

# Diagnosis of hyperthyroidism by using some biomarkers and immune markers

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## Abstract:

Thyroid hormones play important roles in maintaining energy homeostasis and regulating energy expenditure. Their physiologic effects, mediated at multiple target organs, are primarily to stimulate cell metabolism and activity. Thyroid disease is a medical condition that affects the function of the thyroid gland (the endocrine organ found at the front of the neck that produces thyroid hormones). The symptoms of thyroid disease vary depending on the type of Thyroidism. Hyperthyroidism is excessive functional activity of the thyroid gland, characterized by increased basal metabolism and disturbances in the autonomic nervous system because of excess thyroid hormone production. The incidence is higher in women (2%) than in men (0.02%). Several conditions can lead to hyperthyroidism: diffuse toxic goiter or Graves disease, toxic nodular goiter, toxic adenoma, therapy-induced hyperthyroidism (eg, excess T<sub>4</sub> or T<sub>3</sub> substitution), excess iodine intake, thyroiditis, follicular carcinoma, and TSH-producing tumor of the pituitary, However, the most common cause of hyperthyroidism in adults is diffuse toxic goiter or Graves' disease. The Synapsin 1 (SYN 1) is a member in which that work as neuronal phosphoprotein, which interface with the cytoplasmic surface of synaptic vesicles. Analysis was carried TTR is a 55kDa homo-tetramer with a dimer of dimers quaternary structure that is composed in the liver, choroid plexus and retinal shade epithelium for outpouring into the course system, cerebrospinal liquid and the eye, independently, Analysis was carried out using SPSS version 23 The mean differences between T<sub>3</sub>, T<sub>4</sub>, TSH, Tg, Anti-Tg, TPO, Anti-TPO, Synapsin 1 and Transthyretin with control .

## 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 indispensable segment related with the amalgamation of thyroid hormones is iodine, which is normally put resources into the little stomach related tract in sort of iodides. Iodine transport to the follicular cells of the thyroid organ is the first and rate-obliging development in the union strategy. (Anetoh nnaemeka Gabriel 2008). The follicles are stacked with 'colloid' the releases of the epithelial cells. A dynamic thyroid has cuboidal or columnar epithelial cells, however by no colloid in the follicles in light of the way that the releases are released quickly into the dissemination framework (Boron WF. 2003). Hyperthyroidism is the condition that happens as a result of over the best age of thyroid hormone by the thyroid organ, Thyrotoxicosis is the condition that happens due to extreme thyroid hormone of any reason and therefore joins hyperthyroidism Some (Bahn Chair et al., 2011), regardless, use the terms equally, Signs and reactions change among people and may fuse crabbiness, muscle deficiency, resting issues, a fast heartbeat, warm bias, free guts, expansion of the thyroid, and weight decrease, Symptoms are customarily less in the old and in the midst of pregnancy (Erik D Schraga 2014). An unprecedented multifaceted nature is thyroid whirlwind in which an event, for instance, a sickness achieves aggravating symptoms, for instance, confuse and a high temperature and as often as possible realizes demise (Devereaux, D. likewise, Tewelde, SZ. 2014). The opposite is hypothyroidism, when the thyroid organ does not make enough thyroid hormone. (Brent and Gregory A. 2008). Graves' illness is the reason for around half to 80% of the instances of hyperthyroidism in the United States (Brent and Gregory A. 2008). Different causes incorporate multinodular goiter, harmful adenoma, irritation of the thyroid, eating excessively iodine, and an excess of engineered thyroid hormone (Erik D Schraga 2014). A less regular reason is a pituitary adenoma, The analysis might be suspected in view of signs and side effects and after that affirmed with blood tests, Typically blood tests demonstrate a low thyroid animating hormone (TSH) and raised T<sub>3</sub> or T<sub>4</sub>, Radioiodine take-up by the

thyroid, thyroid output, and TSI antibodies may help decide the cause (Brent and Gregory A. 2008).

Thyroid-Stimulating hormone (TSH), otherwise called thyrotropin, is a 28-kDa glycoprotein that is blended and emitted by the phones in the foremost pituitary called Thyrotrophs, TSH animates most if not all parts of intrathyroidal iodide and thyroglobulin digestion, TSH is the physiologic controller of T<sub>4</sub> and T<sub>3</sub> combination and discharge by the thyroid organ, the thyroid hormones control the body's basal metabolic rate, warm creation, protein amalgamation, development, and CNS improvement (Sevilay D. et al., 2016). T<sub>4</sub> and T<sub>3</sub> shaped from the hydrolysis of Tg are discharged from the follicular cell and enter the close-by hairlike flow; notwithstanding, the system of transport of T<sub>4</sub> and T<sub>3</sub> over the basal plasma film has not been characterized. The DIT and MIT created by the hydrolysis of Tg are quickly deiodinated in the follicular cell, Evidence for the generation of TSH by cells of the invulnerable framework was first shown more than twenty years prior (Smith EM et al., 1982). TSH has been appeared to be delivered by a sub-populace of bone marrow hematopoietic cells. This has been shown by intracellular recoloring in mix with CD45 (leukocyte-regular antigen [LCA]) or CD11b recoloring. TSH+ bone marrow cells were solely connected with the LCA+ cells (Wang. et al., 2003). TSH incitement of splenic DCs brings about a more grounded phagocytic reaction in vitro, and expands the cytokine action of IL-1 $\beta$  and IL-12 within the sight of phagocytic boosts (Bagriacik. furthermore, Klein. 2000). Thyroglobulin (Tg) is a 660 kDa, dimeric protein delivered by the follicular cells of the thyroid and utilized totally inside the thyroid organ, thyroglobulin protein represents around half of the protein substance of the thyroid organ (Boron WF. 2003). The combination of the protein antecedent for Tg is the initial phase in the development of T<sub>4</sub> and T<sub>3</sub>. This substance is a 660-kDa glycoprotein made out of two comparative 330-kDa subunits held together by disulfide spans, Tg autoantibodies of IgG write have a commonness of 80-85% in patients with Hashimoto thyroiditis and furthermore a lower predominance of 30-80% in patients with Graves' ailment (Mondal, and Mugesh, 2015). Otherwise called incessant lymphocytic thyroiditis and Hashimoto's infection, is an immune system illness in which the thyroid organ is step by step wrecked

(Akamizu, et al., 2000). Over time the thyroid may grow shaping an effortless goiter (Nalbandian G1 and Kovats S. 2005). Likewise called thyroperoxidase (TPO) or iodide peroxidase, is a protein communicated principally in the thyroid where it is discharged into colloid (Chardès et al., 2002). In immune system ailments, in any case, the resistant framework glitches, erroneously assaulting solid organs and tissues as if they were outside trespassers. In individuals with a thyroid-related immune system condition, the blood level of TPO antibodies may rise (Taurog and Alvin 1999). Against TPO antibodies are the most widely recognized hostile to thyroid autoantibody, show in roughly 90% of Hashimoto's thyroiditis, 75% of Graves' malady and 10-20% of nodular goiter or thyroid carcinoma. Additionally, 10-15% of ordinary people can have abnormal state hostile to TPO counter acting agent titers (Trbojević and Djurica, 2005) and (Melmed et al., 2011). The Synapsin 1 (SYN 1) protein is a member from the Synapsin family that are neuronal phosphoprotein, which interface with the cytoplasmic surface of synaptic vesicles. (Udhof 1990). This phosphoprotein is as an endogenous substrate bound to the vesicular film. It is phosphorylated by four known classes of protein kinases including those actuated by cAMP (Jaffrey, et al., 2002), calcium/calmodulin mitogen, and cyclin. Both isoforms have a similar six-phosphorylation destinations. Changes in thyroid hormone level accomplish separating articulation of mitochondrial qualities. Changes in these mitochondrial qualities instigate especially observed issue of encephalomyopathy, myopathy, and multisystem issue (Pablo, et al., 2016). Human Transthyretin (TTR) is a 55kDa homo-tetramer with a dimer of dimers quaternary structure that is integrated in the liver, choroid plexus and retinal shade epithelium for emission into the circulatory system, cerebrospinal liquid and the eye (Boron. 2003). Diverse point changes in TTR increment, diminish, or don't influence TTR's partiality for T4. The capacity of TTR to frame amyloid fibrils does not give off an impression of being identified with its proclivity for T4. Additionally think about is required to characterize the atomic premise of adjustments in T4 restricting instigated by point transformations situated along the TTR tetramer (Lans, et al., 1993).

**METHODS**

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 Thyroglobin (TG) Ab.
- Measurement the level of Anti-Thyroglobin (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 Thyriodism 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.

**1.Total Triiodothyronine (tT3), Total Thyroxin (tT4 ) and Thyrotropin (TSH) Tests System:**

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

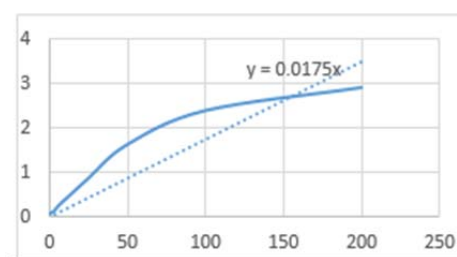
The Kit is the Biomerx marker made in France; the test was done according to principle and manual procedure of Biomerx Company (Tietz 2006).

**2. 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.

**3.HumanATGA/TGAB(Anti–Thyroid-Globulin- Antibody) (Elabscince, 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 Elabscince Company with Catalog No. E-EL-H0436.



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

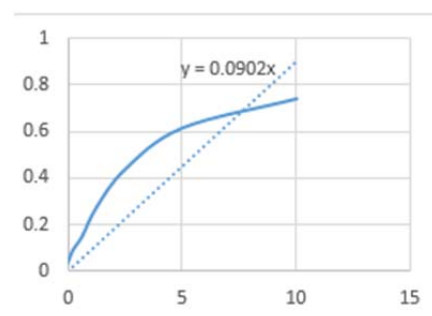
**4. Thyroid peroxidase IgG (TPO) (Calbiotech, U.S.A makes TPO IgG marker Kit):**

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/MI :Negative ,45-55 IU/MI :Borderline positive and >55 IU/MI :positive.

**5. Human Anti-TPO-Ab(Anti-Thyroid-Peroxidase Antibody) (Elabscince, 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 Elabscince Company with Catalog No. E-EL-H0437.



**Figure (2) Stander curve of Anti-Thyroid-Peroxidase Antibody(Anti-TPO-Ab).**

**6. Human Synapsin 1 (Human SYN 1) (Elabscince, China makes SYN 1 marker Kit.)**

The principle of this kit like the principle of the Anti – Thyroid-Globulin Antibody kit that discussed previously but that differ in some points like the preparation of stock solution of 200 ng/mL and make serial dilutions .the recommended are as follows :200, 100, 50, 12.5, 6.25, 3.13 , 0 ng/mL as present in catalog NO. E-

EL-H2502. The results were calculated by forming stander curve by plotting the mean OD value for each standard on the Y-axis against the concentration on the X- axis and draw a best-fit curve through the points on the graph as the following .

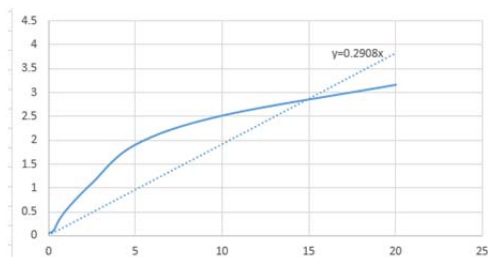


Figure (3) Stander curve of Human Synapsin 1(SYN 1)

**7. 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 peasent in Elabscince company catalog NO.E-EL-H2502.

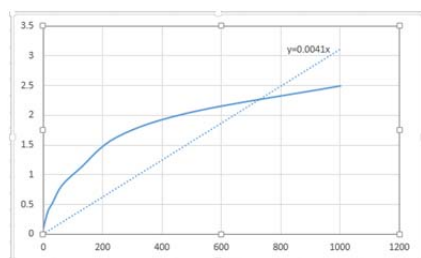


Figure (4) Stander curve of Human Transthyretin (TTR)

**RESULTS**

1. The clinical characteristic features of patients &control group: A total sample of this study consist of (n=30) hypothyroidism patients consist of 54. % & 30 control, 27 % of the total patient's samples have no family history for Thyriodism.

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 .

**1.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 Hyperthyroidism(4.951±1.576) and control(1.797±0.552) at p. value < 0.05 as shown in figure ( 5 ).

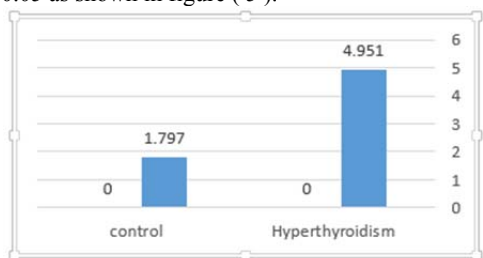


Figure (5) The concentration of T3 hormone levels (nmol/L) among patients and control

**2.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 Hyperthyroidism(143.81±29.64)and control(74.49±11.76) at p. value < 0.05 as shown in Figure ( 6 ).

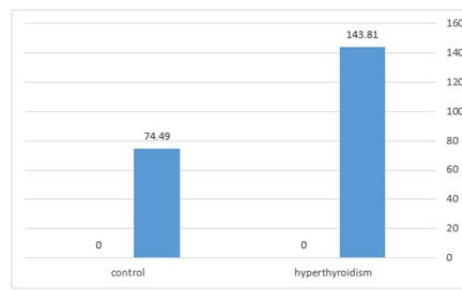


Figure (6) The concentration of T4 hormone levels (nmol/L) among patients and control

**3.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 Hyperthyroidism(0.205±0.077) and control (0.863±1.109) at p. value < 0.05 as shown in Figure ( 7 ).

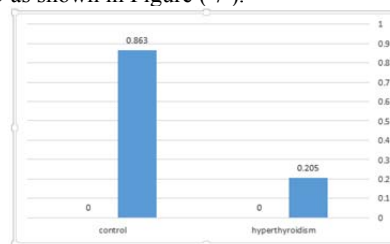


Figure (7) The concentration of TSH hormone levels (µU/mL) among patients and control.

**4.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 Hyperthyroidism (122.75 ±27.67) and control (70.53±5.87) at p. value < 0.05 as shown in Figure ( 8 ).

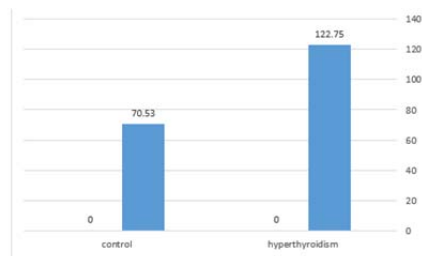


Figure (8) the concentration of Tg Ab levels (IU/ml) in patients and control.

**5.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 Hyperthyroidism(60.165±8.879) and control (10.61 ± 2.22) at p. value < 0.05 as shown in Figure ( 9 ).

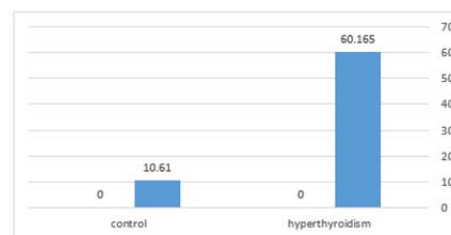
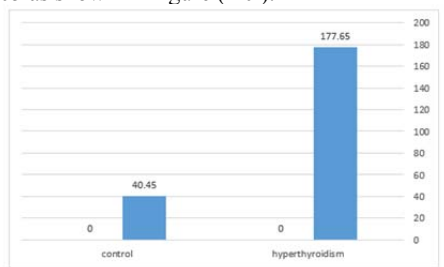


Figure (9) the concentration of Anti-Tg Ab (ng/ml) in patients and control.

**6. The level of TPO Ab (IU/ml) in patients and control.**

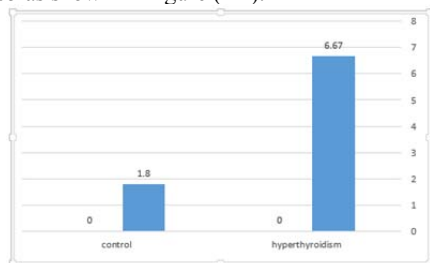
The results of statistical analysis by using Z-test , showed significant differences between TPO-Ab in comparison between Hyperthyroidism( $177.65 \pm 18.77$ ) and control ( $40.45 \pm 13.76$ ) at p. value  $< 0.05$  as shown in Figure ( 10 ).



**Figure (10) the concentration of TPO Ab (IU/ml) in patients and control.**

**7. The level of anti-TPO-Ab (ng/ml) in patients and control.**

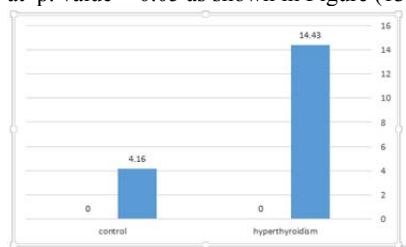
The results of statistical analysis by using Z-test , showed significant differences between Anti-TPO-Ab in comparison between Hyperthyroidism( $6.67 \pm 0.43$ ) and control ( $1.8 \pm 0.08$ ) at p. value  $< 0.05$  as shown in Figure ( 12 ).



**Figure (11) The concentration of Anti-TPO-Ab (ng/ml) in patients and control.**

**8. The level of Synapsin I (SYN I) (ng/ml) in patients and control**

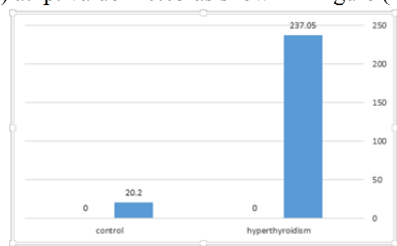
The results of statistical analysis by using Z-test , showed significant differences between Synapsin I (SYN I) in comparison between Hyperthyroidism( $14.43 \pm 0.98$ ) and control ( $4.16 \pm 0.16$ ) at p. value  $< 0.05$  as shown in Figure (13).



**Figure(12) The concentration of Synapsin I (SYN I) (ng/ml) in patients and control.**

**9. 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 Hyperthyroidism( $237.05 \pm 39.10$ ) and control ( $20.20 \pm 1.29$ ) at p. value  $< 0.05$  as shown in Figure ( 13 ).



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

**DISCUSSION**

The investigation was intended to assessing the hormonal levels and also autoantibodies in which that related with hyper and Euthyroid male patients. The particular criteria of male instead of female because of the clinical contrasts between resistance in male and female in relationship with thyroid infections. The invulnerable framework additionally contrasts to some degree amongst male and female. For instance, females have more grounded humeral and cell insusceptibility than male (Nalbandian and Kovats S 2005) , This is showed by more elevated amounts of circling antibodies, higher quantities of coursing CD4 T cells, improved cytokine creation in light of disease, and fast dismissal of allografts (Anatoly et al., 2010). The consequences of measurable examination of T3 level , demonstrate that noteworthy contrasts after correlation between Hyperthyroidism , and control, This outcome may be demonstrate that the T3 level in the hyper. Gatherings have fringe of typical esteem may be because of the primary analysis relying upon TSH level as opposed to T3. As specified by Reid and Wheeler ( 2005) they said that , Because the thyroid's capacities are confused, this single test may not give any complete answers about what isn't right , However, anomalous outcomes can help point them the correct way. So play out a T4 or TSH test to pick up a clearer photo of the thyroid capacity. This outcome may be demonstrate that the aftereffect of T4 as like as T3 in which that the unusual outcomes can help point them the correct way yet more essential than T3. (Tooliss and Eastman , 2014). Estimation of aggregate T4 gives a dependable impression of clinical thyroid status without protein-restricting irregularities and nonthyroidal disease. Be that as it may, changes in restricting proteins can happen that influence the level of aggregate T4, yet leave the level of unbound hormone unaltered.( Wilson et al.,1999). Thyroid brokenness demonstrate that strange TSH level , as a high in ( Hypo. ) and Low in ( Hyper.) may be utilized to affirm the determination of all patients in the present examination and may be demonstrate that high TSH proposes a thyroid that is underactive (hypothyroid) and not doing its activity of creating enough thyroid hormone. As delineated by (Bahn, R. et al., 2011) they said that , the overabundance TSH is attempting to fortify the thyroid organ to create more thyroid hormone .additionally demonstrate that thyroglobulin antibodies (Tg Ab) are diminished in hypothyroidism patients' since this counter acting agent is invulnerable proteins delivered that objective thyroglobulin and thyroid tissue is the main wellspring of flowing Tg Ab. Serum Tg levels might be high in thyrotoxicosis, thyroiditis, iodine insufficiency, and amiable thyroid adenomas and additionally in thyroid malignancy ( Spencer CA, et al.,1999). Hence, it isn't a screening test for thyroid malignancy; as a result of the thyroid follicular cells, Tg levels are not expanded in medullary or anaplastic thyroid carcinomas ( Spencer. et al ., 2014). Serum thyroglobulin testing is touchy (91%) and particular (99%) for recognizing patients with relentless or repetitive separated thyroid tumor. Serum thyroglobulin levels are most exact when patients are hypothyroid (high TSH) and might be questionable in patients with subterranean insect thyroglobulin antibodies. We prescribe TSH-empowered thyroglobulin testing for all patients after aggregate thyroidectomy for separated thyroid tumor of follicular cell source paying little heed to tolerant age or hazard gathering (Duren. et al., 1999). The Thyroglobulin and Anti-Thyroglobulin – Ab are imperative in hypo and hyperthyroidism patients yet the Anti-thyroglobulin-Ab is more essential in hypothyroidism finding than Thyroglobin , on the grounds that the last one is ordinary item in thyroid organ however the Anti-Tg-Ab is lifted hypothyroidism because of immunological reason. Against thyroglobulin immunizer (hostile to Tg) was first exhibited as an autoantibody in the serum of patients with Hashimoto's thyroiditis and this discovering first settled the idea of organ-particular immune system sickness (Tozzoli R.et al., 2006). Autoimmunity

happens when antibodies against TPO cause continuous pulverization of follicles in the thyroid organ, and decline osmosis of thyroid hormones into the cells (Guo . et al., 2001) and (Roddiger SJ, et al.,2002). The height in the immune system hyperthyroidism in light of the fact that the nearness of hostile to TPO antibodies means that the insusceptible framework isn't working appropriately , these antibodies speak to the body mixing up the thyroid organ as "foe" tissue and an endeavor to pulverize that tissue , Anti-TPO-Ab hoisted can likewise allude to other immune system thyroid condition, for example, (Graves' illnesses). (Müssig et al., 2012 ) . The phosphorylation province of Synapsin I was appeared to control the arrival of neurotransmitter in the squid monster axon . The proclivity of phosphorylated Synapsin I for both actin and synaptic vesicles is diminished and calms the imperative on the synaptic vesicles. This outcomes in increments in the quantity of synaptic vesicles in the pools accessible for combination with the cytoplasmic film and arrival of neurotransmitter in which that influenced certain organs, for example, thyroid tissues ( Michael RR 2017). Synapsin I impacts synaptic pliancy by controlling pre-and post-synaptic vesicular discharge. Changes in Synapsin I have been connected to balance of thyroid hormone discharge , and conduct issue, which is a neurologic issue described by factor mixes of epilepsy, learning challenges, macrocephaly, and forceful conduct (Garcia et al. 2004). Certain investigations in which that proposed the TTR may have a urgent part amid early improvement, TTR is the main thyroid conveyance protein ( THDP) incorporated also in the CNS (Schreiber G and Richardson SJ 1997). Each TTR subunit has type of symmetrical  $\beta$ -barrel structure with a twofold trumpeted hydrophobic channel that navigates the particle shaping the two iodothyronines restricting destinations; TTR more often than not ties just a single T4 atom in light of the fact that the coupling liking of the second site is extraordinarily decreased through a negative agreeable impact. The TTR tetramer can tie four atoms 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 (Samuel Refetoff, 2015) . Distinctive ailment related changes have been found in TTR quality , with variable indication. Most ordinarily, they prompt amyloid testimony in the cardiovascular tissue as well as fringe nerves, without influencing the status of thyroid hormones ( Cooper *et al.*, 2017).

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