Diagnosis of Hypothyroidism by Using Some Biomarkers and Immune Markers

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Abstract

Thyroid hormones play vital roles in maintaining energy physiological state and regulation energy expenditure. Their physical effects, mediate at multiple target organs, primarily to stimulate cell metabolism and activity. Thyroid illness may be a medical condition that affects the perform of the ductless gland (the endocrine organ found at the front of the neck that produces thyroid hormones). The symptoms of thyroid illness vary reckoning on the sort of Thyroidism. In individuals with gland disease, the thyroid doesn't build enough endocrine to stay the body running usually. Common causes of hypothyroidism, autoimmune disorder, surgical removal of the thyroid, and radiation treatment. Patients typically have enlargement of the ductless gland (goiter) and either traditional (euthyroid) or gently attenuated (hypothyroid) metabolic state. The T₃, T₄, and thyrotrophic hormone levels is also high in what seems to be a counteractive mechanism to confirm maintenance of euthyroid and eumetabolic states. The Synapsin one (SYN 1) may be a member within which that job as neural protein, that interface with the living substance surface of conjunction vesicles. Analysis was carried TTR may be a 55kDa homo-tetramer with a compound of dimers quaternary structure that's composed within the liver, plexus choroideus and retinal shade animal tissue for outpouring into the course system, neural structure liquid and also the eye, severally. Analysis was dole out victimisation SPSS version 23 The mean variations between T₃, T₄, TSH, Tg, Anti-Tg, TPO, Anti-TPO, Synapsin one and Transthyretin with management.

Introduction

The thyroid is a two-inch long endocrine organ, measuring shy of what one ounce, arranged between the larynx and the collarbones, just under the skin and muscle layer of the front of the neck.

The follicles are filled with ‘colloid the secretions of the epithelial cells. An active thyroid has cuboidal or columnar epithelial cells, but very little colloid in the follicles because the secretions are released promptly into the bloodstream [1].

Hypothyroidism is a disorder of the endocrine system, also called underactive thyroid or low thyroid, in which the thyroid gland does not generate adequate thyroid hormone, It can cause a number of signs, such as poor ability to tolerate cold, a feeling of tiredness, constipation, despondency, and weight gain. Occasionally there may be swelling of the front part of the neck due to thyromegaly [2]. Thyroid-Stimulating hormone (TSH), also known as thyrotropin, is a 28-kDa glycoprotein that is by the cells in the anterior pituitary called Thyrotoph is synthesized and secreted, TSH stimulates most if not all aspects of intrathyroidal iodide and thyroglobulin metabolism, TSH is the physiologic controller of T4 and T3 amalgamation and release by the thyroid organ, the thyroid hormones control the body's basal metabolic rate, warm yield, protein amalgam, advancement, and CNS change [3].

T₄ and T₃ make from the hydrolysis of T₄ are avoided from the follicular cell and enter the nearby by hair like course; regardless, the segment of transport of T₄ and T₃ over the basal plasma film has not been described, Evidence for the manufacture of TSH by cells of the safe system was first indicated over twenty years back [4].

TSH has been had all the earmarks of being made by a sub-masses of bone marrow hematopoietic cells. This has been appeared
by intracellular recoloring in mix with CD45 (leukocyte-ordinary antigen [LCA]) or CD11b recoloring. TSH+ bone marrow cells were exclusively associated with the LCA+ cells [5].

TSH instigation of splenic DCs achieves a more grounded phagocytic response in vitro, and extends the cytokine activity of IL-1β and IL-12 inside seeing phagocytic lifts [6]. Thyroglobulin (Tg) could be a 660 kDa, dimeric super molecule made by the vesicle cells of the thyroid and used out and out inside the endocrine organ, super molecule speaks to around 1/2 the protein substance of the endocrine organ [1].

The amalgamation of the protein precursor for Tg is the underlying stage in the advancement of T4 and T3.

This substance is a660-kDa glycoprotein made out of two near 330-kDa subunits held together by disulfide ranges, Tg autoantibodies of IgG make have a normality out of 80-85% in patients with Hashimoto thyroiditis and besides a lower transcendence of 30-80% in patients with Graves' sickness [7].

Moreover see as ceaseless lymphocytic thyroiditis and Hashimoto's sickness, is a resistant framework disease in which the thyroid organ is gently lost [8]

Over time the thyroid may escalate forming an easy goiter [9]. Furthermore called thyroperoxidase (TPO) or iodide peroxidase, is an impetus communicated generally in the thyroid where it is released into colloid [10].

In resistant framework issue, in any case, the immune system frustrations, erroneously striking sound organs and tissues as though they were remote trespassers. In people with a thyroid-related insusceptible framework condition, the blood level of TPO antibodies may rise [11].

Antagonistic to TPO antibodies are vital fundamental against thyroid immunizer, favoring in close ninetieth of Hashimoto's thyroiditis, seventy fifth of thyromegaly | hyperthyroidism | thyrotoxicosis | exophthalmos and 10-20% of nodular goiter or thyroid undermining neoplastic disease.

Furthermore, 10-15% of standard people will have unusual state unfriendly to TPO protein titers [12, 13].

The Synapsin 1 (SYN 1) protein is a man from the Synapsin family that is neuronal phosphoprotein, which interface with the cytoplasmic surface of synaptic vesicles [14].

This phosphoprotein is as an endogenic substrate bound to the vesicular film. It is phosphorylated by four known classes of protein kinases including that switch on by CAMP [15], calcium/calmodulin mitogen, and cyclin. Both isoforms have comparable six-phosphorylation goals. Changes in thyroid hormone level achieve isolating explanation of mitochondrial qualities.

Changes in these mitochondrial qualities induce particularly watched issue of encephalomyopathy, myopathy, and multisystem issue. Human Transthyretin (TTR) is a 55kDa homo-tetramer with a dimer of dimers quaternary structure that is coordinated in the liver, choroid plexus and retinal shade epithelium for release into the course framework, cerebrospinal fluid and the eye [1]. Unmistakable point changes in TTR augment, decrease, or don't impact TTR's proclivity for T4.

The limit of TTR to outline amyloid fibrils does not have every one of the reserves of being related to its affection for T4. Moreover consider is required to portray the nuclear introduce of modifications in T4 confining actuated by point changes arranged along the TTR tetramer [16].

Materials and Methods

Patients and Conditions of Study

The plans design of this study to measure some parameters in subjects suffer from Thyriodism patients and compared it with control, this parameters include:

- Measurement the level of Triiodothyronine (T3 )
- Measurement the level of Thyroxin (T4)
- Measurement the level of Thyroid-stimulating hormone (TSH)
- Measurement the level of Thyroglobulin (TG) Ab.
- Measurement the level of Anti-Thyroglobulin (ATGA) Ab.
- Measurement the level of Thyroid peroxidase (TPO) Ab.
• Measurement the level of Anti-Thyroid peroxidase (ATPO) Ab.
• Measurement the level of Human Synapsin 1(SYN 1).
• Measurement the level of Human Transthyretin (TTR)

This study was done in Merjan teaching hospital and al- Zahrawi laboratory in Babylon province. The collection of samples was conducted during the period from January to February / 2017.

The samples was taken from 60 patients with Thyroidism and 30 healthy subjects were taken as control (total persons 60), all patients (n=30) hypothyroidism and the results of each group were compared with control group. All patients and control were suffered from males only. The ages of patients and controls were ranges between 20-60 years old and the weight were ranges between 65-100 K.gm.

Total Triiodothyronine (T3), Total Thyroxin (T4) and Thyrotropin (TSH) Tests System

The quantitative determination of total Triiodothyronine Concentration, Total Thyroxin and Thyrotropin in human serum by Vidus technique.

The Kit is the Biomex marker made in France; the test was done according to principle and manual procedure of Biomex Company.

**Thyroglobin (Tg) Ab :- ( Calbiotech, U.S.A Makes Tg-Ab Marker Kit)**

Bring all specimens and kit reagents to temperature (20-25 C) and gently combine the principle and the procedure that present in the leaflet of the Tg-Ab marker kit of Calbiotech Company with Catalog No. TG070T.

**HumanATGA/TGAB (Anti-Thyroid-Globulin- Antibody) (Elabscience, China makes anti-TG –Ab Marker Kit)**

The principle and the procedure that present in the leaflet of the Anti-Tg-Ab marker kit of Elabscience Company with Catalog No. E-EL-H0436.

**Figure 1: Stander curve of Anti-Thyroid-Globulin Antibody (ATGA/TGAB)**

**Thyroid Peroxidase (TPO Ab) (Calbiotech, U. S. A Makes TPO IgG Marker Kit)**

This Elisa kit have the same procedure and calculation method of Thyroglobin (TG) Ab kit that and present in Calbiotech company catalog NO. TP069G.

The index value of TPO Ab is: <45 IU/Ml: Negative, 45-55 IU/Ml: Borderline positive an >55 IU/Ml: positive.

**Human Anti-TPO-Ab (Anti-Thyroid-Peroxidase Antibody) (Elabscience, China makes Anti-TPO-Ab marker Kit).**

The principle and the procedure that present in the leaflet of the Anti-TPO-Ab marker kit of Elabscience Company with Catalog No.E-EL-H0437.
Human Synapsin 1 (Human SYN 1) (Elabscince, China makes SYN 1 marker Kit.)

The guideline of this unit like the rule of the Anti – Thyroid-Globulin Antibody pack that talked about before and yet that vary in a few focuses like the readiness of stock arrangement of 200 ng/mL and make serial weakening. The suggested are as per the following: 200, 100, 50, 12.5, 6.25, 3.13, 0 ng/mL as present in index NO.E-EL-H2502. The outcomes were computed by framing stander bend by plotting the mean OD esteem for every standard on the Y-hub against the fixation on the X-pivot and draw a best-fit bend through the focuses on the diagram as the accompanying.

Human Transthyretin (TTR) (Elabscince, China makes TTR marker Kit)

The principle and the procedure of this kit like the principle of the Anti-Thyroid-Globulin Antibody kit and Synapsin 1 kit that discussed previously that peasant in Elabscince company catalog NO.E-EL-H2502.
Results
The clinical characteristic features of patients & control group:

A total sample of this study consist of (n=30) hypothyroidism patients consist of 54. % & 30 controls, 27 % of the total patient’s samples have no family history for Thyroidism. In this study the patients (only males) were 30 hypothyroidisms patients and the results of this group were compared with control group 30 persons that have normal range of thyroid hormone.

**The Level of T3 (nmol/L) in Patients and Control**
The results of statistical analysis by using Z-test, showed significant differences between T3 in comparison between Hypothyroidism (0.974±0.333) and control (1.797±0.552) at p. value < 0.05 as shown in Figure (5).

![Figure 5: The concentration of T3 hormone levels (nmol/L) among patients and control](image)

**The Level of T4 (nmol/L) in patients and control**
The results of statistical analysis by using Z-test showed significant differences between T4 in comparison between Hypothyroidism (46.7±9.32) and control (74.49±11.76) at p. value < 0.05 as shown in Figure (6).

![Figure 6: The concentration of T4 hormone levels (nmol/L) among patients and control](image)

**The Level of TSH (µU/mL) in Patients and Control**
The results of statistical analysis by using Z-test, showed significant differences between TSH in comparison between Hypothyroidism (8.248±0.111) and control (0.863±1.109) at p. value < 0.05 as shown in Figure (7).
The Level of Thyroglobulin Antibody (TG) Ab (IU/ml) in Patients and Control

The results of statistical analysis by using Z-test, showed significant differences between Tg in comparison between Hypothyroidism (97.38 ±13.01) and control (70.53±5.87) at p. value < 0.05 as shown in Figure (8).

The Level of Anti-thyroglobulin Antibody (Anti-Tg Ab) (ng/ml) in Patients and Control

The results of statistical analysis by using Z-test, showed significant differences between Anti-Tg Ab in comparison between Hypothyroidism (97.75 ± 7.09) and control (10.61 ± 2.22) at p. value < 0.05 as shown in Figure (9).

The Level of TPO Ab (IU/ml) in Patients and Control

The results of statistical analysis by using Z-test, showed significant differences between TPO-Ab in comparison between Hypothyroidism(150.12±19.20) and control (40.45±13.76) at p. value < 0.05 as shown in Figure (10).
The Level of Anti-TPO-Ab (ng/ml) in Patients and Control

The results of statistical analysis by using Z-test, showed significant differences between Anti-TPO-Ab in comparison between Hypothyroidism (1.607 ±0.09) and control (1.8±0.08) at p. value < 0.05 as shown in Figure (12).

The Level of Synapsin I (SYN I) (ng/ml) in Patients and Control

The results of statistical analysis by using Z-test, showed significant differences between Synapsin I (SYN I) in comparison between Hypothyroidism (8.91 ±0.90) and control (4.16±0.16) at p. value < 0.05 as shown in Figure (13).

The Level of Human Transthyretin (TTR) (ng/ml) in Patients and Control

The results of statistical analysis by using Z-test, showed significant differences between
Human Transthyretin (TTR) in comparison between Hypothyroidism (188.81 ±28.27) and control (20.20±1.29) at p. value < 0.05 as shown in Figure (14).

**Figure 13:** The concentration of Human Transthyretin (TTR) (ng/ml) in patients and control

**Discussion**

The study was planned to evaluating the hormonal levels as well as autoantibodies in which that associated with hypo and Euthyroid male patients. The selective criteria of male rather than female due to the clinical differences between immunity in male and female in association with thyroid diseases. The system of immunity differs to some extent between male and female. For instance, females have stronger humeral and cellular immunity than male [9], this is often manifested by higher levels of current antibodies, higher numbers of current CD4 T cells, increased protein production in response to infection, and fast rejection of allograft [17].

The results of statistical analysis of T3 level, show that significant differences after comparison between Hyperthyroidism, Hypothyroidism and control. This result might be show that the T3 level in the hypo. Groups have borderline of normal value might be due to the main diagnosis depending on TSH level rather than T3. As mentioned by Reid and Wheeler [18] they said that, since the thyroid's abilities are perplexed, this single test may not give any indisputable answers about what isn't right, however, surprising results can help point them the right way. So play out a T4 or TSH test to get a clearer photograph of the thyroid limit.

This result might be exhibit that the delayed consequence of T4 as like as T3 in which that the abnormal results can help point them the right way yet more basic than T3 [19]. Estimation of total T4 gives a tried and true impression of clinical thyroid status without protein-confining varieties from the standard and non thyroidal infection. Regardless, changes in limiting proteins can happen that impact the level of total T4, yet leave the level of unbound hormone unaltered [20].Thyroid brokenness exhibit that bizarre TSH level, as a high in (Hypo) and Low in (Hyper) might be used to attest the examination of all patients in the present examination and might be show that high TSH proposes a thyroid that is underactive (hypothyroid) and not doing its action of conveying enough thyroid hormone.

As spoke to by [21] they said that, the excess TSH is endeavoring to empower the thyroid organ to make more thyroid hormone .also exhibit that thyroglobulin antibodies (Tg Ab) are lessened in hypothyroidism patients' since this checking operator is immune proteins conveyed that target thyroglobulin and thyroid tissue is the principle wellspring of orbiting Tg Ab. Serum Tg levels may be high in thyrotoxicosis, thyroiditis, iodine deficiency, and compassionate thyroid adenomas and also in thyroid development [22].

Along these lines, it isn't a screening test for thyroid growth; as a result of the thyroid follicular cells, Tg levels are not expanded in medullary or anaplastic thyroid carcinomas [22]. Serum thyroglobulin testing is touchy (91%) and particular (99%) for distinguishing patients with steady or repetitive separated thyroid growth. Serum thyroglobulin levels are most exact when patients are hypothyroid (high TSH) and might be problematic in patients with subterranean insect thyroglobulin antibodies. We prescribe TSH-empowered thyroglobulin testing for all
patients after aggregate thyroidectomy for separated thyroid malignancy of follicular cell starting point paying little respect to quiet age or hazard gathering [23]. The Thyroglobulin and Anti-Thyroglobulin –Ab are important in hypo and hyperthyroidism patients but the Anti-thyroglobulin-Ab is more important in hypothyroidism diagnosis than Thyroglobin, because the last one is normal product in thyroid gland but the Anti-Tg-Ab is elevated hypothyroidism as a result of immunological cause. Anti-thyroglobulin antibody (anti-Tg) was first shown as an autoantibody in the serum of patients with Hashimoto’s thyroiditis and this discovering first settled the idea of organ-particular immune system infection [24].

Hoisted antibodies to TPO are frequently connected with a hypothyroid condition called Hashimoto’s Disease. It is imperative to take note of that TPO antibodies can be distinguished available for use some time before the difference in thyroid capacity can be seen by changes in the TSH levels [25]. Autoimmunity happens when antibodies against TPO cause continuous pulverization of follicles in the thyroid organ, and reduction absorption of thyroid hormones into the cells [26, 27].

The height in the immune system hypothyroidism in light of the fact that the nearness of hostile to TPO antibodies means that the invulnerable framework isn't working legitimately , these antibodies speak to the body mixing up the thyroid organ as "adversary" tissue and an endeavor to demolish that tissue , The nearness of against TPO's in patients' circulatory system show the nearness of autoimmunity and irritation this condition ought to be separated from customary hypothyroidism which implies that the treatment for this condition is unique [28]. The phosphorylation province of Synapsin1 was appeared to manage the unharness of neurochemical in the squid enormous nerve fiber The partiality of phosphorylated Synapsin I for every basic protein and intersection vesicles is remittent and mitigates the imperative on the intersection vesicles. This winds up in will increment inside the scope of intersection vesicles in the pools realistic for combination with the living substance layer and unharness of neurochemical amid which that influenced beyond any doubt organs like thyroid tissues [29]. Synapsin1 impacts synaptic pliancy by controlling pre-and post-synaptic vesicular discharge. Changes in Synapsin I have been connected to regulation of thyroid hormone discharge, and conduct issue, which is a neurologic issue portrayed by factor blends of epilepsy, learning challenges, macrocephaly, and forceful conduct [30].

Certain investigations in which that proposed the TTR may have a vital part amid early improvement, TTR is the main thyroid dispersion protein (THDP) combined furthermore in the CNS [31]. Each TTR subunit has type of symmetrical β-barrel structure with a twofold trumpeted hydrophobic channel that navigates the atom shaping the two iodothyronines restricting locales; TTR normally ties just a single T4 particle on the grounds that the coupling proclivity of the second site is significantly diminished through a negative helpful impact.

The TTR tetramer can tie four particles of Retinol-restricting protein (RBP) that don't meddle with T4-authoritative, and the other way around. TTR can be estimated by densitometry after its partition from the other serum proteins by electrophoresis, by hormone immersion, and by immunoassays [32]. Distinctive malady related changes have been found in TTR quality, with variable indication. Most ordinarily, they prompt amyloid statement in the heart tissue and additionally fringe nerves, without influencing the status of thyroid hormones [33].

References


antibodies in a large, unselected population.


