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Evaluation the correlation between IL-17 level and autoimmune antibodies in hypo and hyper thyroidisms Iraqi patients

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Abstract

The current study included measuring the level of IL-17A and IL-17F and some autoimmune antibodies related to the thyroid gland in case of hypo and hyperthyroid in Iraqi patients to evaluate the correlation between all the measured parameters in this study. The study has been carried out in AL-Kindey Endocrine Gland and Diabetic Center in Baghdad during the period between February 2018 and May 2018, included: 88 patients were divided into three groups, The first group composed of 30 patients of Hypothyroidism that included (26) of them were females, (4) of them males. The second group composed of 30 of patients of Hyperthyroidism that included (20) of them were females, (10) of them were males . and second groups were conducted with hypo and hyperthyroidism respectively. While the third group composed of 28 of healthy individuals (Control) that composed of (25) of them were females, (3) of them were males was represented as control .The results revealed a significant ($P < 0.05$) correlation between TSH concentration and age in patients groups. When the age is more than 40 years, the concentration of TSH was noticed to be higher than that in patients aged less than 40 years. The results show that the level of antibodies was significantly increased ($P < 0.01$) in hypo and hyperthyroid patients in comparison with the control group. At the same time the level of Anti-TG antibodies was higher significantly in the hypothyroid patients than its level in hyperthyroid patients group. No significant differences have been found between hypo and hyperthyroid patients in the level of Anti-TPO and Anti TSH-R antibodies. But its noticed that the higher level of Anti-TPO Ab. was found in the hypothyroid group where as higher level of Anti TSH-R Ab. was found in the hyperthyroid group and simultaneously both types were significantly higher than their levels in the control group As well as the results demonstrated a highly significant increase ($P < 0.01$) in the levels of IL-17 Types (A and F) in hypo and hyperthyroid patients with bias toward the comparison with the control group. The statistical study showed a significant negative correlation between the level of TSH and Anti-TG levels ($r = -0.33$). While a significant positive correlation between the level of Anti-TG and level of IL-17F ($r = 0.39$) in hypothyroid patients. Also in hypothyroid patients, there is a significant positive correlation between the level of Anti-TPO and IL-17A ($r = 0.33$). In conclusion: The positive correlation between IL-17F and Anti-TG and between IL-17A and Anti-TPO in hypothyroid patients refer to increase in the concentration of IL-17 may lead to increase the thyroid autoantibodies, which affect thyroid tissue potency, decreasing thyroid hormone production, according to feedback inhibition loop.

Keywords: Hypothyroidism, Hyperthyroidism, Anti-TPO, Anti-TG,

تقييم الارتباط بين مستوى الأنترلوكين -17 والأضداد الذاتية لدى مرضى قصور و فرط الدرقية
العراقيين

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الخلاصة

شملت الدراسة الحالية قياس لمستوى الانتروكين 17 أي و الانتروكين 17 أف ومستوى بعض الاجسام المضادة المرتبطة بالدرقية لدى مرضى قصور الدرقية و فرط الدرقية العراقيين لتقييم العلاقة بين المؤشرات التي تم قياسها. أجريت الدراسة في مركز الكندي للغدد الصم و السكري في بغداد للمدة بين شهر شباط ولغاية شهر ايار للعام 2018 ، وشملت 88 مريض قسموا الى ثلاث مجاميع ،الاولى تضمنت 30 من مرضى قصور الدرقية 26 منهم اناث و 4 ذكورو الثانية تضمنت 30 من مرضى فرط الدرقية 20 منهم اناث و 10 ذكورو الثالثة شملت 28 من الاشخاص الاصحاء تمثل مجموعة السيطرة تضمنت 25 من الاناث و 3 من الذكور. أظهرت النتائج ارتباط معنوي بين مستوى ال TSH و العمر في مجموعة المرضى ، اذ يزداد مستوى ال TSH في الاعمار الاكثر من الاربعين سنة بالمقارنة مع مستواه لدى الاعمار الاقل من 40 سنة . وجد ارتفاع معنوي لمستوى الاجسام المضادة للكوليبولين الدرقي Anti-TG لدى كل من مرضى قصور و فرط الدرقية بالمقارنة مع مستواه في مجموعة الاصحاء. اما بالنسبة لمستوى اضداد البيروكسيد الدرقي Anti-TPO و اضداد مستقبل هرمون محفز الدرقية Anti TSH-R فقد لوحظ عدم وجود فروق معنوية في مستوياتها بين مرضى قصور و فرط الدرقية ولكن المستوى الاعلى لاضداد Anti-TPO وجد لدى مرضى قصور الدرقية و المستوى الاعلى لاضداد Anti-TSH-R وجد لدى مرضى فرط الدرقية و مستوى كلاهما كان اعلى بشكل معنوي من مستواه لدى الاشخاص الاصحاء. أظهرت النتائج زيادة معنوية لمستوى الانتروكين 17 أي و أف لدى كل من مرضى قصور الدرقية و فرط الدرقي بالمقارنة مع مستواه لدى الاشخاص الاصحاء ولكن المستوى الاعلى للانتروكين 17 أي وجد لدى مرضى فرط الدرقية. أظهرت الدراسة الاحصائية لمعامل الارتباط بين مؤشرات الدراسة وجود ارتباط معنوي سالب بين مستوى هرمون محفز الدرقية و اضداد الكوليبولين الدرقي و ارتباط معنوي ايجابي بين مستوى اضداد الكوليبولين الدرقي ومستوى الانتروكين 17 أف ، ايضا وجد ارتباط معنوي ايجابي بين مستوى اضداد البيروكسيد الدرقي ومستوى الانتروكين 17 أي ، و جميع الارتباطات ظهرت لدى مرضى القصور الدرقي.

الاستنتاج: ان وجود ارتباط ايجابي بين مستوى الانتروكين 17 أف و اضداد الكوليبولين الدرقي و الانتروكين 17 أي و اضداد البيروكسيد الدرقي لدى مرضى قصور الدرقية يشير الى ان زيادة مستوى الانتروكين 17 قد يؤدي الى زيادة انتاج الاضداد الدرقية مما يؤثر في فعالية الغدة ويقل بالنتيجة انتاج هرمونات T3 و T4 ووفق الية التغذية الراجعة السالبة.

Introduction

Hypothyroidism is a common endocrine disorder resulting from deficiency of thyroid hormone or, more rarely, from their impaired activity at the tissue level. Deficiency of the hormone has a wide range of effects, because all metabolically active cells require thyroid hormone [1]. The common forms of autoimmune Thyroiditis are Hashimoto's thyroiditis (HT) is characterized by progressive loss of follicular cells and replacement of lymphoid infiltrates and fibrosis [2].

Hyperthyroidism is a pathological disorder characterized by excess thyroid hormone synthesis and secreted by the thyroid gland [3]. Graves' Disease, the common cause of hyperthyroidism is an autoimmune disease that thyroid stimulating antibodies activate TSH- Receptors and induce synthesis of thyroid hormones [4].

Autoimmune thyroid disease (AITD) is the most common specific autoimmune disorder which affects mostly the middle-aged women. About (2-4) % of women and up to 1% of men are affected, and the prevalence rate increases with advancing age [5]. Autoimmune thyroid diseases (AITD) is considerable evidence implicating that the actual destruction of thyroid cells in AITD may be caused by different and multiple mechanisms, including autoreactive T-lymphocytes, natural killer (NK) cells, and cytokines [6].

The disease was diagnosed by the presence of serum autoantibodies, anti-thyroid peroxidase is considered the best serological marker for the diagnosis of HT , approximately 95% of patients with HT are positive for Anti-TPO ,while Anti-Thyroglobulin is positive in nearly 60-70% of HT patients and are found to be positive in higher percentage of healthy people [7].

IL-17 family that involved six members (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (or IL-25), and IL-17F [8]. The main source of IL-17A and IL-17F is type T helper 17 cells (TH17 cells), which also produce cytokines such as IL-22 and IL-21. IL-17 play important role in the pathogenesis of autoimmune diseases. The levels of IL-17 are found elevated in patients with rheumatoid arthritis, systemic lupus erythematosus (SLE) and in Psoriasis [9].

The present study was aimed to evaluate the serum level of IL-17A and IL-17F in hypo and hyperthyroid patients and study its association with the thyroid-related autoimmune antibodies.

Materials and Methods:

The current study has been carried out in AL-Kindeg for Endocrine Gland and Diabetic Center in Baghdad during the period between February 2018 and May 2018, include: 88 subjects were divided into three groups, The first group composed of 30 patients of Hypothyroidism that included (26) of them were females, and (4) of them males. The second group composed of 30 of patients of Hyperthyroidism that included (20) of them were females, and (10) of them were males. While the third group composed of 28 of healthy individuals (Control) that composed of (25) of them were females, and (3) of them were males. The current study includes patients of hyper and hypothyroidism their age range between (20 - 64) year, and healthy individuals represented as the control group, their age range between (23 - 58) years.

Venous blood samples (3ml) were taken from all subjects of the studied groups, centrifuged at 3000 rpm for 5 minutes. The collected serum kept in the freezer (-20 °C) until use to determine The Biochemical, Immunological parameters according to the manufacturer's protocol.

As indicators of thyroid function, T3, T4, TSH level were estimated using Biomerieux (France) kits. Autoantibodies (TPO and TG) were estimated using immunolab (Germany) ELISA kits. Anti TSH-R Ab. were estimated using MyBiosource (USA) ELISA kits and cytokines (IL-17A and IL-17F) were estimated using Diaclone SAS (France) ELISA kits.

Body weight was measured using an analog scale, all calculations were made using a standard statistical package (SPSS for Windows software- V.24), used to test the difference between three groups on the study parameters. Data were expressed as (mean \pm SE) with $P < 0.05$ considered to be significant and $P < 0.01$ considered to be highly significant.

Results and Discussion

The results revealed a significant ($P < 0.05$) association between TSH concentration and age in patients group as shown in (Figure-1). This result agree with the study of (Hoogendoorn *et al.*, 2006), in which TSH concentration decreases when age is increased in hypothyroidism patients. The results in this study elucidated that, when age is less than 40 years the mean concentration of TSH is (34.29 ± 8.20) $\mu\text{IU/ml}$ while in age more than 40 years the mean concentration of TSH is (22.10 ± 3.55) $\mu\text{IU/ml}$.

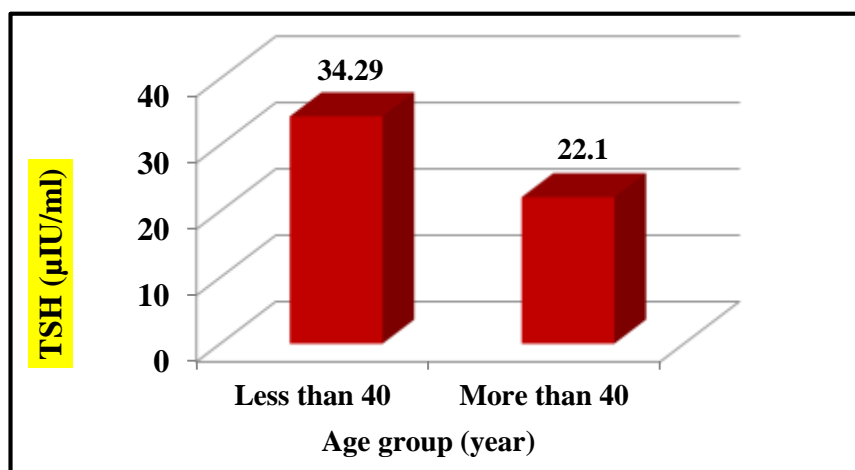


Figure 1 -Effect of age in level of TSH in hypothyroidism patients group.

This study shows that the average body weight of hypothyroid patients was higher than its average in hyperthyroid patients and control, (Figure-2). Body weight related to thyroid hormones, since thyroid hormones regulate basal metabolism, thermogenesis and play a role in lipid and glucose

metabolism, food intake and fat oxidation ,therefore any disturbance in thyroid gland function may cause changes in body weight and composition, body temperature and total and resting energy expenditure (REE) [10].

Thyroid hormones (T3 and T4) accelerate metabolic pathways, therefore, decreasing its level encourage the body to store excess energy in the adipose tissue, that why body weight increase in hypothyroid cases. Besides, it was found that even a slight increase in serum TSH levels are associated with an increase in the increase in obesity [11].

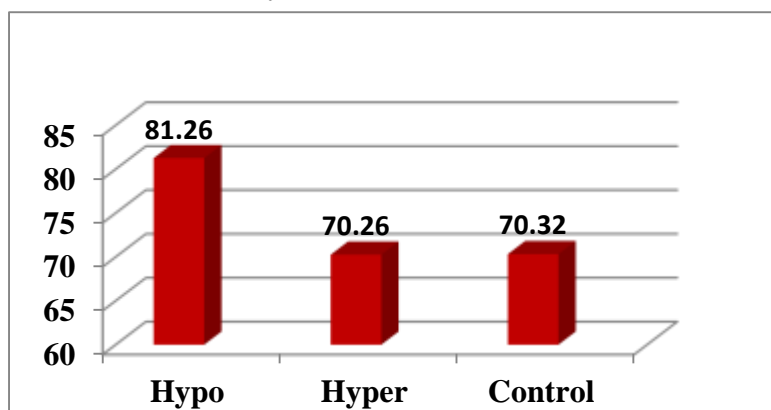


Figure 2-Weight percentage among study groups.

As shown in Table-1, the level of T3 hormone in hypothyroid patients was lower than its level in the other groups, statistically no significant differences have been found in the T3 level between hypothyroid patients and control group. T3 level in hyperthyroid patients was higher significantly than its level in hypothyroid group in comparison with the control group. Otherwise, the T4 hormone level differs significantly between the three groups, the lowest level is found in hypothyroid patients. Significant higher levels of TSH were found in hypothyroid patients compared with the control group. Whereas no significant differences in TSH level between hyperthyroid patients and control group. TSH level in hypothyroid patients higher was significantly than its level in the control group.

Table 1-Levels of thyroid functions tests (T3 , T4 , TSH) in the study groups .

Groups	Mean ± SE		
	T3 (nmol/l)	T4 (nmol/l)	TSH (nmol/l)
Hypothyroidism	1.270 ± 0.07 b	61.18 ± 4.71 c	25.75 ± 3.57 a
Hyperthyroidism	2.986 ± 0.26 a	122.50 ± 8.11 a	0.081 ± 0.02 b
Control	1.721 ± 0.05 b	85.79 ± 1.24 b	2.082 ± 0.13 b
LSD value	0.461 **	15.706 **	5.938 **
P-value	0.0001	0.0001	0.0001

The failing in thyroid gland activity that leads to an increase or decrease in the secretion of these hormones may result in thyroid disorder [12]. Hypothyroidism was the commonest thyroid disorders observed ,characterized by increased serum TSH levels with reduced T3 and T4 hormone production, while the hyperthyroidism was characterized with over-production of T3 and T4 hormones and decreased TSH levels [13]. The elevated of T3 and T4 due to negative feedback mechanisms [14]. About half hypothyroidism cases are due to autoimmune destruction [15]. Hashimoto's thyroiditis, an autoimmune disease often able to progressively destroy the thyroid gland, represents the most common cause of acquired subclinical or overt hypothyroidism in adults in areas of iodine sufficiency [16]. The frequently causes of hyperthyroidism are Graves' disease and toxic nodular goiter [17].

The results show that the level of Anti-TG antibodies was significantly increased ($P > 0.01$) in hypothyroid patients group in comparison with the control group (1909.29 ± 239.71 vs 142.91 ± 56.02) IU/ml, as well as in the hyperthyroid patients group (1223.97 ± 239.58 vs 142.91 ± 56.02) IU/ml. At the same time the level of Anti- TG antibodies was higher significantly in hypothyroid

patients than its level in hyperthyroid patients group (1909.29 ± 239.71 vs 1223.97 ± 239.58) IU/ml as illustrated in (Figure-3).

No significant differences have been found between hypothyroid and hyperthyroid patients in the level of Anti-TPO and Anti TSH-R antibodies. But its noticed that the higher level of Anti-TPO Ab. was found in hypothyroid group (1989.68 ± 254.53 vs 191.16 ± 47.96) IU/ml and the higher level of Anti TSH-R Ab. was found in hyperthyroid group (11.23 ± 0.56 vs 5.034 ± 0.41) IU/ml and both types were higher significantly than their levels in the control group Figures-(4, 5)

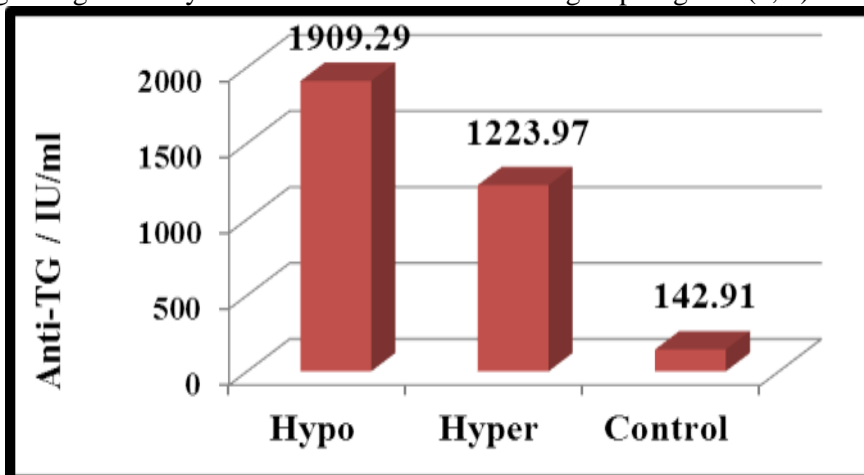


Figure 3-The levels of Anti-thyroglobulin antibodies in groups of this study

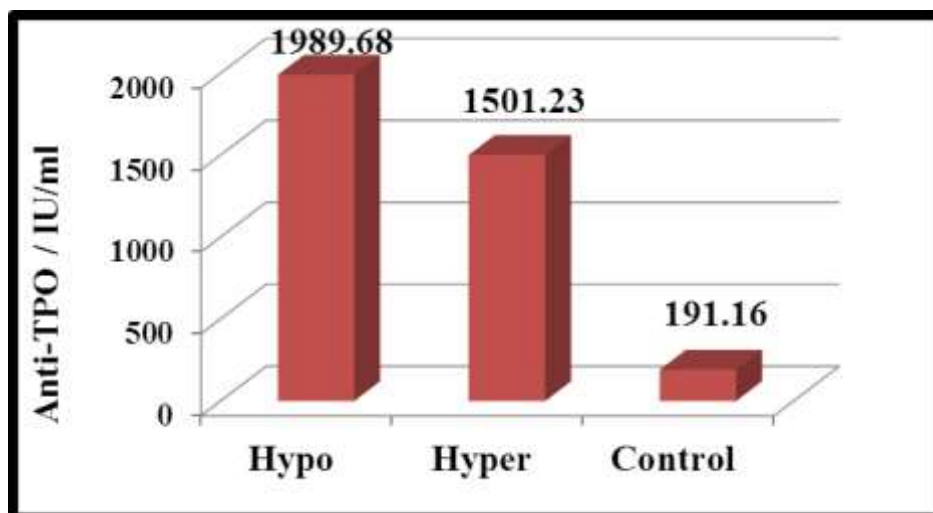


Figure 4-The level of Anti-Thyroid Peroxidase in groups of this study

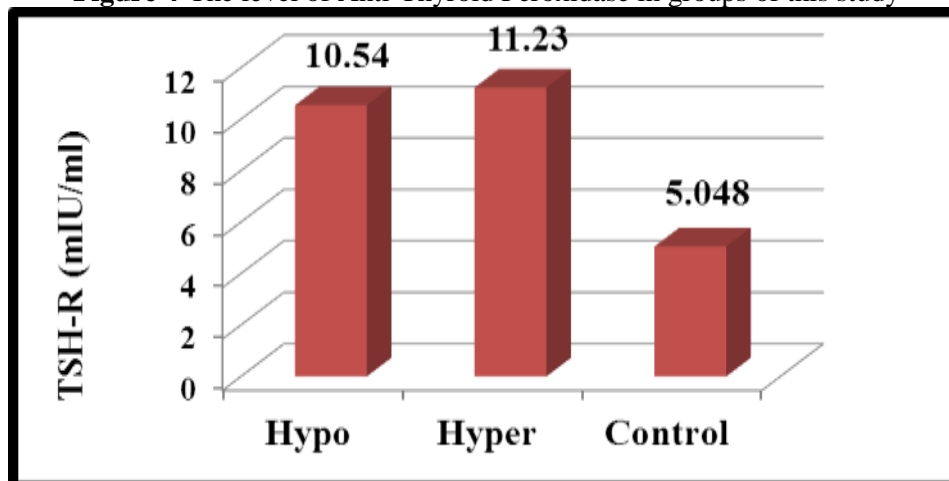


Figure 5-The level of TSH-Receptor antibodies in groups of this study

The elevated of autoantibodies that increased probability of patients that have an autoimmune disease (Graves' disease and Hashimoto's Thyroiditis) because patients produced high levels of thyroid autoantibody and contain lymphoid tissue that resembles secondary lymphoid follicles [18] (Armengol *et al.* , 2001).

The result of the present study disagree with the results of Elmugadam *et al.* , (2010) [19] study which revealed insignificant differences between hypothyroidism 14% and hyperthyroidism 7.5% and control group, However observed 27.8% and 66.7% were positive to Anti-TG in Graves' and Hashimoto's diseases patients respectively .

Chronic hypothyroidism is most closely associated with Hashimoto's thyroiditis, all types of thyroiditis may progress to permanent hypothyroidism .The outcome is more likely in patients with higher serum concentrations of thyroid antibodies or in patients whom a more severe hypothyroid phase develops. The hypothyroid phase of thyroiditis results from the gradual depletion of stored thyroid hormones [20].

Anti-TG it is an intra-follicular antibody that can bind to immune cells and antigens with or without tissue destruction. Massive destruction of thyroid gland induces a structural change in TG leading to antibody production against TG. Major T-cell epitopes on TG require iodination for recognition by autoreactive T-cell [21] .The study of Sultana *et al.* , (2016) [22] showed elevation of Anti-TG in hypothyroidism and hyperthyroidism patients about 41.81% and 40% was positive to Anti-TG respectively .

Dham *et al.* , (1995) [23] observed that Anti-TPO positive in 58.3% of primary hypothyroidism and in 70% of primary thyrotoxicosis and in 100% of Hashimoto's disease and the study showed a significant elevation in IgG in Graves' disease. Another study by Sultana *et al.* , (2016) [22] revealed that seropositivity of Anti-TPO in patients with hypothyroidism is about (54.54%) ,while in patients with hyperthyroidism is about (53.33%) and approximately agreed with the current study , also its noticed that prevalence of Anti-TPO ,as well as Anti-TG together, comprise 69.09% in hypothyroidism and 66.66% in hyperthyroidism cases which indicate to high seropositivity when these autoantibodies are elevated together in patients. Swain *et al.* , (2005) [24] observed that Anti-TPO is above 60% in hypothyroidism .

Thyroid autoantibodies are the markers of autoimmunity in autoimmune thyroid diseases [25] (Al-Naqdy *et al.* , 2003). another study in Iraqi patients by AL-A'araji , (2016) [26] that revealed high occurrence of Anti-TPO in hypothyroidism and hyperthyroidism patients groups .Another study observed that 66.7% and 50% are positive to Anti-TPO in hypothyroidism and hyperthyroidism patients respectively [27] .

Anti-TPO was considered to be a sensitive marker for autoimmune thyroid disease [28]. In the study performed in Norway reveal an association between Anti-TPO level and abnormally low and high TSH concentration [29]. Jayashankar *et al.* , 2015 [30] in their study found that about 80% and 50% of clinical and subclinical respectively positive to Anti-TPO. While the study of Mohanty *et al.* , 2008 [31] noted that 73.78% of subclinical hypothyroidism patient were positive to Anti-TPO. About 90% of Brazilian women patients with autoimmune thyroiditis have elevated levels of Anti-TPO [32].

TPO is an enzyme that responsible for iodination of tyrosine residues and coupling of iodinated residues to synthesis thyroid hormone [33]. Higher seropositivity in TPO that compared to TG because of a variable is differences in antigenic characteristic of these two thyroid antigens [22]. Figueroa-Vega *et al.*, (2010) [34] found an elevation of TSH-R in Graves' patients but not in Hashimoto's patients.

The results demonstrated a highly significant increase ($P < 0.01$) in the levels of IL-17A in hypo and hyperthyroid patients in comparison with the control group (9.24 ± 1.17 vs 3.40 ± 0.34) Pg/ml and (14.21 ± 1.08 vs 3.40 ± 0.34) Pg/ml respectively. On the other hand Hyperthyroid patients have a higher level of IL-17A (Figure-6). Also, the study demonstrated a highly significant increase ($P < 0.01$) in the levels of IL-17F in hypo and hyperthyroid patients in comparison with the control group (22.82 ± 1.87 vs 3.60 ± 0.31) Pg/ml and (26.22 ± 1.36 vs 3.60 ± 0.31) Pg/ml respectively. No Significant differences have been found in the levels of IL-17F between groups of patients in comparison with the control group (22.82 ± 1.87 vs 3.60 ± 0.31) Pg/ml. (Figure-7).Also, Hyperthyroid patients have a higher level of IL-17F.(Figure-6)

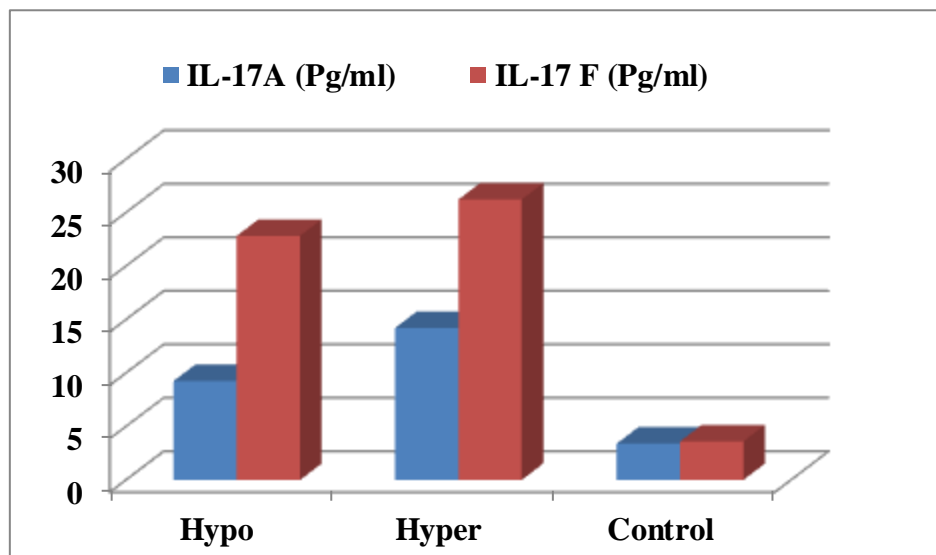


Figure 6-he levels of serum IL-17A and IL-17F in groups of patients and control group of this study.

The study of Cautha *et al.*,(2018) [35] showed increased levels of IL-17A in children with Hashimoto's disease, and suggested the possible role of IL-17A in the pathogenesis of this disease. Figueroa –vega *et al.*, (2010) [34] revealed an elevation in the levels of IL-17⁺ and IL-22⁺ lymphocytes and IL-17 mRNA levels in the blood and thyroid tissue of patients with Hashimoto's disease as compared to Graves' disease patients and control group. In China, Qin *et al* (2012) [36] found higher mRNA levels of IL-17A and INF- γ in both peripheral blood mononuclear cells and thyroid tissue of Hashimoto's Thyroiditis patients than healthy control and higher also in Graves' disease patients but not significant concluded that similarly high levels of IL-17A suggested a mixed response of Th17 and Th1 in Hashimoto's Thyroiditis where both cells may play important roles in the destruction process by cell mediated cytotoxicity. However, our results agree with the Iranian study, in which the higher levels of serum IL-17A include all Hashimoto's disease patients and total hypothyroidism (6.71 ± 4.35), overt hypothyroidism patients (7.51 ± 4.85) and in subclinical hypothyroidism (5.97 ± 3.79) in comparisons with the control (3.48 ± 2.14) [37]. The levels of IL-17 were increased in Graves patients in comparison to control group [38].

The statistical study for the correlation between measured parameters, showed a significant negative correlation between the level of TSH and Anti-TG levels ($r = -0.33$), as shown in (Figure-7). A significant positive correlation in hypothyroid patients between the concentration of Anti-TG and concentration of IL-17F ($r = 0.39$), as showed in (Figure-8). Also in hypothyroid patients, there is a significant positive correlation between level of Anti-TPO and IL-17A ($r = 0.33$), as illustrated in (Figure-9).

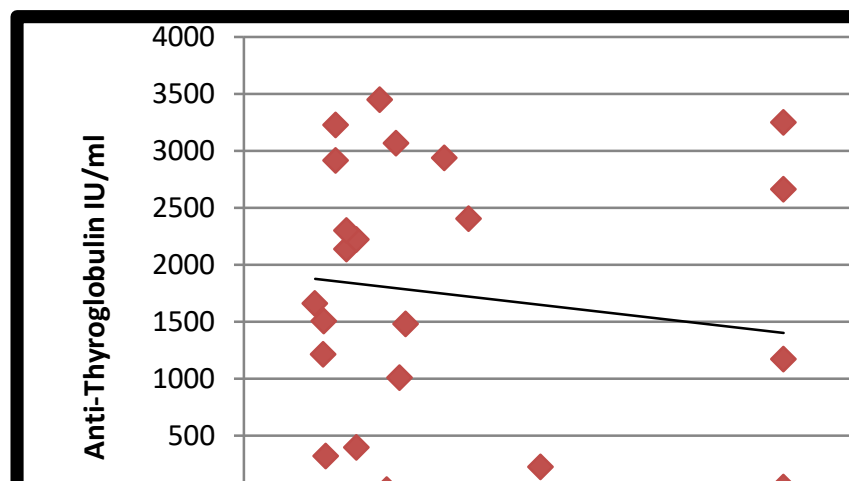


Figure 7-Relationship between level of TSH and Anti-Thyroglobulin in hypothyroidism patients ($r = -0.33$).

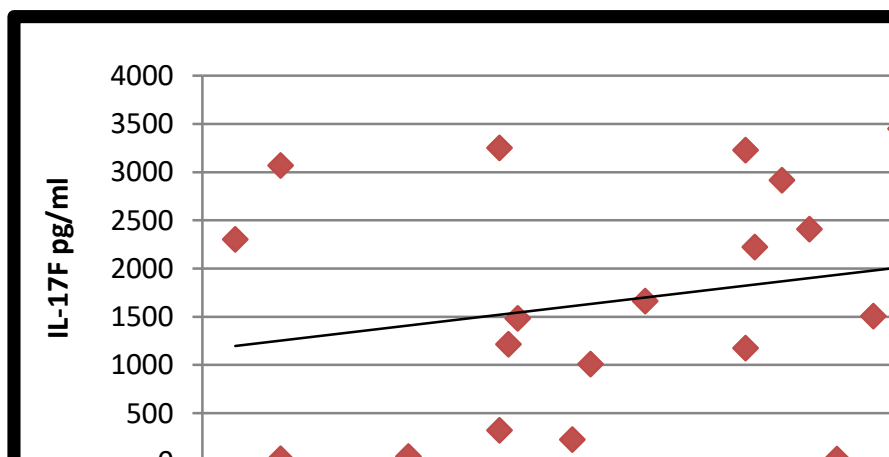


Figure 8-Relationship between level of Anti-TG and IL-17F in hypothyroid patients ($r= 0.39$).

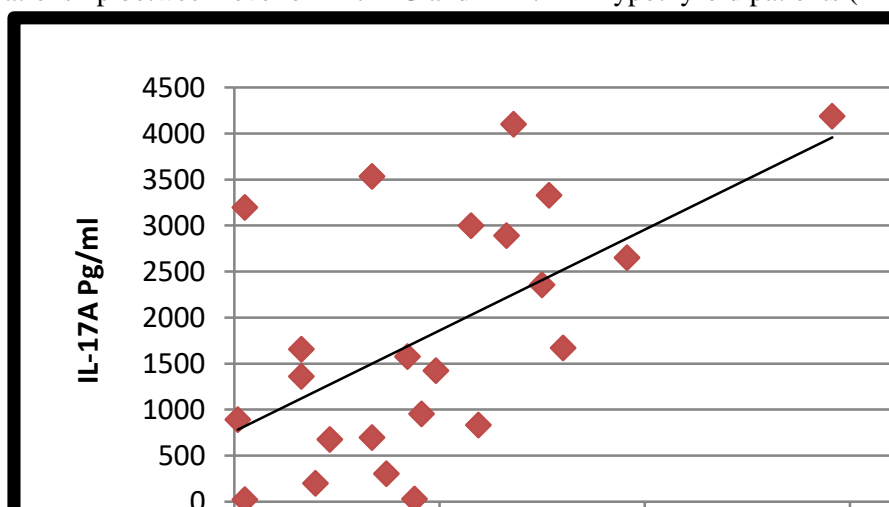


Figure 9-Relationship between level of Anti-TPO and IL-17A in hypothyroid patients. ($r= 0.33$).

Hashimoto's thyroiditis is the main cause of hypothyroidism, considered a Th-1 mediated disease [39]. The new subset of Th cell discovered called Th17 cell that implicated in different types of autoimmune diseases. Th17 cells mainly secrete of IL-17A and IL-17F which play an active role in the pathogenesis of HT [35, 37].

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