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REVIEW

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Different novel biomarkers involved in diagnosing hypothyroidism

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Abstract

Hypothyroidism is a clinical condition caused by the deficiency of thyroid hormones that are T_4 and T_3 due to an increase in serum TSH level. The upper and lower limit of T_4 and TSH helps to identify the disease. The metabolic pathways are important to know for diagnostic tests. By conducting different biochemical tests, a proper diagnosis can be performed when hypothyroidism is suspected clinically. Previously, many assays were performed just to detect the disease but recent tests are in both direct and indirect categories. Direct tests are purposely enough to detect the deficiency of thyroid hormones just like T_3, T_4, serum TSH, free-T_4, free-T_3, T_4 resin uptake, free T_4 index, T_4 binding globulin, and anti-TPO. All these tests are performed considering the reference ranges of hormones and the discovery that lower and higher TSH readings, even within the standard range, could predict future hypothyroidism, respectively, while the connection is stronger for hypothyroidism, supports the significance of diagnosing moderate thyroid status problems. If needed, one can also consider the radiological test that is a radioactive iodine uptake test. Other biochemical tests are considered as indirect because these tests actually confirm other changes in the body due to hypothyroidism such as lipid profile tests (TC, TAG, HDL-C, TC/HDL-C, and TAG/HDL-C), cytokine tests (interleukin-6, TNF-α, visfatin, and leptin) and other regular tests like iron deficiency test, hemoglobin test, ferritin, and TIBC. This article carries brief information regarding all the tests mentioned above and their purpose of conduction in hypothyroidism disease.

Keywords Hypothyroidism, Tests, Diagnosis, Thyroxine, Triiodothyronine

Introduction

Endocrine disorder and particularly thyroid diseases are the subject of argument. Though it is common among various endocrine disorders, the term specifies the causes related to the immune system, management, prevention, and treatment [1]. With the exception of some areas of India that are endemic for iodine-deficient illnesses, the profile of thyroid problems seen in children and adolescents is comparable to that seen in most other parts of the world. A projection based on numerous studies on thyroid problems indicates that approximately 42 million people in India have one or more thyroid abnormalities. The overall prevalence of thyroid disorders shown by studies is 1.9% hyperthyroidism, 5.4% hypothyroidism, and 7.5% of autoimmune thyroiditis [2]. Among the five main thyroid diseases, hypothyroidism has a large variance in clinical presentation and usual manifestation. Hypothyroidism is a clinical condition that is caused by a deficiency of circular thyroid hormones (T4 and T3) due to functional or structural changes in the thyroid gland. Either mild or subclinical in condition, untreated hypothyroidism can be severe at last [3]. According to a 2016 study about hypothyroidism, different lower and upper limits of 0.65–3.81 mIU/L and 0.27–4.87 mIU/L for the same population were determined using the Hoffman and Tukey statistical methods, respectively. Though there is a matter of discussion about whether thyroid dysfunction should be classified using the current reference levels

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for TSH and free thyroxine or not. The fact that the reference ranges are typically utilized as a treatment threshold makes this issue of clinical significance [4].

In most cases, hypothyroidism develops slowly, and symptoms might appear later in the course of the condition. Though women are more prone to hypothyroidism than men, the symptoms are far similar in both. According to previous reports, women have a lifetime risk of 3.5% and males have a risk of 1.0% of getting overt hypothyroidism. The most prevalent symptoms, which are often non-specific, are weariness, cold intolerance, and constipation. Numerous studies that also found a higher prevalence of hypothyroidism of symptoms like exhaustion or tiredness, shortness of breath, dry skin, mental anguish, and constipation in women corroborate the findings of a higher level of complaints in the female normal population [5]. Since symptoms are not unique to hypothyroidism, there is a wide range in clinical presentation and the presence of symptoms has a low sensitivity and positive predictive value for diagnosis [6, 7].

In order to view diagnostic purposes, the production, regulation, and metabolism are important parts that should be considered. Thyroid hormones are produced by the thyroid gland and regulated strictly via the hypothalamus–pituitary–thyroid axis. By briefing the production, thyrotropin-releasing hormone (TRH) is produced by the hypothalamus and travels through the hypophyseal portal circulation to the anterior pituitary. The thyroid gland's follicular cells' own receptors are activated by the secretion of TSH, which is stimulated by the activation of TRH receptors. Activating the enzyme thyroid peroxidase results in greater cellular uptake of iodine from the blood, increased synthesis of thyroglobulin, and secretion of triiodothyronine (T3) and thyroxine (T4) into the bloodstream (TPO) [8]. A variety of pathways consists of the metabolism of prohormone, i.e., thyroxine for circulating thyroid hormone leading to triiodothyronine production, deactivation of both hormones (T_3 and T_4), and excretion process of thyroxine with subsequent metabolites. The main pathways of metabolism of thyroid hormone are deiodination, glucuronidation, sulfation, and ether-link cleavage. Deiodination is the pathway in which for the availability of T_3, thyroxine is converted peripherally with the help of the deiodinase enzyme which removes the iodine atom from the thyroid isoform ring [9]. Glucuronidation involves the thyroxine hormone undergoing the conjugation of the phenolic hydroxyl group with glucuronic acid and resulting in T4/T3-glucuronide (T4G/T3G) production as a byproduct of this coupling reaction, which is catalyzed by UDP-glucuronosyltransferase (UGTs) with UDP-glucuronic acid (UDPGA) as a cofactor. The water solubility of compounds as a result of glucuronidation of thyroid hormone increases making them easier to pass through the gut and bile [10]. Sulfation involves the phenolic hydroxyl group conjugated with sulfates which further results in the catalysis of a reaction by the enzyme sulfotransferase. It is important for the preference of 3,3'T_2 in humans. Several new therapeutic targets for metabolic illnesses have emerged as a result of the function of TH in controlling metabolic pathways, and another thing is if the metabolism of thyroid hormone gets affected by the influence of disease, then it changes the normal functions in the human body as well which makes the diagnosis easier [11].

Although the diagnosis was confirmed by the presence of symptoms including bradycardia, low basal metabolic rate, and delayed ankle reflexes, both moderate and severe forms of hypothyroidism were probably underdiagnosed. Many assays were also being used to confirm thyroid disorders. In fact, the root causes were identified with risk factors [12]. Later by following the physiology of the thyroid gland, it was found that thyroid hormones actually play a key role and its deficiency as well as excess may affect organs which are the kidney, bone, liver, heart, and intestine. Currently, the most common method for determining TH status is to measure serum thyroid-stimulating hormone (TSH), although it is debatable if TSH accurately reflects TH levels across the entire body [13, 14]. And not only in accordance with the risks related to thyroid hormone but also like atherosclerosis, depression of metabolism symptoms are considered for testing. By conducting different biochemical tests, a proper diagnosis can be performed when hypothyroidism is suspected clinically. The aim of this review is to highlight the various biomarkers related to hypothyroidism discussed in detail.

Different biomarkers for confirming hypothyroidism

Biochemically, due to a high level of TSH from the pituitary in primary hypothyroidism, there is a hyposecretion in the concentration of T_4 and T_3 with raise in serum TSH [15]. Different bodily processes are regulated by thyroid hormones, just as increasing heat generation and resting metabolic rate, have an impact on cell division and growth and control how other hormones are responded to, and changes in proteins, carbohydrates, and fats metabolization [16] (Tables 1 and 2).

Thyroid profile

For determining the changes in the level of hormones, accurate diagnosis is way essential through laboratory tests. It is due to subtle symptoms of thyroid dysfunction in most of the patients and so hormone tests or thyroid

Table 1 Summary of different biomarkers

Biomarkers		
Variables	**Tests**	**Action**
1. Thyroid profile	T_3 (nmol/l)	All tests confirm the changes in hormones affecting normal thyroid function
	T_4 (nmol/l)	
	TSH (mIU/L)	
	FT3 (ρmol/l)	
	FT4 (ρmol/l)	
	T_3 resin uptake	
	Free T_4 index	
	T_4-binding globulin	
	Anti-TPO	
	Radioactive iodine uptake	
2. Lipid profile	TC (mg/dl)	Reduced activity of HMG-CoA reductase shows the changes in cholesterol levels in the body due to hypothyroidism
	TAG (mg/dl)	
	HDL-C (mg/dl)	
	TC/HDL-C	
	TAG/HDL-C	
3. Cytokines	IL-6	Confirming changes in autoimmunity of thyroid
	TNF-α	
	Visfatin (ng/ml)	
	Leptin (ng/ml)	
4. Others	Hb	Hemoglobine level
	Iron and zinc	For iron and zinc deficiency
	Ferritin	For iron
	TIBC	For iron

Table 2 Reference ranges of hormone profile tests

Tests	**Range**
T_3	0.92–2.78 nmol/L
T_4	58–140 nmol/L
TSH	0.5–4.7 mIU/L
FT_3	0.22–6.78 pmol/l
FT_4	10.3–35 pmol/l
T_3 resin uptake	24–37%
Free T_4 index	4.8–12.7 mcg/dL
Thyroxine-binding globulin	150–360 nmol/L
Anti-TPO	0–65 IU/ml

function tests are being performed to measure in peripheral blood [17].

T3

It is the more active hormone intracellularly processed to maintain stable plasma T3 levels. About 0.2 to 0.3% of T_3 circulates in its free form or unbound state in blood plasma. T_3 is carried by thyroxine-binding globulin whereas a minor fraction is coupled to transthyretin [18]. The levels of T_3 are less convenient in diagnosing hypothyroidism because according to the conversion from T_4 to T_3, increased levels of T_4 serum levels within its normal range always show a maintained level of T_3 until the severe condition of hypothyroidism. Patients with TSH-secreting pituitary tumors and those with TH syndrome resistance frequently have high T3 levels. It is measured in nmol/l [19, 20].

T4

The thyroid gland's main hormone to be secreted is T4. The creation of an indirect total T4 test in the 1950s marked the beginning of thyroid function testing. The peripheral conversion of T4 via 5′-mono-deiodination in diverse tissues accounts for about 80% of the serum T3 levels. So it can be said that it is a barely active form that has to be converted into T_3 for exerting its action. Only 0.02% of T_4 and 0.2% of T_3 circulate in the bloodstream free; the majority of thyroid hormones are linked to plasma proteins [21, 22]. Utilizing radioimmunoassay, serum total T_4 concentration is determined. T_4, a tiny molecule, cannot be utilized as an antigen on its own; however, antibodies can be generated utilizing it as a hapten coupled to albumin or as the native thyroglobulin

conjugate. Generally speaking, the dextro isomer of T4 binds to antibodies just as well as the naturally occurring levo isomer, although it is not detectably present in biological fluids [23]. Overt hypothyroidism, iodine deficiency, decreased energy expenditure, weight gain, and elevated cholesterol are all linked to low T_4 [24]. L-thyronine, 3 monoiodothyronine, monoiodotyrosine, diiodotyrosine, and 3,5 L-diiodothyronineare derivatives or precursors of T4 barely react with the antibody. The T4 antibody interaction is mainly unaffected by iodide. I-T4 is used to designate the antibody that binds to T4, and procedures used in all immunoassays are used to separate the bound from the free. Females typically have a slightly greater concentration of total T4 than males, with the range of the radioimmunoassay being between 5 and 12 g/dl. The intra-assay coefficient of variation is around 5%, and the inter-assay variation is around 7% [25].

TSH

It is secreted by the anterior pituitary gland and an important hormone for thyroid function because by activating receptors of its own on follicular cells present in the thyroid gland, it increases intracellular delivery of iodine from blood with the secretion of T_3 and T_4 (with the activation of enzyme thyroid peroxidase). The most important feature of the control of thyroid function with regard to the diagnosis of thyroid disorders is the link between the magnitude of changes in serum TSH and the consequent magnitude of changes in circulating thyroid hormones [26]. According to the current status for treatment and diagnosis of hypothyroidism, it is the most sensitive and specific marker of systemic thyroid status with test findings being interpreted in accordance with established reference ranges [27]. Thyroid-stimulating hormone (TSH) is used by many medical professionals as the initial screening blood test for patients suspected of having thyroid disorders like for identifying hypothyroidism, and it is because TSH plays a key role in the negative feedback system which results in substantial alterations in its secretion when there is even a small change in function of the thyroid. The chemiluminescent assay of TSH can currently detect the levels as low as 0.1 mU/L and elevated as well [28, 29]. 0.5–4.5 mU/L is the normal range, but assuming a lower limit of the reference range has been 0.3 mIU/l and the upper limit of the reference range has been estimated from 2.1 to 7.5mIU/l [30].

FT3 and FT4

The free form or the unbound form of T_3 and T_4 are way more relevant than the levels of total T_3 and T_4 due to its active state and independent of thyroid status. In an adult human being, the range of normal values for FT_4 is from 13 to 39ρmol/l [31]. Triiodothyronine that is not attached to any other molecules is measured by the compound FT3. It is helpful in detecting hyperthyroidism or thyroxine overproduction in women who are expecting or on any potent medications that alter TBG, such as estrogen [32]. To evaluate thyroid function and track hyper- and hypothyroidism therapy, TSH and FT4 are frequently employed. TH transporter expression alterations do not affect FT4, and it has very little intra-individual variation [18]. The reference limit of FT_3 in adults is 35 to 77ρmol/l and for FT_4 is 9 to 23ρmol/l. By using the absolute direct method of equilibrium dialysis, the upper value of normal for FT_4 is 32ρmol/l [33, 34].

T3 resin uptake

The triiodothyronine resin uptake (T_3 uptake) or thyroid hormone binding ratio is a method for empirically quantifying thyroid hormone binding sites in unsaturated serum proteins. Less T_3 is taken up by the erythrocytes in hypothyroidism because there is an increase above normal in the unsaturated protein-binding sites [35]. The T3RU is a proximate indicator of TBG binding strength. The resin uptake is high if there is a low level of TBG and vice versa [36]. An aliquot of the patient's serum that has a trace amount of 125 1-labeled T3 is combined with a solid phase T3 resin binder that has a relatively low affinity. The thyroid-binding protein's quantity or composition, as well as variations in the blood's level of thyroid hormone, have an impact on the T3 absorption. However, unless the substance supplied impacts thyroid hormone protein binding or alters the patient's thyroid status, it should not be impacted by the delivery of organic or inorganic iodine to a patient. It has been demonstrated that the P.B.I. xT3 uptake index has great relevance in determining the thyroid function of thyrotoxic patients receiving 131I [37].

Free T4 index

Total T4 and T3 resin uptake can be used to calculate the free T4 index. The amount of labeled thyroid hormone that binds to an insoluble substance (like resin) is known as T3 resin uptake, which indirectly measures the amount of thyroid hormone bound to blood proteins [38]. The T3RU has been utilized to adjust total T4 since changes in the T3RU brought on by binding abnormalities are inconsistent with changes in T4. For calculating the corrected free T_4 index, a formula has been in process:

$$Free\ T_4\ index = Total\ T_4\ sample \times \frac{T_3RU\ sample}{T_3RU\ mean\ control}$$

Though it is unitless, the T_4 and T_3RU aid to get the values which are having its unit. In hypothyroidism, lower T_4 and lower T_3RU state the condition [39].

Thyroxine-binding globulin (TBG)

The TBG blood test detects your body's level of a protein that transports thyroid hormone. Thyroxine-binding globulin is the name of this protein (TBG). Human TBG radioimmunoassay has been shown to be sensitive and specific. The assay offers ease by needing less than 10 µl of serum and enabling the testing of 100 samples at once. It is especially well suited for population investigations of variation in TBG concentration because of these benefits [40]. The highest TBG binding capacity for T4 was found to be proportional to the TBG concentration as assessed by immunoassay and was tested by reverse flow electrophoresis of whole serum. All the confirmatory tests using FT_4, TSH, and TBG help to diagnose thyroid dysfunction [41, 42].

Anti-TPO

Thyroid autoantibodies are the defining feature of thyroid autoimmunity. Autoantibodies have demonstrated useful outcomes as early diagnostic markers in several diseases in recent years. Nevertheless, thyroid autoantibodies are only frequently assessed when abnormalities in thyroid hormones, particularly FT4 and TSH, are discovered. However, their presence even before the principal marker, the TSH marker, has not been recognized [43, 44]. Two promising methods for detecting anti-TPO antibodies are radioimmunoassay and enzyme-linked immunosorbent assay (MELISA). With thyroiditis, increased levels of anti-TPO are usually seen [45].

Radioactive iodine uptake

In the past, measurements of thyroidal radioactive iodine uptake (RAIU) were frequently used as a gauge of thyroid activity. The requirement for in vivo radioiodine tests was significantly reduced after the development of accurate technologies for determining serum thyroid hormone and TSH concentrations, and many people now wonder whether in vivo RAIU measures are still required for the evaluation of thyroid function. However, at least in Europe, about two thirds of thyroid clinics continue to perform RAIU measurements [46]. While diagnosing, the test helps to get numerous values mainly when patients are of hypothyroidism. And the variability actually depends on the severity of the disease. Though thyroid hormone tests have significance more than RAIU, it acts as a direct indicator to show the activity of the thyroid gland [47]. It was discovered that patients with reversible hypothyroidism and those with irreversible hypothyroidism could be distinguished from one another using the RAIU test. RAIU values change in a healthy person according to the thyroid gland's iodine reserves, which are directly influenced by the intake of iodine for a long time duration. The RAIU values are anticipated to vary as a result of regional variations in dietary iodine content. In order to correctly diagnose various thyroid diseases and correctly interpret laboratory results, it is crucial to establish local RAIU reference values [48, 49]. The test is somewhat similar to a thyroid scan, but it has its procedure: first, a pill with a very little amount of radioactive iodine is administered, and after ingestion, one must watch as the iodine gathers in the thyroid. Usually, 4 to 6 h after taking the iodine pill, the first uptake occurs. A second uptake is often performed 24 h later, and a device known as a gamma probe is used over the area where the thyroid gland is located. That probe measures the strength of the radiation emitted by the radioactive substance and confirms the quantity of tracer absorbed by the thyroid by showing on the screen [50].

Lipid profile

Thyroid hormones control the metabolism of lipids and cholesterol, whereas thyroid conditions, such as overt and subclinical hypothyroidism (SCH), significantly affect the lipid profile and increase the risk of cardiovascular disease. By directly interfering with lipid metabolism, it is seen that hypothyroidism patients have a high risk of atherosclerosis in which usually weight gain as a symptom can be observed easily. A decrease in low-density lipoprotein (LDL) receptors and a weakening of T_3's control over SREBP2, which is necessary for the development of LDL receptors, are the main contributors to hypercholesterolemia in hypothyroidism [51]. The thyroid hormone's impact on bile acids has lately been recognized as having a distinct hypocholesterolemic effect. Hypothyroidism shows multiple changes like decrease the binding of LDL to its receptor, reduce degradation of LDL in fibroblasts, increase the half-life of LDL, reduce the number of receptors, reduce the expression of mRNA of receptors, and increase the residence in serum [52, 53]. Hyperlipidaemia, a constant biochemical characteristic of hypothyroidism, is said to cause high levels of LPO.

Increased level of total and low-density lipoprotein cholesterol, higher plasma-oxidized LDL-cholesterol levels, and a slight change in serum high-density lipoprotein cholesterol causes cardiovascular abnormalities as a complication in hypothyroidism. Blood lipid and lipoprotein levels may also be negatively impacted by high-normal serum TSH readings [54]. As a useful marker, it can be used to identify the effects of thyroid hormone shortage at the tissue level in patients with overt as well as mild thyroid failure. There is mounting evidence that greater TSH levels are linked to deteriorating cholesterol and blood pressure levels. Over the reference range, this difference is similar to between 33 and 50% of the blood pressure change seen with antihypertensive monotherapy, with an increase in both systolic and diastolic blood

pressure of roughly 2 mm Hg per 1 mU/L elevation in TSH. These connections are also shown in youngsters, demonstrating the long-term effects of TSH on cardiovascular risk factors [55, 56].

Total cholesterol

It is measured by an enzymatic colorimetric cholesterol esterase method. The normal level of total cholesterol is about > 200 mg/dL, and the range should be like 200 to 239 mg/dL. A high cholesterol level indicates a higher risk of cardiac disease. This test confirms the total cholesterol in the body and indirectly confirms the change in the level of TSH in the body [57, 58].

Triacylglycerol regioisomers

TAG presents in fat-lipid droplets which is responsible for the storage of fat and indicates mainly the existence of high lipid in the body. High TAG in plasma indicates the risk of cardiovascular diseases [59].

High-density lipoprotein

A lower risk of cardiovascular disease is frequently indicated by high levels of HDL cholesterol. HDL cholesterol levels less than 40 mg/dL are regarded as lower than desired, whereas values of 60 mg/dL or greater are considered excellent. There are no medication that decreases your chance of suffering a cardiovascular event by increasing HDL levels [60]. Low HDL cholesterol frequently coexists with high triglyceride levels, particularly in insulin-resistant people. There could be a mechanism whereby HDL particles that are present in high triglyceride concentrations are more easily metabolized [61].

TC-HDL

The normal value of the cholesterol ratio should be below 5:1 [62].

TAG/HDL

This value can be found with the help of high-density lipoprotein and triacylglycerol regioisomer.

Reduced receptor activity, which results in less lipoprotein catabolism, is the cause of the rise in cholesterol levels. The decrease in HDL levels in hypothyroid patients is brought on by increased hepatic lipase and CETP (cholesteryl ester transport protein) activity. Similar to how decreased lipoprotein lipase activation causes increased TG levels in hypothyroid individuals due to impaired breakdown of TG-rich lipoproteins. Apolipoprotein AV is elevated as a result of thyroid hormone (ApoAV). ApoAV is crucial to the control of TG. Levels of TGs may potentially be explained by decreased ApoAV activity in hypothyroidism [63].

Cytokines

A diverse set of polypeptides known as cytokines have a variety of effects on cells other than immune cells in addition to being crucial in initiating and directing inflammatory, and immune responses. The majority of cytokines originates from diverse biological origins, including immune and non-immune cells, and frequently functions in an autocrine or paracrine manner. As a result, they rarely reach measurable levels in circulation [64]. The idea of a cytokine network was developed as a result of the fact that many cytokines have overlapping roles or have effects that are either enhanced or decreased by other cytokines. The defining feature of autoimmune thyroid disease is lymphocytic infiltration of the thyroid, and various studies have investigated the cytokine profile in thyroid disease using immunohistochemistry or analysis of mRNA isolated from entire tissue retrieved during surgery [65, 66].

Interleukin 6

IL-6 has a significant impact on metabolism, weight, and sleep. Multidisciplinary studies combining researchers from the fields of endocrinology, cardiology, immunology, and sleep disorders must address the substantial correlation between IL-6, obesity, and sleep apnea (with potential repercussions on the cardiovascular system) [67]. It is crucial to the development of the euthyroid ill syndrome because it inhibits the enzyme 5′-deiodinase, which turns T4 into T3 and T3 back into diiodothyronine. Additionally, it has been demonstrated that IL-6 affects intermediary metabolism, secretion of the hormone is regulated by the circadian rhythm of sleepiness, and acute sleep loss stimulates its production [68]. Usually, this test is performed in the presence of lowering T3 and increasing IL-6 at the same time [69].

TNF

Initially, tumor necrosis factor (TNF) was thought to cause tumor necrosis following a bacterial infection. Nevertheless, this cytokine also has a role in fever, septic shock, replication, and inflammation [70]. TNF-α stimulates lipolysis and decreases the transport of fatty acids into adipocytes, which causes an increase in circulating free fatty acids that may induce insulin resistance. So indirectly, it should be tested in obese or weight-gaining patients. In most of the metaanalysis studies, it is shown that TNF-α is seen as normal while testing in hypothyroidism patients [71].

Visfatin

Nicotinamide phosphoribosyltransferase or visfatin which is secreted through lymphocytes is functionally a

mimic of insulin, and it is expressed as a 52-kDa cytokine. It helps to reduce glucose levels by binding to insulin receptors and stimulates the utility of glucose [72, 73]. While previous studies showed that T3 was reported to inhibit visfatin mRNA expression in 3T3-L1 adipocytes, experimental studies have shown conflicting results suggesting that T3 could accelerate adipocyte differentiation with the elevation of visfatin levels [74]. Patients with hyperthyroidism had visfatin levels that were substantially lower than those with hypothyroidism. Individuals with hypothyroidism experienced a significant decrease in plasma visfatin levels following treatment, but patients with hyperthyroidism experienced a considerable increase. Visfatin levels were found to have a substantial negative association with fT3 and fT4 values and a significant positive correlation with TSH levels [75].

Leptin

As a peptide hormone, leptin is expressed as a 16-kDa cytokine secreted from fat cells called as adipocytes. It functionally regulates the body weight and in inflammation. It actually affects the weight of the body and not linked with any type of disease [76]. Patients with autoimmune diseases show that elevated serum leptin levels may either cause the disease or act as a diagnostic sign in clinical settings. Leptin's potential as a therapeutic target for the treatment of autoimmune illnesses in humans is still being determined [77]. According to earlier research, a rise in blood leptin levels may be used to prevent weight gain brought on by hypothyroidism rather than only reflecting changes in body weight as a result of hypothyroidism [78].

Other tests

Different other parameters to measure for the assessment of iron, ferritin, selenium, zinc, and other trace elements are considered in the tests of thyroid dysfunction. As the selenium deficiency is observed and by treating the patient with oral administration of sodium selenite, the level returns to normal [79]. The biosynthetic enzyme for thyrotropin-releasing hormone has been found to require zinc as a cofactor, and according to some current studies, it has been found that the serum zinc level decreases in hypothyroid patients so zinc deficiency actually affects thyroid hormone function [80, 81]. If iron is tested then total iron binding capacity is checked with lower hemoglobin in the blood [82]. Though the lower level of iron is directly related with anemia and ferritin as it is its storage form, mostly, iron test is enough for confirming deficiency as a complication [83, 84]. The TIBC test is as same as iron and ferritin so the normal range in this test is 240 to 450mcg/dL [85].

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