

## THE UNDERLYING PATHOLOGY OF AUTOIMMUNE THYROID DISEASE IS IL-22 INDEPENDENT

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The thyroid gland is frequently associated with autoimmune disease. It produces thyroid hormones responsible for controlling cellular metabolism. The current case control study involved ninety subjects which were assigned into two equal-numbered groups of patients and apparently healthy controls. For laboratory evaluation, five millilitres of venous blood were withdrawn from individual participants, serum were collected and stored at  $-20^{\circ}\text{C}$  to be analysed. Immunoassay technique was used to measure the serum level of thyroid stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3). While ELISA technique was used for measuring the serum levels of anti-thyroid peroxidase (anti-TPO) antibodies and IL-22. The results of the current study showed that, in the patient group, thirty eight (84.44 %) subjects were diagnosed with hypothyroidism, represented by thirty five female (77.77 %) and three male (6.67 %); furthermore, seven individuals (15.56 %) were grouped as hyperthyroid patients and represented by five females (11.11 %) and two males (4.45 %). The results also demonstrated that the serum TSH levels ( $12.04 \pm 2.76$ ) for the patients were significantly ( $p < 0.05$ ) higher than that of the control group ( $1.87 \pm 0.15$ ). Whereas, T3 and T4 mean serum levels  $\pm$  SE were  $2.05 \text{ nmol/l} \pm 0.14$ ;  $100.66 \text{ nmol/l} \pm 4.76$  and  $2.14 \text{ nmol/l} \pm 0.07$ ;  $105.37 \text{ nmol/l} \pm 2.92$  for patient and control categories, respectively. The findings of this work showed that mean serum level (IU/ml) of anti-thyroidperoxidase antibody in patient group differed significantly ( $P < 0.05$ ) in comparison to control group (represented by  $259.08 \pm 59.99$  and  $8.71 \pm 1.23$ , respectively). No statistical difference was non-significant when comparison involved mean serum concentration levels of IL-22 for patients ( $157.22 \text{ ng/ml} \pm 24.81$ ) and controls ( $157.08 \text{ ng/ml} \pm 24.80$ ). In conclusion: IL-22 cannot be proposed as an essential factor participating the development and/or the progression of autoimmune thyroid disease (AITD).

**KEYWORDS:** Autoimmune diseases, Anti-TPO, ELISA, IL-22**1. INTRODUCTION**

Autoimmune diseases are an exaggerated general dilemma affecting nowadays community (Przybylik-Mazurek *et al.*, 2007). Epidemiological investigations performed over the last several years ascertained that 3-8% of population from the entire world develop autoimmune diseases (Szczeblowska *et al.*, 2011).

The thyroid is a hormone-producing gland most likely had an autoimmune disorder than any organ (Shevach, 2009). Clinically, the most abundant manifestations of thyroid autoimmune diseases are Graves' disease and Hashimoto's thyroiditis; both conditions are accompanied by a varying degrees of lymphocytic infiltration of the thyroid tissue as well as the development of self-reactive autoantibodies against thyroid antigens such as thyroglobulin (TG), thyroid peroxidase (TPO), and thyroid stimulating hormone receptor (TSHR) (Guo *et al.*, 2018).

Thyroid autoimmune disorders ensue as a consequence of the interactions between environmental and genetic elements and are manifested by generating an immune response against thyroid antigens which is attributed to autoreactive immune cells bypassing immunological tolerance. Both humoral immune responses and cell-mediated immunity result in tissue damage in autoimmune thyroid disorder. Due to dominance of specific immune mediators in particular setting, CD4+ cells differentiation culminates in the development of variable T cell subsets. Identification of T cells has revealed a mosaic pattern of cytokine secretion emphasizing that both, T helper 1 and T

helper 2, activation participated in all kinds of autoimmune thyroid diseases. More recently, research denoted T cell subtypes involving Treg and Th17 as having profound roles in the establishment of AITD (Rydzewska *et al.*, 2018).

The involvement of Th17 cells in the immune responses against extracellular microbes is well established beside their capability to liberate cytokines: IL-17, IL-17F, IL-21, IL-22 and IL-23 (Bossowski *et al.*, 2013), which are described as a pro-inflammatory cytokines that contribute to the development of autoimmune disease (Peng *et al.*, 2013).

Cytokines are involved in the pathogenesis of autoimmune phenomena. The actual initiator of the self-tolerance disturbances with the resultant outcomes culminating in the triggering of pathologic autoimmune reaction remain to be elucidated for the majority of the existing autoimmune disorders. Recently, the interleukin IL-17/IL-23 axis has been shown as the new paradigm which led significantly to re-evaluate the cytokine-induced immune circumstances in the initiation, propagation, and treatment of autoimmune disorders (Moudgi and Choubey, 2011).

In this study, we aimed to determine and compare serum concentration of IL-22 in autoimmune thyroid patients versus controls healthy subjects in Duhok province.

**2. MATERIALS AND METHODS****2.1. Participants:**

Ninety patients and healthy control subjects (8-80 years old), 45 Patients and 45 Control; were enrolled in the present study.

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A questionnaire form was submitted to every participant which reported information such as sex, age, geographical location, and marital status. Candidate subjected were selected from those who had been referred to Duhok central health laboratory from November 2018 to May 2019. Preliminary investigation was performed as the candidate patients suffered clinical symptoms suggestive of malfunctioning thyroid gland. A document verifying preliminary diagnosis was submitted to each patient.

## 2.2. Collection Of Blood Samples:

Five milliliters (ml) of venous blood were collected from each individual (patient and control), left at room temperature for 20-25 minute to clot, and then centrifuged for ten minutes at 3000 round per minute (rpm); serum was stored at  $-20^{\circ}\text{C}$  to be analysed. Cobas e411 instrument was used to measure the serum concentration of TSH, T4 and T3, Electrochemiluminescence (ECL) is Roche's technology for immunoassay detection. ELISA method was used for measuring the serum levels of anti-TPO antibodies and IL-22. The manufacturer's instructions were precisely implemented.

## 3. STATISTICAL ANALYSIS

Data were represented as the mean  $\pm$  standard error. Comparisons between groups were conducted using the student t test (independent samples).  $P < 0.05$  was considered to describe a statistically significant difference. Statistical results were obtained using SPSS (IBM Corporation, New York, NY, USA, Version 26.0).

## 4. RESULTS

The results of this work demonstrated that, in the patient group, thirty eight (84.44 %) subject were diagnosed with hypothyroidism as per their TSH serum level, represented by thirty five females (77.77 %) and three males (6.67 %); furthermore, seven individuals (15.56 %) were grouped as hyperthyroid patient and represented by five females (11.11 %) and two males (4.45 %) (Table 1).

Table 1. The frequency and gender of hyper and hypothyroid patients.

Gender	Hypothyroid		Hyperthyroid		Total	
	Frequency	Percentage (%)	Frequency	Percentage (%)	Frequency	Percentage (%)
Male	3	6.67	2	4.45	5	11.12
Female	35	77.77	5	11.11	40	88.88
Total	38	84.44	7	15.56	45	100.0

The findings of the present study demonstrated that mean TSH serum concentration (uIU/ml) and standard error ( $\pm$ SE) of 12.04  $\pm$  2.76 for the patients which profoundly exceeded that of the controls 1.87  $\pm$  0.15; and statistically significant difference ( $P < 0.05$ ) was obtained. Whereas, T3 and T4 mean serum levels (nmol/l)  $\pm$ SE were 2.05  $\pm$  0.14; 100.66  $\pm$  4.76 and 2.14  $\pm$  0.07; 105.37  $\pm$  2.92 for patient and control groups, respectively. The comparison of the two categories indicated no significant difference ( $p < 0.05$ ) (Table 2).

Table 2: The means of TSH and thyroid hormones serum levels for patient and controls.

Hormones	Group	Frequency	Mean serum level $\pm$ SE	p value
TSH (uIU/ml)	Patient	45	12.04 $\pm$ 2.76	.000*
	Control	45	1.87 $\pm$ 0.15	
T3 (nmol/l)	Patient	45	2.05 $\pm$ 0.14	0.11
	Control	45	2.14 $\pm$ 0.07	
T4 (nmol/l)	Patient	45	100.66 $\pm$ 4.76	0.06
	Control	45	105.37 $\pm$ 2.92	

\*Significance at  $P < 0.05$

In this study, the mean serum concentration of anti-TPO antibody differed significantly ( $P < 0.05$ ) when the two candidate categories were compared (Table 3).

Table 3. Comparison of the mean Anti-TPO serum concentration for patients and controls.

Category	Frequency	Mean Anti-thyroidperoxidase serum level $\pm$ SE	p value
Anti-TPO (IU/ml)	Patients	259.08 $\pm$ 59.99	.000*
	Controls	8.71 $\pm$ 1.23	

\*Significant at  $P < 0.05$

Furthermore, when comparison involved mean serum concentration (ng/l) levels of IL-22 of patients (157.22  $\pm$  24.81) and controls (157.08  $\pm$  24.80), no statistical significant difference ( $p < 0.05$ ) appeared (Table 4).

Table 4. Mean serum conc.  $\pm$ SE of Interleukin-22 for both patient and control.

Interleukin	Patients (mean serum conc. ng/l $\pm$ SE)	Controls (mean serum conc. $\pm$ SE)	p value
Interleukin-22 (ng/l)	157.22 $\pm$ 24.81	157.08 $\pm$ 24.80	0.06

## 5. DISCUSSION

The anterior pituitary hormone (thyroid stimulating hormone) is considered a fundamental biomarker for assessment of normal thyroid status and is useful in directing treatment strategies for hypothyroid and hyperthyroid subjects. The amplified response of TSH to minor alterations in thyroid hormone profiles provides a meaningful and directly estimated signal in the routine care setting. Laboratories submit reference ranges for TSH with lower and upper limits to define normal thyroid function (Razvi, *et al.*, 2019). Thyroid dysfunction is often characterized by nonspecific symptoms, and diagnosis is ascertained by laboratory testing for T3, T4, and TSH. Nowadays systematic approach for the diagnosis and treatment of thyroid disorders focuses mainly on the measurement of TSH, as the highly sensitive and specific indicator of systemic thyroid status, with test findings interpreted as per defined reference range (Jonklaas *et al.*, 2014; Okosieme *et al.*, 2016). Majority of autoimmune cases are more prevalent in females than males; conservative estimates announce that approximately 80% of autoimmune diseases victims are women (Viswanath, 2013). Hashimoto's thyroiditis generally affects women between forty-five and sixty-five years old, with a female to male ratio of 10:1. Graves' disease is ten times more frequent in females than males (Vanderpump, 2005). The above-mentioned gender differences seem to indicate the

profound impact of sex hormones on the normal functioning of immune system, and investigations have explored this matter to demonstrate the influence on autoimmune diseases (Cutolo *et al.*, 2006). Former studies revealed that the inactivated Barr body in women is still partially active, and up to 15% of X-chromosome genes can thus be expressed by both chromosomes (Rubtsov *et al.*, 2010). The genetic mutations and the skewed DNA may influence the second X-chromosome activation, a condition which has been manifested more frequently in individuals with an autoimmune disorder, compared to healthy subjects (Brown, 1999; Oliver and Silman, 2009). Not surprisingly, the findings of this work were consistent with the general consensus which stated that females are much more prone to thyroid autoimmunity than males and the most affected people are those at the adulthood age and older (Canaris *et al.*, 2000; Hollowell *et al.*, 2002; Gussekloo *et al.*, 2004; Flynn *et al.*, 2004; Leese *et al.*, 2008)

The observations obtained in this study were in accordance with others who found 79 out of 215 patients had elevated serum thyroid stimulating hormone levels, but contradict the same research findings when consider the 136 patients out of the same total to have TSH serum level slightly above normal value (Sridevi *et al.*, 2018). It was found that patients had significantly higher TSH concentrations compared to controls ( $p < 0.001$ ) but no difference between the two groups when fT4 levels were considered, the later mentioned findings were in line with those of the present work.

From the population of 5,962 subjects, 24.5% showed changing TSH values (19.0% with elevated TSH concentrations suggestive of hypothyroidism); despite with a higher percentages value of hypothyroid individuals due to the inclusion criteria (Carmona *et al.*, 2018), the above results mimic those of the present study.

In accordance with the results of the current study, after evaluating 472 subjects attending occupational health centers in Chile, it was noted that frequency of thyroid alterations was two times greater in women than men (Fardella *et al.*, 2001).

The presence of a high anti-TPO Abs is a distinctive feature of autoimmune thyroid disorders, especially Hashimoto's thyroiditis, but also being highly frequent in Graves' disease and postpartum thyroiditis (Prummel and Wiersinga, 2005). However, the existence of anti-TPO Abs is not uncommon in normal thyroid subjects. For example, in the NHANES III study, 8.0% and 14.6% of euthyroid men and women had such antibodies (Hollowell *et al.*, 2002).

The results of the present study were consistent with these of other authors when they demonstrated that anti-TPO antibodies are most likely detected in subjects with deranged TSH in comparison to those with the usual TSH ( $p$  value 0.001) (Tipu *et al.*, 2018). Similarly, Al Rabia, (2017) has obtained similar results indicating TSH is more often deranged if anti-TPO or anti-TG antibodies are positive. Moreover, it has been found that anti-TPO positivity is associated with 60% increase in TSH (Brown *et al.*, 2016). In Iran, consistent findings have been observed by Ghorraishian *et al.*, (2006), they studied relationship of anti-TPO with T3, T4, and TSH in nearly two thousand five hundred individuals and found these to be significantly altered in antibody positive group.

IL-22, mainly synthesized by Th17 and Th22 cells, has an important role in the pathogenesis of several inflammatory and autoimmune conditions (Figueroa-Vega *et al.*, 2010; Pan *et al.*, 2009; Pan *et al.*, 2013)

The outcomes of the current investigation were different from others who measured the serum concentration of IL-22 in a large series of recently diagnosed, euthyroid HT patients and compared these findings with those estimated in euthyroid non-HT subjects with nodular goiter and in healthy individuals without thyroid disorders. All HT patients revealed serum IL-22 levels significantly higher than those of non-HT patients with nodular goiter or healthy controls, but no differences

emerged between goitrous patients and healthy controls. These data indicated that IL-22, whether it is produced by Th17 or Th22 or both, plays special role in the initiation of HT. This is an interesting finding because HT is commonly considered to be a Th1-mediated disease (Ruggeri *et al.*, 2014).

## 6. CONCLUSION

It has been concluded that autoimmune thyroid diseases affected a considerable number of participants with hypothyroidism being the more prevalent over hyperthyroidism and that IL-22 had no evident role in the development or progression of the disease process.

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