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A correlation study between hyperthyroidism and some apoptosis markers among Iraqi patients

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Abstract

This study was carried out in the Center of Endocrinology and Diabetes in Baghdad during the period between October 2019 to February 2020. The aim was to measure the level of some apoptosis markers and some autoimmune antibodies related to the thyroid gland in Iraqi patients with hyperthyroidism and evaluate the correlation between all the measured parameters. The study included 88 patients who were divided into three groups; group 1 included 30 newly diagnosed hyperthyroidism patients (24 females, 6 males); group 2 included 30 patients of hyperthyroidism who were under treatment (28, 2 males); group 3 included 28 healthy individuals as control group (22 females, 6 males).

Most of the patient's ages ranged between 40 to 60 years (73.3%), while60.7% of the control group were within the same age category. The highest rate of disease was in females compared with males (**86.7% vs. 13.3%)**. The current study included 30% of newly diagnosed hyperthyroid patients and 30% of patients undergoing treatment for a while. The majority of the hyperthyroidism patients, both newly diagnosed and treated, were overweight, and they accounted for **53.3%** of each group.

Highly significant differences (p=0.001) were found in the level of TNF-α in the newly diagnosed and under treatment patient groups in comparison with the level in the control group. The results show a significant decrease in TNF-α level in the treated patients as compared to its levels in the other groups, which indicates that this factor is affected by the given therapy.

It was found that 25% of the patients with hyperthyroidism were suffering from diabetes, with a significant correlation (p=0.009) between hyperthyroidism and diabetes mellitus. It was observed that these patients have a significant increase (p=0.038) in the level of p53 as compared to its level in patients with non-diabetic hyperthyroidism patients and healthy subjects.

This study shows a non-significant negative correlation between TNF-α and TSH levels (r= -0.06) and a non-significant positive correlation between TNF-α and p53 levels (r= 0.17) in hyperthyroidism patients.

The positive correlations between some apoptosis markers and anti-TSHR antibodies and between TSH and these antibodies in hyperthyroidism patients refers to an increase in the concentration of apoptosis markers, which may lead to an increase in the levels of thyroid autoantibodies, which affects thyroid tissue potency and increases thyroid hormone production.

Keywords: Hyperthyroidism, Antibody-Thyroid Stimulating Hormone Receptor, Apoptosis, Tumor Necrosis Factor-Alpha, Tumor Protein 53, B-cell Lymphoma 2, and Factor Related Apoptosis Ligand.

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دراسة العلاقة بين فرط نشاط الغدة الدرقية وبعض مؤشرات الذوي الخلوي لدى المرضى العراقيين

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الخلاصة

أجريت هذه الدراسة في مركز الغدد الصماء والسكري في بغداد خلال الفترة ما بين تشرين الأول 2019 إلى شباط 2020 لقياس مستوى بعض مؤشرات الذوي الخلوي وبعض الأجسام المضادة المناعية ذات الصلة بالغدة الدرقية لدى مرضى فرط نشاط الغدة الدرقية العراقيين وتقييم العلاقة الارتباطية بين جميع المؤشرات المقاسة ، شملت الدراسة : 88 مريضاً تم تقسيمهم إلى ثلاث مجموعات ، المجموعة الأولى تتكون من 30 مريضاً تم تشخيص فرط نشاطهم في الغدة الدرقية (24 إناث ، 6 ذكور). تتكون المجموعة الثانية من 30 مريضا من فرط نشاط الغدة الدرقية تحت العلاج (28 ، 2 من الذكور). بينما تتكون المجموعة الثالثة من 28 من الأفراد الأصحاء (ممثلة بالسيطرة) التي تضم 22 أنثى و 6 ذكور.

تراوحت أعمار معظم المرضى بين 40 إلى 60 عامًا (73.3٪) والمجموعة الضابطة التي شكلت (60.7٪) ضمن نفس الفئة العمرية. وكانت أعلى نسبة للإصابة بين الإناث وأقل عند الذكور (68.7٪ مقابل 13.3٪) على التوالي. اشتملت الدراسة الحالية على 30٪ من مرضى فرط نشاط الغدة الدرقية المشخصين حديثًا و 30٪ من المرضى الذين يخضعون للعلاج لفترة من الوقت، وكان معظم مرضى فرط نشاط الغدة الدرقية، سواء كانوا تحت العلاج أو بدون علاج، يعانون من زيادة الوزن، وكانوا يمثلون (53.3٪) من كل مجموعة.

تم العثور على اختلافات كبيرة للغاية ($P = 0.001$) في مستوى TNF−α بين مجموعات المرضى الذين تم تشخيصهم حديثًا وتحت العلاج مقارنة بمستواها في مجموعة السيطرة. أظهرت النتائج انخفاضًا كبيرًا في مستوى TNF−α في المرضى المعالجين مقارنة بمستواه مع المجموعات الأخرى ، وهذا يشير إلى أن هذا العامل يتأثر بالعلاج المعطى. وجد أن 25٪ من مرضى فرط نشاط الغدة الدرقية يعانون من مرض السكري ، وأظهرت الدراسة الإحصائية وجود علاقة معنوية ($p = 0.009$) بين فرط نشاط الغدة الدرقية ومرض السكري. وقد لوحظ أن هؤلاء الأشخاص لديهم زيادة كبيرة ($P = 0.038$) في مستوى p53 مقارنة بمستواه في المرضى الذين يعانون من مرضى فرط نشاط الغدة الدرقية غير المصابين بالسكري والأشخاص الأصحاء.

توضح دراسة الارتباط عدم وجود علاقة بين مستوى TSH ومستوى مصل TNF−α ($r = -0.06$) في مرضى فرط نشاط الغدة الدرقية ، وعدم وجود علاقة ارتباط بين مستويات p53 و TNF−α ($r = 0.17$). يشير الارتباط الإيجابي بين بعض بروتينات الذوي الخلوي المبرمج والاجسام المضادة لمستقبلات الهرمون المحفز للدرقية TSH R وبين TSH و Anti−TSH R في مرضى فرط نشاط الغدة الدرقية إلى زيادة تركيز بروتين الذوي الخلوي المبرمج قد يؤدي إلى زيادة الأجسام المضادة للغدة الدرقية ، مما يؤثر على قوة أنسجة الغدة الدرقية ، وزيادة إنتاج هرمون الغدة الدرقية .

Introduction

Hyperthyroidism is described as an abnormal excessive production and/or secretion of thyroid hormones by the thyroid gland. Thyrotoxicosis is the medical problem that involves systemic clinical manifestations by the impact of high thyroid hormone levels in tissues [1].

Hyperthyroidism is the major clinical characteristic of Graves' disease (GD) which occurs because of the excessive production of thyroid hormones via follicular cells in response to autoantibody attacks on the thyroid - stimulating hormone (TSH) receptors. As with all autoimmune diseases, cells are activated against self - antigens when self-tolerance is compromised, and B cells generate antibodies that attack the host cells. Autoantibodies are in this case guided toward TSH receptor (TSHR). Association of these antibodies results in an over-function of the follicular cells and, therefore, thyroid hormones such as thyroglobulin, triiodothyronin (T3) and thyroxin (T4), iodotyrosin, and iodinated albumin-like protein are released into the circulation at higher levels [2].

Cell death is a vital step in the growth of multicellular organisms, their integrity, and tissue

homeostasis. The unnecessary cells are removed during metamorphosis, embryogenesis, pathogenesis, and tissue turnover [3].

Cell death typically involves two broadly defined mechanisms: programmed cell death and necrosis. Cell death which includes a genetically programmed process of cell suicide in response to particular signals is called programmed cell death. Usually, programmed cell death is controlled by a variety of extracellular and intracellular signals which are directed by the environment of the cell. Programmed cell death is distinguished from cell necrosis as it has distinct morphological characteristics, maintains tissue homeostasis, and regulates the proper number of cells in multicellular organisms by eliminating unwanted cells [4].

The apoptosis process consists mainly of two central pathways involved in apoptosis induction: the extrinsic pathway and intrinsic pathway. The extrinsic pathway is mediated by the death receptor (DR) and the intrinsic pathway is mitochondrial - mediated. Both apoptotic pathways might lead to the same terminal event (execution pathway) [5]. Apoptotic signaling by the extrinsic pathway is triggered when extracellular ligands, such as TNF (tumor necrosis factor) and Fas-L (Fas ligand), are bound to the DR's (a transmembrane receptor) extracellular domain. The order of events in the extrinsic apoptosis process is characterized by the FasL / FasR and TNF - α / TNFR1 models [6].

The intrinsic pathway points to an apoptotic pathway that is primarily regulated by the mitochondria. This pathway is caused by various extra and intracellular pressures, including cytotoxic drug treatment, irradiation, and oxidative stress [7].

Some proteins were officially established in the intrinsic pathway, with Bcl-2 (B-cell lymphoma protein 2) being one of them [8]. The tumor protein p53 promotes apoptosis, independent of transcription via direct interaction with anti-apoptotic proteins. The activity of p53 is regulated by its protein abundance as well as by its posttranslational modifications [9].

This research aimed to assess the serum levels of apoptosis proteins in hyperthyroid patient's serum and study its association with the thyroid-related autoimmune antibodies.

Materials and Methods

This cross-sectional study included 28 healthy controls in addition to 30 patients who were under methimazole treatment and 30 patients who were newly diagnosed with thyrotoxicosis; increased T3 and/or T4 and decreased TSH levels. Those patients attended at the hormonal unit at the Specialized Center for Endocrinology and Diabetes in Rusafa, Baghdad, –Iraq, for the period between October, 2019 and February, 2020. The first group was composed of 30 patients of newly diagnosed hyperthyroidism (24 females, 6 males). The second group was composed of 30 under-treatment patients of hyperthyroidism (28 Females, 2 males). While the third group was composed of 28 healthy individuals (control) that included 22 females and 6 males. The age of the individuals in both groups (patients and control) ranged between 20 and 75 years.

Three milliliters of venous blood samples were collected from every subject in the studied groups and centrifuged for 5 minutes at 3000 rpm. The serum was collected and preserved in the freezer (-20 ℃) until the analysis date.

As for thyroid performance indicators, Biomerieux (France) kits were used to estimate T3, T4, and TSH levels. ELISA package MyBiosource (USA) was used to estimate anti-TSH-R and apoptosis markers (TNF-α, P53, Fas-L and Bcl-2).

Bodyweight was measured using an analog scale. Statistical analysis was made using a standard Windows software statistical package (SPSS-V.24) for testing the differences in the study parameters among the three groups. The data was demonstrated as mean ± SE, with a probability limit of $p<0.05$ being considered significant, whereas that of $p<0.01$ was considered highly significant.

Results and Discussion

Table -1 shows the differences between newly diagnosed patients, treated patients, and control group regarding levels of thyroid relate hormones and antibodies. The results showed highly significant differences ($p=0.001$) for T3, T4, TSH, and anti-TSHR antibodies. These results showed high levels of T3, T4, and anti-TSHR antibodies, with a low level of TSH.

Table 1-Comparison between hyperthyroidism patients and control group

Thyroid Hormone	Study group			p-value
	Newly Diagnosed	Treated	Control	
T3	3.24 ± 1.67	3.04 ± 1.78	1.51 ± 0.33	0.001**
T4	198.2 ± 105.7	189.6 ± 108.3	90.8 ± 10.0	0.001**
TSH	0.054 ± 0.017	0.424 ± 0.085	1.95 ± 1.01	0.001**
Anti-TSHR	1.16 ± 1.08	1.35 ± 1.26	0.41 ± 0.39	0.001**

Subjects were divided into three categories according to their age: <40, 41 to 60, and >60 (Figure 1). The results showed that the percentage of patients whose age ranged between 40 to 60 years is the highest (73.3%) in patients with hyperthyroidism, whether they are newly diagnosed or undergoing treatment, compared to their percentage in healthy participants (60.7%). The statistical study indicated a significant difference (p=0.017) between the groups according to age. On the other hand, it was noted that the disease is common in ages over 40 and it that the highest rate of disease was in females than males (86.7% vs. 13.3%. The proportion of males in the control group was 21.4% while 78% were females. These findings, as shown in Figure 2, are compatible with other studies in female patients with thyroid disorder which reported a significant correlation (p=0.009) with the age of over 40 years old [10]. The prevalence of hyperthyroidism is higher in females than males, and higher in iodine-deficient areas for both genders. Thus, it is concluded that females are more likely to suffer from thyroid autoimmunity [11]. It can be assumed that hormones have a role in the fact that females are more susceptible to disease than males, although both have the same hormones (i.e., estrogens, progesterone, and testosterone). The difference lies in the location of secretion, its concentration in the blood, and its interaction with the tissues and organs of the body [12].

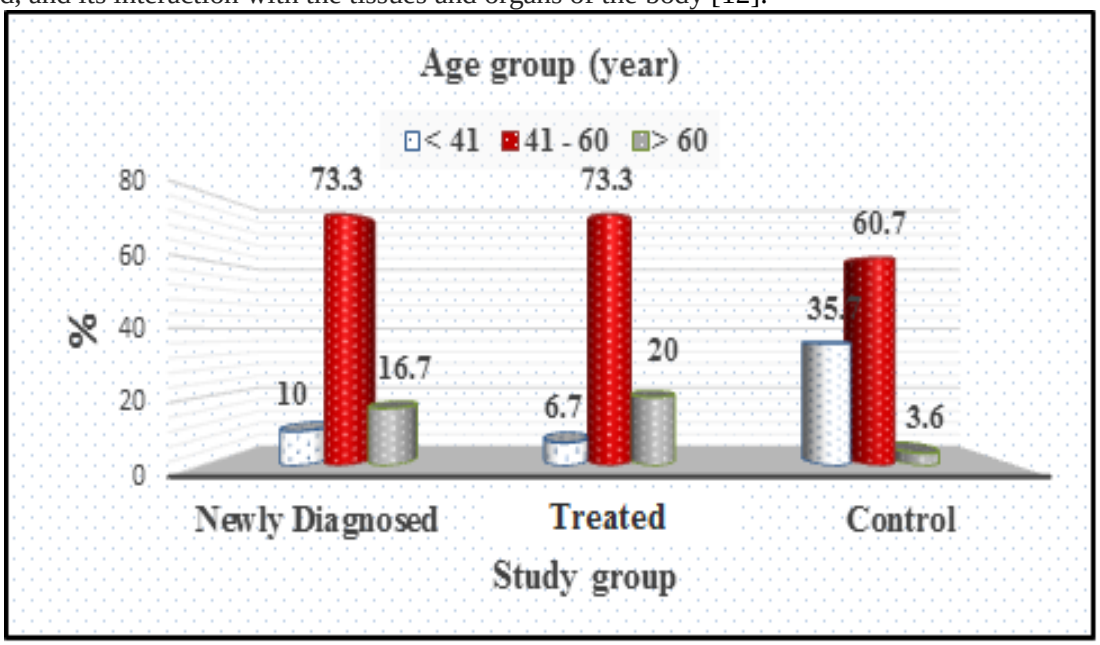

Figure 1-Age distribution among the study Groups

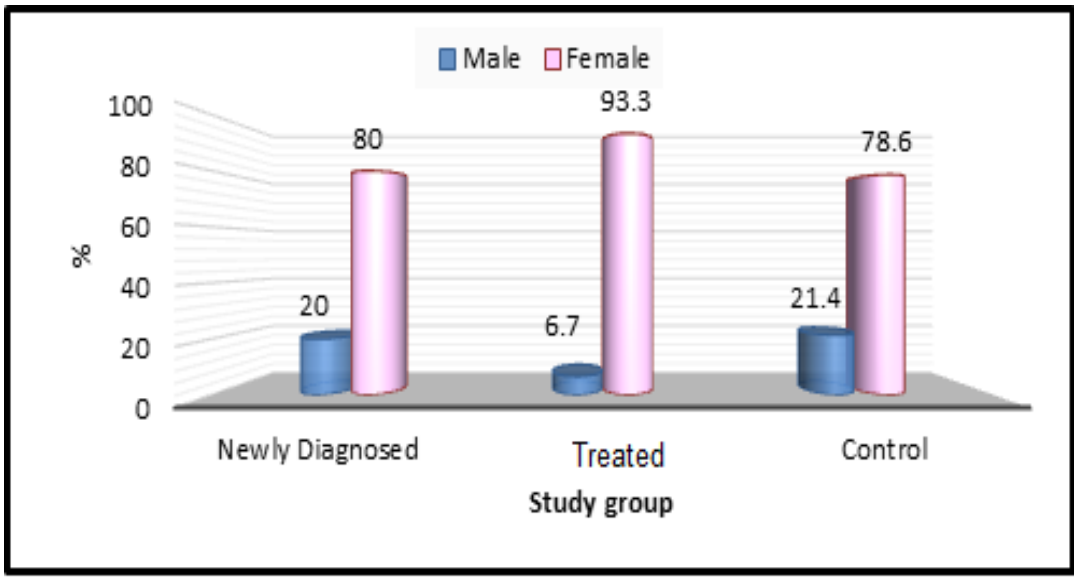

Figure 2- Gender-differences among the study groups.

Figure-3 shows that the majority of the hyperthyroid patients, both treated and un-treated, were overweight. They accounted for 53.3% of each group and were significantly higher than other categories within each group. While it was found that the largest proportion of the healthy subjects were in the categories of overweight (46.4%) and the normal weight (42.9%).

In this study, normal-weight patients were mostly among those newly diagnosed and, from their history, they showed signs of weight loss, because hyperthyroidism leads to increased basal energy consumption, causing a reduction in lean and fat body mass, leading to weight loss [13]. The study of Holm *et al*., 2005, reported that obesity may reduce the risk of hyperthyroidism [14].

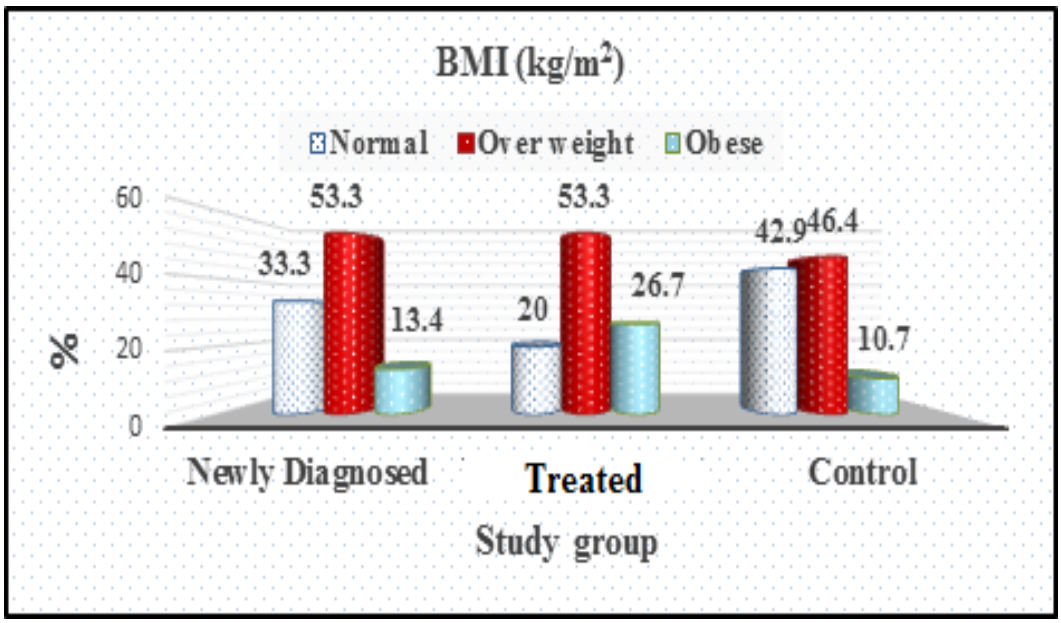

Figure 3-BMI distribution among the study groups.

In the current study, 20% of the newly diagnosed hyperthyroid patients were diabetic, while 30% of the treated patients were diabetic, as shown in Figure 4. The statistical study showed a significant correlation (p=0.009) between hyperthyroidism and diabetes mellitus.

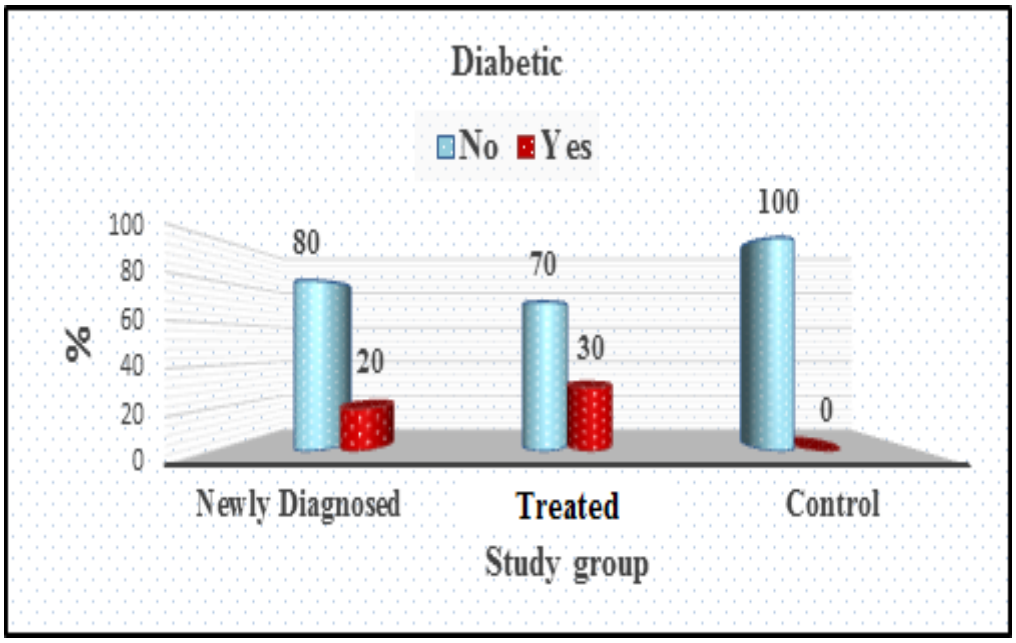

Figure 4-Distribution of DM among the study groups.

Hyperthyroidism was linked to insulin resistance associated with increased glucose turnover, increased hepatic glucose intake, increased intestinal glucose absorption, increased fasting and/or postprandial insulin and proinsulin rates, increased peripheral transport of glucose associated with glucose usage, and increased free fatty acid concentrations. Type 2 diabetic patients with thyroid dysfunction demonstrated a greater susceptibility to ketosis and ketogenesis [15].

The results of this study revealed a highly significant difference (p=0.001) in the level of TNF-α between the newly diagnosed and treated patient's groups in comparison with its level in the control group, while the other markers (P53, Fas-L, and Bcl-2) showed insignificant difference as compared to the control group (Table 2).

Table 2-Results of the apoptosis markers tests for the study groups

Apoptosis Markers	Study groups			p-value
	Newly Diagnosed	Treated	Control	
TNF-α	488.5 ± 294.6	242.2 ± 202.3	48.91 ± 35.56	0.001 **
P53	246.4 ± 627.7	258.8 ± 579.4	208.8 ± 373.4	0.936 N.S
Fas L	76.3 ± 191.0	74.15 ± 177.9	66.8 ± 68.3	0.972 N.S
B cl-2	17.0 ± 53.8	8.64 ± 26.9	3.03 ± 2.93	0.316 N.S

The results of the present study are consistent with those of other studies in patients with thyroid dysfunction, which confirmed the activation of the TNF-α system. In both patients with hypo- and hyperthyroidism, previous studies reported significant plasma concentrations of TNF-α due to its multiple immunological mechanisms [16]. Evidence in some literature showed that cell exposure to TNF-α was previously linked to apoptosis and inflammation through the pathway of the nuclear factor kappa B (NFκB) and the activation pathway of caspase-3 [17]. Higher serum Fas-L in hyperthyroidism patients than the control group (not achieving statistical significance, however) can confirm thyrocyte co-apoptosis parallel to high activation of TSH receptor- autoantibodies [18].

Numerous researches have investigated the levels of cytokines in patients with thyroid disorders [19, 20]. Some of these studies showed significantly elevated levels of IL-6 and TNF-α before treatment, while after undergoing treatment, they began to decrease [21]. This is consistent with our results shown in Table 3, which indicate a highly significant increase in the level of TNF- α (p=0.001).

Hypothyroidism, as well as hyperthyroid, patients were reported to have substantially higher TNF-α levels compared to controls. The effective treatment led to the optimization of TNF-α levels in

hyperthyroid patients, which is in agreement with a previous study [22]. Kumar *et al*., 2007, investigated the role of TNF-α in the hepatic impairment associated with thyrotoxicosis. They found an increase in the expression and activation of one of the types of death receptors, known as p75 neurotrophin receptor (p75NTR) [23].

The significant elevation of TNF-α level in the hyperthyroidism patients, unlike other types of measured apoptotic proteins, may indicate that the extrinsic cell death pathway is common in the cases of hyperthyroidism, especially those of immunological cause. This result cannot be considered conclusive, and therefore, further studies are needed.

TNF-α was the only apoptotic indicator affected by the therapy among the other measured indicators, as shown in Table 3. The results show a significant decrease in its level in treated patients as compared to the other groups.

Table 3-Newly diagnosed vs. treated patient.

Apoptosis Markers	Patient		p-value
	Newly	Treated	
TNF-α	488.5 ± 294.6	242.1 ± 202.2	0.001**
P53	246.4 ± 627.7	258.8 ± 579.4	0.937 N.S
Fas-L	76.3 ± 191.0	74.2 ± 177.9	0.965 N.S
Bcl-2	17.04 ± 53.8	8.64 ± 26.90	0.448 N.S

Therapy for hyperthyroid patients depends on the underlying cause. Treatment strategies include antithyroid drugs, radioactive iodine, thyroid surgery, and medications for symptom control. The most commonly used antithyroid drugs are the thionamides, propylthiouracil (PTU), and methimazole (MMI) [24]. The MMI-treated thyrocytes induce FasL-dependent apoptosis in co-cultured lymphocytes. Therefore, the results of the present study showed a correlation between MMI and the increment of apoptotic cells, confirming that this drug can induce lymphocyte apoptosis. MMI seems to act not only by the Fas-FasL pathway, but also by the interaction with the Bcl-2 expression and may contribute to the immunomodulatory effects of thionamides in this disease [25].

It was found that 25% of the patients with hyperthyroidism (20% of the newly diagnosed plus 30% of the treated patients) were suffering from diabetes. It was observed that these patients have a significant increase (p=0.038) in p53 level as compared to its level in patients with non-diabetic hyperthyroid patients and control group, while there was no significant difference in the other apoptotic indicators (TNF-α, Fas L and Bcl-2), as shown in Table-4.

Table 4-Non-diabetic and diabetic hyperthyroidism patient

Apoptosis Markers	Patient		p-value ¥
	Non-Diabetic©	Diabetic	
TNF-α	243.6 ± 275.6	367.1 ± 253.8	0.113N.S
P53	265.1 ± 583.5	110.2 ± 105.7	0.038*
Fas L	83.4 ± 168.6	19.62 ± 21.07	0.003N.S
Bcl-2	10.61 ± 38.37	5.38 ± 11.52	0.335N.S

A recent study suggested that p53 plays a significant role in the development of metabolic diseases, including DM, and further that p53 may also be consequential to tumor suppression [26]. Sliwinska *et al*., 2017, suggested that TP53 may be linked with type 2 DM [27]. The fluctuations of serum TP53 level may reflect metabolic and oxidative stress associated with chronic hyperglycemia.

The results showed a non-significant negative correlation between serum TNF-α and TSH levels (r= - 0.06) in hyperthyroidism patients, as shown in Figure (5).

The results of Roman *et al*., 2018, revealed no significant difference for the other evaluated parameters. Also, they found a non-significant negative correlation between TNF-α and TSH levels. An increased level of TNF-α was associated with a lower serum level of TSH, whereas TNF-α was found to inhibit the effect of TSH on the thyroid gland. However, after the normalization of the thyroid function, a reduction of serum TNF-α level was seen in patients with hyperthyroidism [28].

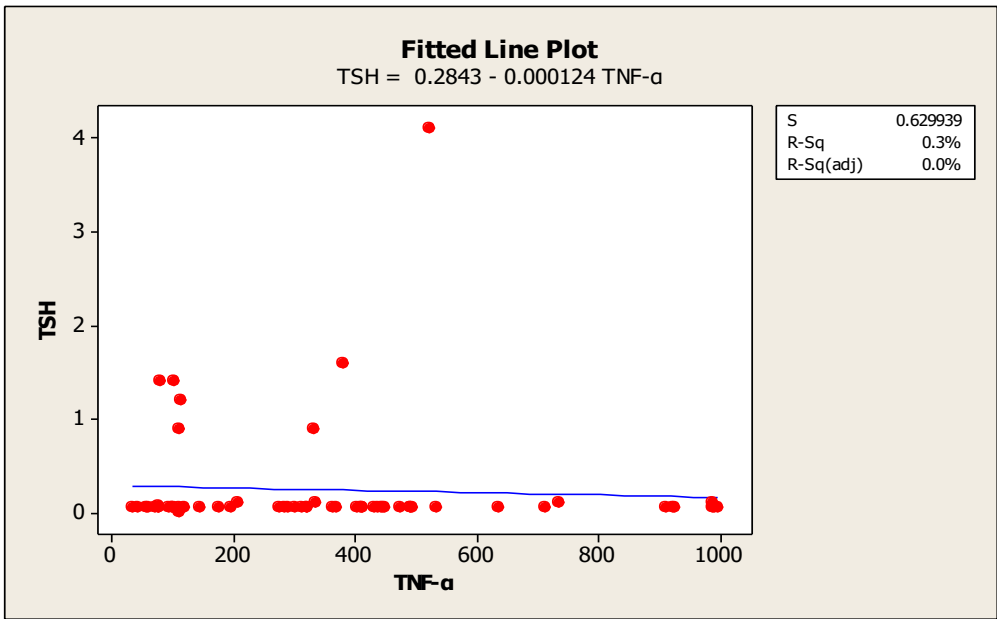

Figure (5): The relationship between TNF-α and TSH levels in hyperthyroidism patients groups.

The statistical study for the correlations among the measured parameters showed a non-significant positive correlation between the level of p53 and TNF-α levels (r= 0.17), as shown in Figure (6). This is not in agreement with the study of Ghandehari-Alavijeh *et al*., 2019, who obtained a significant correlation between the expression of TNF-a with both hypoxia markers due to the inflammatory condition. Therefore, both inflammatory and hypoxia pathways may play a role in hyperthyroidism [29].

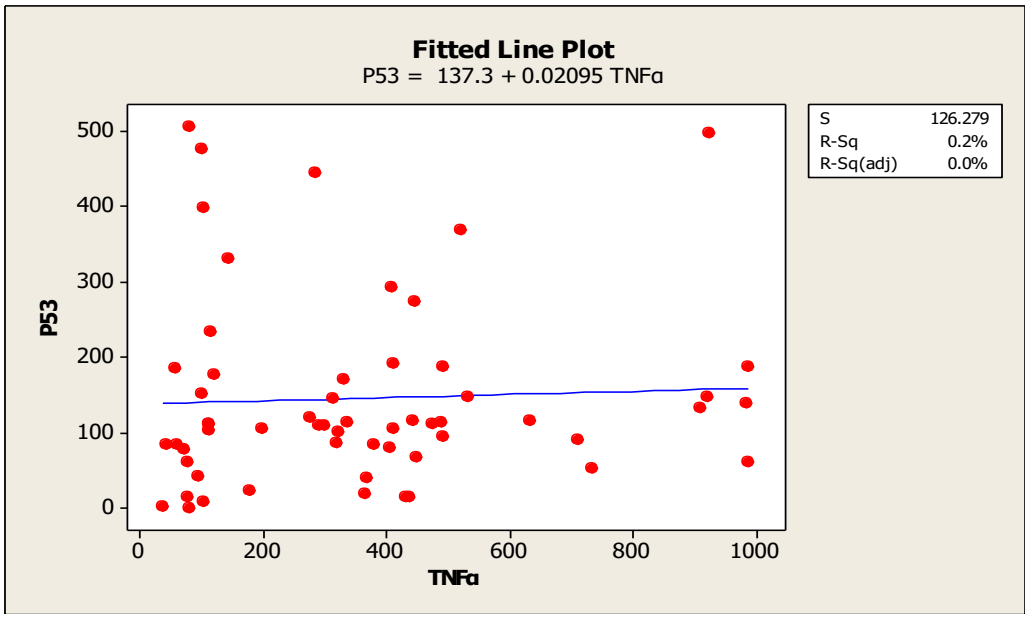

Figure 6-The relationship between TNF-α and p53 levels in hyperthyroidism patients groups

Hyperthyroidism patients showed a statistically significant correlation between antibodies against

the TSH receptor and Bcl-2 expression (r= 0.47, p < 0.03). Bossowski *et al*., 2008, assumed that the attenuated activity of Bcl-2 leads to spontaneous destruction of thyrocytes due to the predominance of enhanced apoptotic signal coming from pro-apoptotic proteins [30]. This is in agreement with the present study that showed a correlation value of r = 0.42 , as illustrated in Figure (7).

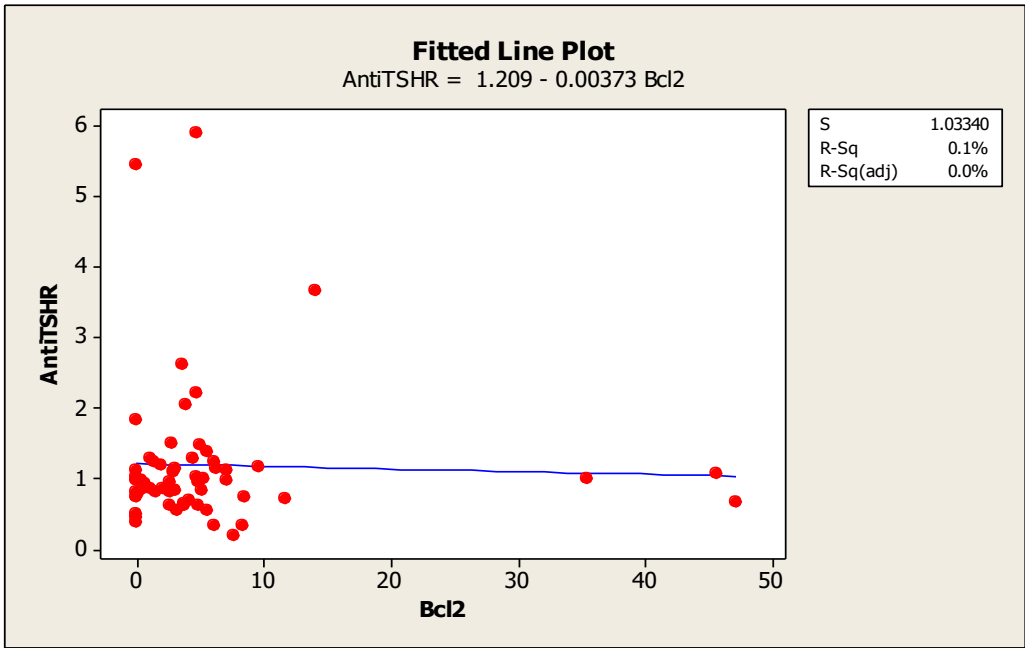

Figure 7-The relationship between the level of anti-TSH R and Bcl-2 in hyperthyroidism patients groups.

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