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Detection of TPO antibody and TFT among Thyroiditis patients in Thi-Qar Governorate, Iraq

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Abstract: **Thyroiditis is a general term that refers to thyroid gland inflammation. The present research was conducted to identify the role of thyroid peroxidase antibodies (TPO-Ab) in Thyroiditis patients and assaying the levels of thyroid function tests (TFT), which include levels of serum triiodothyronine hormone (T3), thyroxin hormone (T4), and thyroid stimulating hormone (TSH). A total of 86 serum samples (76 thyroiditis patients and 10 controls) from August 2023 to November 2023, at Al-Haboby Teaching Hospital and some private clinics in Thi-Qar Governorate were enrolled in the present study. The sandwich-ELISA technique assayed the levels of TPO-Ab, and the levels of (TFT) T3,T4, and TSH by using a competitive binding assay. Thyroiditis patients were divided into hypothyroidism and hyperthyroidism type with 47(61.84%) and 29(38.16%) patients, respectively (P≤0.05). Hypothyroidism and hyperthyroidism groups showed elevated mean serum levels of TPO-Ab (29.78%) and (20.68%) for members of each group separately, with a mean of (478.8) and (1070), respectively. While the mean of TPO-Ab in the control group was (319.5). The means of TFT in hypothyroidism group for T3,T4 and TSH were (0.9215), (5.402), and (22.86), respectively. The means of TFT in hyperthyroidism group for T3,T4 and TSH were (1.003), (7.409) and (0.3893), respectively, compared to the control. The mean of TFT for T3,T4 and TSH was (0.781), (7.374), and (1.603), respectively. As a demographic profile, Thyroiditis, with a high significant differences, tends to infect females (92.11%), middle-ages (21-40 years), housewives (81.58%), and urban residences (73.69%) ($p \leq 0.001$). The findings of the current study could illuminate the function of TPO-Ab and TFT as serological and diagnostic instruments in thyroiditis, and offer valuable contributions to the disease's classification and evaluation.**

***Keywords*: Thyroiditis, Hypothyroidism, TPO, TFT Hyperthyroidism, AITD.**

I. INTRODUCTION

Thyroiditis, inflammation of the thyroid gland, encompasses a spectrum of conditions that may disrupt the gland's normal functioning. This condition is categorized based on the nature of clinical manifestations (whether symptomatic or asymptomatic), the temporal progression of the disorder (acute, sub-acute, or chronic), and the etiological factors involved (such as autoimmune processes, infectious agents, pharmacological interventions, or radiation exposure) [1]. Indeed, thyroiditis has the potential to induce both transient and enduring forms of thyroid dysfunction, including hypothyroidism and hyperthyroidism. The condition's impact on thyroid hormone levels can vary, leading to a decrease (hypothyroidism) or an increase (hyperthyroidism) in their production and release, which may be temporary or lasting depending on the underlying pathology and its treatment [2]. Autoimmune thyroid diseases (AITD), including Graves' disease and Hashimoto's thyroiditis, represent the predominant category of organ-specific autoimmune pathologies. AITDs are multifactorial, polygenic conditions that arise from an interplay between genetic susceptibilities—about both thyroid-centric and immune-regulatory genes—and a host of environmental factors, including but not limited to iodine intake, selenium levels, pharmaceuticals, exposure to radiation, tobacco use, infections, and psychological stress. These disorders are hallmarked by the infiltration of lymphocytes into the thyroid parenchyma and the subsequent generation of autoantibodies targeting thyroid antigens [6]. The enzyme thyroid peroxidase (TPO) plays a crucial role in the biosynthesis of thyroid hormones. Disruptions in TPO activity can lead to abnormalities in levels of thyroid hormones. The detection of TPO-Ab is indicative of AITD conditions and serves as a significant biomarker for their diagnosis [3]. Laboratory analyses reveal that more than 90% of individuals with Hashimoto's thyroiditis and between 40%-70% of those with Graves' disease have detectable auto-antibodies, regardless of the thyroid gland's functional state [4]. TPO-Ab specifically attacks the enzyme complexes within the thyroid gland. Consequently, these auto-antibodies are valuable indicators for the presence of AITD, as they reflect an immune response against the thyroid tissue itself [5]. The present study was conducted to shed light on the role of thyroid peroxidase antibodies (TPO-Ab) in Thyroiditis patients and assaying the levels of thyroid function tests (TFT), which include levels of serum triiodothyronine hormone (T3), thyroxin hormone (T4), and thyroid stimulating hormone (TSH).

II. PATIENTS AND METHODS

Samples Collection: A total of (86) serum samples were collected from 76 Iraqi thyroiditis patients and 10 individuals deemed healthy, serving as a control group, from Al-Haboby Teaching Hospital and some private clinics

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starting from August2023 to November 2023, In Thi-Qar Governorate. The following patient data was collected: sex, age, occupation, and place of residence. The samples were centrifuged for 10 minutes at 3000 rpm. Until the supernatant was used to measure the amount of TPO-Ab and TFT, it was frozen at -20 ºC.

The study obtained ethical permission from the Thi-Qar Health Directorate, endorsed through their coded agreement 174/2023.

Thyroid function tests and thyroid peroxidase antibody determination:

All samples were subjected for TFT and were conducted using a fully automated quantitative assay compatible with the Auto Analyzer Cl900i system (Mindray, Korea), which employs competitive binding technology. for the accurate measurement of hormone levels in human serum by using a ready kit for human TPO-Ab (Sun-Long Biotech, China). The serum levels of TPO-Ab were quantitatively measured in the serum of thyroiditis patients and control subjects using enzyme linked immunosorbent assay (ELISA) technique (Fig. 1). Statistical evaluations were conducted across all study groups using descriptive statistics, employing the Chi-square test and independent t-tests for analysis, and *p*-values ≤0.05 and 0.01 were considered as significant.

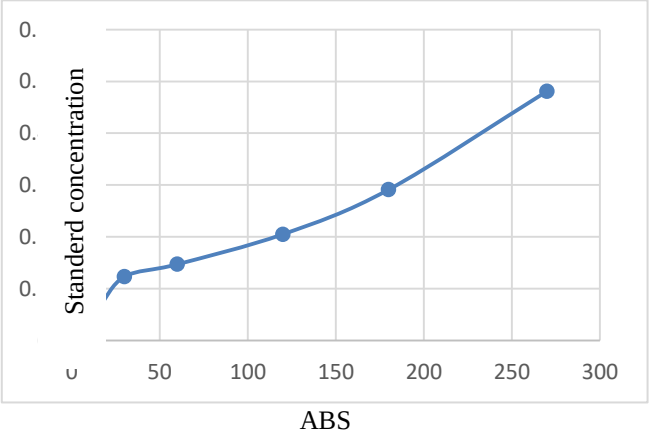

Figure (1): Standard curve of TPO-Ab concentration in serum.

III. RESULTS AND. DISUSSION

Thyroiditis patients were distributed to hypothyroidism and hyperthyroidism types with 47 (61.84%) and 29 (38.16%) patients, respectively ($P \leq 0.05$) as in table1.

Table (1): Classification of Thyroiditis patients based on the type.

Thyroiditis type	No. (%)
Hypothyroidism	47 (61.84%)
Hyperthyroidism	29 (38.16%)
Total	76 (100%)

Geographically, thyroid disorders are more pronounced in iodine-deficient areas. Inadequate consumption of iodine is linked to reduced production of thyroid hormones and an increased prevalence of hypothyroidism [22]. Hypothyroidism is a common pathological condition worldwide. In Iraq, approximately (14.5%) of women suffer from hypothyroidism. Both iodine deficiency and excessive iodine intake can have adverse health consequences [23_24]. High iodine intake leads to symptoms similar to iodine insufficiency, including hypothyroidism, elevated TSH levels and goiter. An overabundance of iodine impedes the synthesis of thyroid hormones in susceptible individuals, resulting in increased TSH stimulation and potential goiter development. Nutritional habits, such as a vegan diet and low consumption of iodine rich foods, contribute to iodine deficiency [25]. A family history of thyroid disorders is a significant risk factor associated with an increased likelihood of thyroid disease development. The typical model for the progression of AITD involves a combination of inherent genetic susceptibility and an external precipitating factor that sets off a series of reactions, ultimately leading to either hypofunction or hyperfunction of the thyroid. It is estimated that genetic factors account for approximately (70%-80%) of the predisposition to AITD, while the residual (20%-30%) is attributed to environmental exposures or triggers [37].

The present findings indicated that patients with hypothyroidism were in the lead among thyroid disorders, were in consistent with a locally related studies conducted in Baghdad and Nasiriyah cities, with hypothyroidism rates of (53.48%) and (68.75%), compared to hyperthyroidism (46.52%) and (31.25%) [12_13]. Similarly outside Iraq, the same hypothyroidism preponderance against hyperthyroidism (70.83% vs. 29.17%) was reported [14].

As shown in table (2), 42 (55.26%) of the patients had elevated levels of TPO-Ab, 17 (40%) and 25 (59.52%) suffered from hyperthyroidism and hypothyroidism, respectively. The low and high concentrations of TPO-Ab were 29 (69.05%) and 13 (30.95%), respectively. The mean of TPO-Ab was (478.8), (1070) and (319.5) for hypothyroidism, hyperthyroidism and controls groups, respectively.

Table (2): result of TPO-Ab in serum.

Result	TPO-Ab No. (%)
Low positive	29 (69.05%)
High positive	13 (30.95%)
Total	42 (100%)

The immune system is a complex network of cells and molecules designed to defend against infections. AITD falls within the classification of endocrine conditions marked by an aberrant immune system's response to the body's thyroid gland. In AITDs, a significant immunological shift occurs within the thyroid's follicular cells. Surface proteins, receptors and enzymes, notably TPO, TSH receptors, and thyroglobulin becomes autoantigens. The body responds by producing corresponding autoantibodies, leading to cell-mediated cytotoxicity. These endocrine autoimmune disorders are marked by elevated levels of specific

antibodies, such as TPO-Abs and thyroglobulin antibodies, which are synthesized by the body itself [7]. It is noted that AITDs are usually accompanied by the presence of TPO-Ab, among other antibodies [8]. The TPO-Ab is a highly sensitive indicator of AITD. While they are typically linked to Hashimoto's thyroiditis, they may also be found in approximately 5% of cases involving Grave's disease [36]. Elevated levels of TPO-Ab typically indicate that hypothyroidism is likely the result of an autoimmune disorder, such as Hashimoto's thyroiditis [31]. The presence of TPO-Ab is observed in over 90% of individuals with autoimmune hypothyroidism. In Addition, TPO-Ab is found in roughly 10% of the population who do not have thyroid disorders, where they may act as 'markers' of autoimmunity, potentially leading to autoimmune diseases. This pattern may suggest an increased likelihood of developing autoimmune conditions in the future for these individuals [32]. TPO-Ab is a valuable marker for predicting hypothyroidism. They contribute to confirming the diagnosis and, on occasion, can aid in determining the underlying cause of the condition [33]. A Turkish study concluded that Hashimoto's thyroiditis is associated with a significant elevation in the serum concentrations of TPO-Ab, which is the main antithyroid antibody in Hashimoto's disease [34]. Another study concluded that the serum concentrations of TPO-Ab and thyroglobulin antibodies may serve as indicators for early thyroid autoimmunity development prognosis, and they also suggested including these tests in the same list of TFT [35].

These results are consistent with two Iraqi studies in Baghdad. The first showed the concentration of TPO-Ab in serum samples was measured at (235.29IU/ml). This is significantly higher compared to the (20.7 IU/ml) found in healthy controls, which falls within the normal range of less than (35 IU/ml). These findings suggest that elevated TPO-Ab levels are indicative of thyroid autoimmunity and hyperthyroidism [28]. Also, the second one showed the serum of TPO-Ab was also markedly elevated in the hypothyroidism patients compared to healthy controls [29].
Globally, the current research findings corroborate international studies, including a notable demonstration that TPO-Ab levels were significantly elevated in both male and female patients diagnosed with hypothyroidism, in contrast to those with hyperthyroidism.]. Otherwise, the findings of the current study diverge from those of a research conducted in Erbil, Iraq, which revealed that the prevalence of TPO-Abs was greater in patients with hypothyroidism (63.6%) compared to those with hyperthyroidism [30].

The means of TFT in the hypothyroidism group for T3,T4 and TSH were (0.9215), (5.402) and (22.86),respectively. While, the means of TFT in the hyperthyroidism group for T3,T4 and TSH were (1.003), (7.409) and (0.3893), respectively. The controls in the present results showed a means of TFT for T3,T4, and TSH were (0.781), (7.374) and (1.603), respectively (table 3).
Thyroid hormones are essential for the proper regulation and performance of diverse bodily functions. Consequently, understanding the factors that can influence the levels of TSH and thyroid hormones is of paramount importance.

Table (3): The result of TFT in serum.

Hormone	Mean of T3	Mean of T4	Mean of TSH
Hypothyroidism	0.9215	5.402	22.86
Hyperthyroidism	1.003	7.409	0.3893
Control	0.781	7.374	1.603

While genetic determinants account for approximately 65% of the variability in TSH and thyroid hormone concentrations among individuals, a multitude of environmental factors are also known to impact thyroid function. These include lifestyle choices such as smoking, alcohol intake, dietary habits, and physical activity, as well as exposure to environmental pollutants like chemicals and heavy metals. An excessive intake of iodine, a crucial dietary micronutrient, has been observed to increase TSH levels while reducing thyroid hormone levels. Among the various pollutants studied, exposure to perchlorate has been commonly associated with a reduction in thyroid hormone levels [10].

As illustrated in Table 4, the results of the current study according to sex showed that females have a high incidence of infection with 70 (92.11%) while the males have a low incidence of infection with only 6 patients (7.89%). The predominance of female infection was with significant differences ($p \leq 0.001$).

Table (4) : Distribution of thyroiditis patients according to sex.

Sex	No. (%)
Male	6 (7.89%)
Female	70 (92.11%)
Total	76 (100%)

Thyroid problems are prevalent worldwide, particularly among females. This association is linked to imbalances in female sex hormones, such as elevated estrogen during puberty and pregnancy. Additionally, X chromosome inactivation plays a role in thyroid gland function and the immune system, contributing to the female predilection for autoimmune thyroiditis [15]. The present study findings align with local studies in Thi-Qar Governorate, which revealed female/male percentages of (63/47) and (78.82/21.18), respectively []. These results were globally supported by a lot of articles conducted around the world [].

As shown in Fig. 2, The findings of the current research indicate that the second age group (21-40) years are the

most susceptible to infection with 37 patient (48.68%) with an age mean of (38.32).

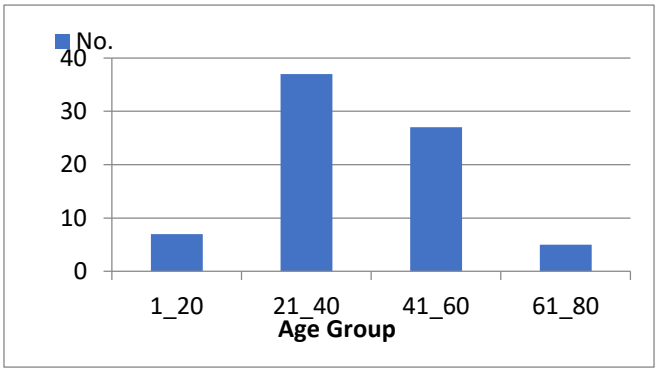

Figure (2): Distribution of Thyroiditis patients according to age.

Middle-aged and older adults, especially females, exhibit a higher susceptibility to thyroid disorders. Thyroid autoimmunity tends to increase with age. Additionally, age-related changes in body functions can impact hormone production, metabolism, tissue responses, and biological rhythms, including the menstrual cycle []. Furthermore, individuals with additional autoimmune disorders, including type 1 diabetes, rheumatoid arthritis, and multiple sclerosis, are associated with an increased likelihood of thyroid dysfunction. Certain medications, including amiodarone, glucocorticoids, dopamine agonists, and somatostatin analogues, can also alter TSH levels [38].

The present study findings according to age seem to be in alignment with a wide range of other local related studies []. Also, and in the same time a lot of globally researched was conducted in Malaysia, India, and Pakistan, respectively [].

The outcomes of the current investigation reveal that, according to occupation, housewives are more susceptible, with high level of significant, to infection than other jobs with (81.58%). While the rest of all jobs (18.42%) ($p \leq$ 0.001).

Socioeconomic factors are correlated with the incidence of thyroid disorders. Individuals with lower income or those who are unemployed exhibit a heightened occurrence of conditions such as hypothyroidism and hyperthyroidism [].

The results of the current study, according to residence, showed that people who live in urban are more susceptible to infection with 56 (73.69%) compared to rural area with 20 (26.31%). The predominance of citizen infection was with high significant differences ($p \leq$ 0.001).

The potential etiological factors could be attributed to environmental influences, particularly the elevated pollution levels in the city. Additionally, dietary habits and the consumption of specific food types may also play a contributing role.

The present study findings agreed with study in Al-Basrah, Iraq, which showed that thyroid disorders were more common among people who live in the city (71.94%) than those who live in the countryside (28.06%) [47_49]

IV. CONCLUSIONS

High levels of TPO-Ab are linked to the immunological alterations in the thyroid gland that characterize AITD. The presence of these antibodies is associated with the severity of the disease and can be used as an important marker for diagnosis and management.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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