential anti-inflammatory function of these biomarkers in GD.

Keywords: Graves' disease, IL-2, IL-17, Radioactive iodine therapy.

INTRODUCTION

Thyroid gland is an endocrine gland that secrete hormones Triiodothyronine (T3) and Thyroxin (T4) into the blood [(1)]. These two hormones are necessary to the human body work normally [(2, 3)].

However, thyroid hormones are involved in regulating metabolism. It has been observed that T3 hormone is more active in body metabolism. A close connection was found between iodine concentrations in food and the activity of thyroid gland. Thyroid gland is involved in many metabolic processes include brain function, lipid and energy metabolism, protein synthesis, tissue maturity and cellular oxidation[(4)]. Thyroid-stimulating hormone (TSH) is one of largest growth factor that is created by the pituitary gland and involved in regulation of both T3 and T4. Graves' disease (GD) is one of autoimmune condition diseases that can cause both inflation and hyperthyroidism. The signs and symptoms of Graves` disease which also is called toxic thyroid enlargement can include skin problems, muscle weakness, weight loss, heart rhythm speed and eye problems in most patients [(5)]. Graves' disease is the most common cause of hyperthyroidism. The reported prevalence is 0.8% in the USA and 1.3% in Europe [(6)].It is a type of autoimmune disease that affect thyroid resulting in an overactive thyroid gland [(7)] .Grave's disease affects elderly females who previously, had normal thyroid function[(8)] .However, the thyroid gland plays a critical role in overall body metabolism, including hematopoiesis[(9)] .

Thyroid- stimulating hormone (TSH) is used often to test the thyroid gland disorders. Radioactive Iodine (RAI) has been recommended as the best treatment option for patients with Graves' disease because of its ease, low cost and low rate of serious complications. It is based on the unique ability of the thyroid follicular cells to trap and organify iodine[(10)]. However, the role of radioactive iodine therapy on regulation of autoimmune disease is still unclear. Cytokines are little proteins have a major role in regulating the activity and development of the immunological and inflammatory responses of immune system.

MATERIALS AND METHODS

The serum levels of interleukin-2 (IL-2) and interleukin-17 (IL-17) were measured using commercially ELISA assay (Sun Long Biotech, China), following the manufacturer's instructions. The blood samples were collected from a total of 70 Iraqi GD patients (aged ranging from 25 to 55 years) were attended Hormonal Unite at Specialized Center for Endocrinology and Baghdad Center for Radiotherapy and Nuclear Medicine, Germany Hospital for Nuclear Medicine, Baghdad, Iraq. The study was conducted during the period between February and April, 2022. They were divided into two groups: 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. Hormonal estimation included free triiodothyronine

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(FT3), thyroxine (FT4), and thyrotropin (TSH) as well as lipid profiles were measured also in this study.

Ethical clearance:

The Ethical Committee of the Department of Biology, College of Science, University of Bagdad, Baghdad, Iraq, gave their stamp of approval to this work. The authorization with the reference number CSEC/0122/0035.

Statistical Analysis

The Statistical analysis was done by using SPSS program (version 23). Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to compare between means. Chi-square test was used to compare between percentages. The result was stated as Mean ± SEM and $P \leq 0.05$ was considered significant.

RESULTS

The results showed that mean age of untreated and treated GD patients with RAI treatment 45.22 ± 2.49 and 47.62 ± 2.13 years, respectively, while healthy control was 37.13 ± 2.47 years. However, there was a significant difference between the mean of two GD patients and healthy control ($P \leq 0.01$). The results of BMI indicated that mean BMI of untreated and treated GD patient with RAI treatment 22.73 ± 0.14 and 24.33 ± 0.24 kg/m^2 respectively, while healthy control was 24.70 ± 0.25 kg/m^2. However, there was a significant difference between the GD patients with RAI treatment and healthy control ($P \leq 0.0001$) but there was no significant differences between untreated GD patients and healthy control as shown in table (1).

Table (1): Age and BMI distribution between different studied groups

Group	Age (years)	BMI (kg/m^2)
	Mean ± SE	
GD Patients	45.22 ± 2.49 a	22.73 ± 0.14 a
GD with RAI Treatment	47.62 ± 2.13 a	24.33 ± 0.24 b
Healthy Control	37.13 ± 2.47 b	21.70 ± 0.25 a
LSD value	6.675 **	0.614 **
P-value	**0.0078**	**0.0001**

Means with different letters in the same column varied significantly. Data are presented as mean ± standard Error (SE). ** ($P \leq 0.01$).

Distribution of GD patients according to their gender are shown in table (2). The majority of untreated GD patients were females (77.14%) and about 22.86% of them were males. Similar distribution was observed with treated GD patients. The mean gender of females and males in GD patients with RAI treatment were 88.57% and 13.33% respectively.

While healthy control females and males were 86.67% and 13.33% respectively. However, there was a significant difference between the mean of two GD patients groups and healthy control.

Table (2): Distribution of GD patients according to gender

Group	No.	Male No. (%)	Female No. (%)	P-value
GD Patients	35	8 (22.86%)	27 (77.14%)	0.0001 **
GD with RAI Treatment	35	4 (11.43%)	31 (88.57%)	0.0001 **
Healthy Control	30	4 (13.33%)	26 (86.67%)	0.0001 **
P-value	--	0.0497 *	0.0497 *	---
*** (P≤0.05), ** (P≤0.01)**				

Table (3) showed serum thyroid hormonal levels in untreated and treated GD patients. A significant ($p \leq 0.0001$) increases in FT3 level were observed in GD patients in comparison with GD patients with RAI treatment and control, while no significant difference was observed between GD patients with RAI treatment and healthy control. Similar significant increases were observed in serum levels of FT4.

Serum level of TSH in GD patients and GD patients with RAI treatment were 0.072 µIU/ml and 13.86 µIU/ml respectively.

While the mean of TSH in healthy control was 1.60 µIU/ml. Moreover, a significant ($p \leq 0.0001$) increase in TSH level were observed in GD patients with RAI treatment in comparison with GD patients and control, while no significant difference was observed between GD patients and healthy control (Table 3).

Table (3): Distribution of hormone levels in different studied groups

Group	FT3 (ng/dL)	FT4 (µg/dL)	TSH (µIU/ml)
	Mean ± SE		
GD Patients	5.01 ±0.51 a	1.59 ±0.09 a	0.072 ±0.01 b
GD with RAI Treatment	3.07 ±0.41 b	1.097 ±0.09 b	13.86 ±3.37 a
Healthy Control	3.13 ±0.09 b	1.21 ±0.03 b	1.597 ±0.16 b
LSD value	1.0357 **	0.236 **	7.337 **
P-value	**0.0003**	**0.0001**	**0.0004**
Means with different letters in the same column varied significantly. Data are presented as mean ± standard Error (SE). ** (P≤0.0001).			

The distribution of lipid profile included cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and very low density lipoprotein (VLDL) in Iraqi Graves' disease patients. The results of cholesterol indicated that there was no significant difference among the different groups of GD patients without and with RAI treatment (153.25 ± 3.87 and 164.35 ± 4.65 mg/dL respectively) and control (160.00 ±4.64 mg/dL) as shown in table (4). Similar results about LDL were found as shown in table (4). The results of triglyceride in this study showed that mean levels of triglyceride in GD patients and GD patients with RAI treatment were 169.85 ± 17.32 mg/dL and 138.14 ±5.09 mg/dL respectively and the mean of triglyceride in healthy control was 120.53 ± 3.93 mg/dL. A significant decrease in triglyceride level was observed in GD patients without RAI treatment in comparison with GD patients treated with RAI and control (Table 4).

Table (4): Comparison of studied parameters in GD patients according to Lipid profile

Group	Mean ± SE (mg/dL)				
	Cholesterol	**Triglyceride**	**LDL**	**HDL**	**VLDL**
GD patients	153.25 ± 3.87	169.85 ± 17.32 a	76.04 ± 4.32	42.02 ±1.01b	34.01 ± 3.46 a
GD with RAI treatment	164.35 ± 4.65	138.14 ± 5.09 b	86.14 ± 4.94	46.35 ±1.45a	30.66 ±2.40 ab
Healthy control	160.00 ± 4.64	120.53 ± 3.93 b	86.75 ±4.49	47.96 ±1.85a	24.59 ±0.98 b
LSD value	12.342 NS	31.455 **	12.970 NS	4.051 **	7.348 *
P-value	**0.188**	**0.0088**	**0.108**	**0.010**	**0.044**
Means with different letters in the same column varied significantly. Data are presented as mean ± standard Error (SE). ** (P≤0.01), ** (P≤0.001).					

The results showed that means of IL-2 in GD patients and GD patients with RAI treatment were 316.92 and 127.73 mg/dL respectively. While, the mean value of IL-2 in healthy control was 90.73 mg/dL. Moreover, a significant ($p \leq 0.0001$) increases in IL-2 levels were observed among GD patients with or without RAI treatment in comparison with control as well as significant differences ($p \leq 0.0001$) was observed between GD patients and GD patients with RAI treatment. The results showed that means of IL-17 in GD patients and GD patients with RAI treatment were 51.36 and 34.01 mg/dL respectively. While, the mean value of IL-17 in healthy control was 37.54 mg/dL. Also, the IL-17 results indicated that there was no significant differences among all studied groups.

Table (5): Distribution of immunological markers in different studied groups

Group	Mean ± SE (mg/dl)	
	IL-2	**IL-17**
GD Patients	316.92 ±10.33 a	51.36 ±1.32
GD with RAI Treatment	127.65 ±9.54 b	43.01 ±8.20
Healthy Control	90.73 ±1.76 c	37.54 ±0.82
LSD value	24.077 **	13.972 NS
P-value	**0.0001**	**0.148**
Means with different letters in the same column varied significantly Data are presented as mean ± standard Error (SE). ** (P≤0.0001).		

DISCUSSION

Graves' disease is often classified as a middle age disease. Age may also be regarded as a surrogate for the various biological changes that occur with age where the risk of GD increases with age and iodine insufficiency. It has been suggested that thyroid gland disorder may be more common in middle age people then the younger age [11] .According to **Gabriel *et al*** [12] .numerous studies the majority of thyroid diseases occur in people aged 30 to 50 year .The results of BMI of this study are in line with previous studies suggests that typically BMI state to be within normal weight in GD According to **Van Veenendaal and Rivkees *et al*** .[13]. Our results of gender are in line with the findings of many studies, which showed that the thyroid diseases affect women more than men According to **Allahabadia *et al*.** [14] mentioned that Graves' disease affecting women 5–10 times more frequently than men.

Because many medicines interfere with protein binding thyroid hormone, which leads to interference with total thyroid hormone levels, free thyroid hormone concentrations are preferred in the diagnosis of thyrotoxicosis [15] .However, the measurement of FT3 and FT4, instead of the total form, is more commonly used in current practice In addition, serum thyrotropin thyroid-stimulating hormone (TSH) level is still used as the most sensitive and special method that used in the diagnosis of primary thyroid disorder According to **Moon and Yi,*etal*** [16].

Our hormonal results are in line with many studies indicated that before radiation, most of GD patients had greater FT3 and FT4 levels, a lower FT3/FT4 ratio, and lower TSH levels than healthy controls According to **Ross *et al*.** [17] .Also, these findings are consistent with **Ahmad *et a*l**. [18]study who discovered that after RAI treatment, patients with toxic nodular goiter exhibited a significant decrease in thyroid hormonal levels. Also, the results of this study are in agreement with other studies, which found no significant difference in FT3 and FT4 levels in GD patients after one months of RAI treatment and that serum levels declined non-significantly after six months of treatment. These findings could be explained by limited uptake of low doses of radioiodine leading to greater doses being advised in radioactive iodine patients to attain euthyroidism symptoms as well as a slowing in FT3 and FT4 as indicated by According to **Kahaly *et al.*** [19].It was reported that FT4 levels were high in all GD patients prior to treatment and then dropped on days 4, 19, and 59 following radioiodine therapy The death of thyroid follicles and, the release of stored thyroid hormones into the circulation may cause an increase in FT4 levels. Study of **Burch *et al*.** [20], indicated that 53% of GD patients had a transient elevation in FT4 after two weeks, putting these patients at risk for long-term hyperthyroidism. The significance of this observation is that the FT4 level begins to rise throughout the first half of the year.

A significant impact of thyroid problems on lipoprotein metabolism are widely established. Hyperthyroidism causes a drop in serum cholesterol according to study of [8]These results are in line with our findings of the current study. A drop in HDL levels are also found in GD patients and increased hepatic triglyceride, lipase activity is thought to be the cause of this reduction). However, changes in LDL levels are the principal source of changes in total cholesterol concentrations. A decrease in HDL2/HDL3 has been documented as a result of thyroid hormones' effects on hepatic lipase. Changes in the HDL2 sub-fraction cause, the most noticeable change in HDL and these findings are in line with previous research agree to **Sütken *et al*. [21].**

Thyroid hormones (FT3 and FT4) affects the production, clearance and transformation of lipid metabolism and thyroid-stimulating hormone (TSH) also participates in lipid regulation independently of FT3 and FT4 agree to **Liu and Peng *et al.*** [22].Variations of altered lipid profile in GD make the control of this disease often to be difficult. However, it is important to keep track of blood lipids and thyroid function after radioiodine therapy agree to **Stancu *et al***.[23].

Interleukin-2 (IL-2) is a cytokine that plays a master role in regulating the proliferation and differentiation of lymphocytes, and the presence of IL-2R. It is a good indicator of T-lymphocyte activation and/or proliferation. The Interleukin-2 is being used in clinical trials for treatment of autoimmune thyroid disease (AITD) including Graves' disease agree to **Jimenez *et al*.**[24] .Type 1 cytokines, including IL-2 promote cell mediated immune responses. However, the IL-2 results are in line with other results indicated that anti- IL-2 drugs induced hypothyroidism in GD patients Study by **Hamamoto *et al*.** [25]found that serum IL-2 receptor (sIL-2R) levels in GD patients were significantly elevated and by using a multiple linear regression analysis. The sIL-2R was significantly associated with triiodothyronine. A little information about the effect of radioactive iodine therapy on IL-2 production in GD suggests that many cytokines including IL-4, IL-6 and IL-10 were significantly reduced in GD patients after treatment with RAI.

Many studies revealed a close correlation between and Th17 cells that produced by IL-17 and GD pathogenesis. A mouse GD model study revealed that improved IL-17 levels and decreased Treg cells are possibly engaged in the pathogenesis of GD According to **Yuan *et al*.**[26]. In addition, the results of According to **Torimoto *et al*.** [27]suggest that Th17 cell activation play an important role in the pathology associated with potential immune abnormalities in GD. As a result IL-17

could be important inflammation that plays a part in the inflammatory phenomena seen in GD. The expression of IL-17 protein levels significantly increased and mRNA of IL-17 were noted in GD patients in comparison with healthy controls, which suggests IL-17 to be a pathogenic factor for GD. Interestingly, the levels of IL-17 showed gradually increasing trends during the process of Hashimoto's thyroiditis malignant transformation According to **L**i *et al*., [(28)]The comparison between untreated GD patients and GD patients treated with radioactive iodine revealed that level of IL-17a dropped after receiving therapy [(20)]

CONCLUSION

The elevation of serum biomarkers including IL-2 and IL-17 levels in Graves' disease particularly in patients with radioactive iodine treatment explain the importance of these parameters in progression of Graves' disease. However, the role of IL-2 and IL-17 in GD remains unclear, and more relevant studies is required to clarify its role in GD development.

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