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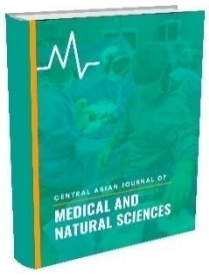

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Analysing the Levels of Various Biochemical Markers (T3, T4, and TSH) in Iraqi Patients with Thyroid Problems

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Key words: Triiodothyronine (T3) and Tetraiodothyronine (T4), Thyroid Stimulating Hormone TSH.

Abstract:

Introduction: Around the world, thyroid disorders are rather common. Thyroid disease is also very prevalent in Iraq. With increased awareness, thyroid illness is being identified more frequently. Although men are not exempt from this disease, women are more affected by chronic non-communicable diseases than men are.

Methods: A total of 100 people (40 controls and 60 patients) from various age groups (51-55) and (56-60) were chosen as the study subjects.

The study's primary goal was to examine blood indicators that can be used to gauge the severity of a thyroid disease.

Result: T3, T4, and TSH levels in the patients' serum were the relevant markers. These are all discovered to have a favorable correlation. The levels of T3 and T4 typically rise when TSH levels fall. The study's goal was to make this discovery, and the parameters were measured in line with that goal. By using a solid phase competitive chemiluminescent immunoassay, the levels of T3 and T4 were examined. Sandwich paramagnetic chemiluminescent immunoassay was used to examine TSH levels.

The findings were found to be significant, particularly for the age categories of 51-55 and 56-60. With the TSH levels declining, it was discovered that the T3 and T4 levels were dramatically rising. The statistical tests back up this conclusion.

Conclusion: The of examine blood indicators (T3, T4, TSH) can be used to gauge the severity of a thyroid disease.

Introduction

The thyroid gland is a component of the endocrine system. It is situated in front of the lower neck and has two lobes, which are connected by a thin band of tissue called an isthmus *(Weetman AP. et al., 1997).*

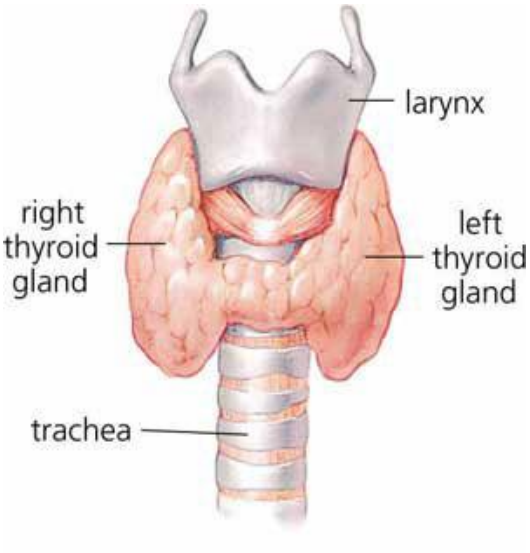

Figure 1: Thyroid gland *(Sacher, et al., 2000)*.

Heart rate, blood pressure, body temperature, and the rate at which food is metabolized into energy are all controlled by hormones that the thyroid gland produces and stores. All bodily cells depend on thyroid hormones to function properly. They aid in controlling metabolism (chemical reactions) and growth in the organism. Children's growth and development are also aided by thyroid hormones *(Sacher, et al., 2000).*

The thyroid gland is positioned around the trachea (windpipe) at the lower portion of the neck, below the Adam's apple. It is shaped like a butterfly, with two wings (lobes) joined at the isthmus in the center (**Figure 1**) *(Kariyawasam, D., et al., 2015).*

The thyroid gland's spherical follicles selectively collect iodine, which is then used to create the iodine-containing hormones thyroxine (T4) and tri iodothyronine (T3). The parafollicular cells (C-cells) situated between the follicles secrete calcitonin, a different hormone. The thyroid hormones are necessary for homeostasis, metabolic functions, and appropriate growth and development in children. Reduced production or quality of these hormones causes one or more organ systems to malfunction and causes mental impairment in children. Production and activity of T3 and T4 The follicular cells produce thyroxine from the tyrosine residues of the protein thyroglobulin (TG) *(Inoue, K., 2015).*

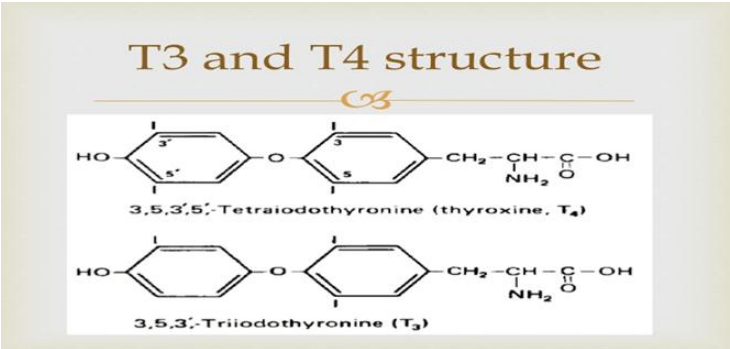

Figure 2: Structure T3 and T4 *(Inoue, K., 2015).*

Iodine that has been trapped via a "iodine trap" is attached to the 3' and 5' locations of the benzene ring of the tyrosine residues on TG by the enzyme thyroid peroxidase (TPO) (Figure 2). The follicular cells are stimulated by TSH to reabsorb TG and proteolytically cleave the iodinated tyrosine from TG, joining it with T4 (T3 has one less iodine than T4), and releasing T3 and T4 into the blood. T4 and T3 are partially bind to albumin, transthyretin, and thyroxine-binding globulin in the blood. Hormonal activity only exists in the free fraction, which is not bound to these proteins *(Inoue, K., 2015).*

The creation of the iodine-containing hormone thyroxine, which is crucial for the metabolism and development of the animal world, requires iodine. Iodine deficiency in the diet can result in endemic goiter and gland enlargement. thyroid conditions The thyroid gland releases thyroid hormones, which are controlled by the pituitary, which is controlled by the hypothalamus *(Mangiullo R, et al,. 2010).* Any of these organs or glands that are overactive produce too much thyroid hormone, or are underactive and produce too little thyroid hormone, which is known as hypothyroidism. The pituitary gland detects how much thyroid hormone is present in the blood and adjusts the thyroid gland's stimulation accordingly, either increasing or decreasing hormone synthesis *(Baskin, et al,. 2002).*

The following conditions commonly affect the thyroid :

1. Hypothyroidism

Occurs as a result of the thyroid gland's reduced activity, poor development at birth, partial or complete surgical removal, or inability to produce the hormone *(Synthroid, et al,. 2012).*

2. Hyperthyroidism

Graves' disease, an autoimmune condition where the body struggles to distinguish between its own tissues and organs and foreign organisms like germs, is the most frequent cause. *(Yue WS, et al,. 2011).*

3. Goiter

It is characterized by the thyroid gland enlarging as a result of Graves disease, hashimotos disease, nutritional inadequacies, etc. *(Erem C, et al,. 2010).*

4. Thyroid cancer.

Cancerous thyroid nodules are rare and more prevalent in younger persons, typically between the ages of 22 and 50 years.

5. Thyroiditis.

Swollen thyroid due to bacteria or virus is known as thyroiditis

(a) Subacute granulomatous thyroiditis: it is very painful around the neck, feeling of tenderness prevails *(Biondi B, et al., 2010).*

(b) Acute infectious thyroiditis: Staphylococcus or streptococcus bacteria are frequently to blame for this. It falls under the category of painful.

(c) Painless thyroiditis: 10% of hyperthyroidism is believed to be caused by this kind. In this case, the thyroid does not expand.

6. Hypothyroidism in pregnancy

Because hypothyroid women do not ovulate or do not generate mature eggs, it is uncommon. However, it raises the fetus's risk of stillbirth or growth retardation.

Adults may experience early signs such as easy weariness, exhaustion, poor tolerance to low temperatures, constipation, and carpal tunnel syndrome (wrist pain and numbness), as well as later signs such as poor appetite, weight gain, dry skin, and other conditions. loss of hair cognitive capacity declines, Depression, puffiness around the eyes, a deeper, hoarser voice, irregular or nonexistent menstrual cycles, and puffiness around the eyes. Hyperthyroidism signs and symptoms Insomnia, Hand trembling, anxiety, feeling overheated in mild or chilly weather frequent bathroom visits, Despite having a regular or heightened appetite, excessive perspiration the menstrual cycle becomes irregular or stops entirely, joint discomfort difficulty paying attention, Eyes seem to be growing bigger. checking for thyroid problems According to the definition of screening, it is "the application of a test to detect a potential disease or condition in a person who has no known signs or symptoms of that condition at the time the test is done." Therefore, screening with thyroid function tests may find asymptomatic people who have minor, nonspecific symptoms like cold sensitivity or feeling "a little tired." *(Brandt F, et al 2011).*

The development of consequences from both hyperthyroidism and hypothyroidism is thought to be at risk due to subclinical thyroid dysfunction, which can be identified before symptoms and complications show up. With the use of screening, patients with subclinical thyroid dysfunction can be found and treated before they experience severe consequences. Early kinds of thyroid malfunction progress to more severe ones. The method used most frequently today is to categorize patients based on the findings of thyroid function tests. Patients who have both a low thyroxine (T4) level and a high thyrotropin (TSH) level are said to have "overt hypothyroidism" in this categorization. Patients who have a low TSH and an increased T4 or triiodothyronine (T3) are said to have "overt hyperthyroidism." *(Thvilum M, et al,. 2012).*

To identify and treat subclinical thyroid impairment, screening is primarily justified. According to this justification, subclinical thyroid dysfunction is a risk factor for later consequences and may present with symptoms that can be managed with medication. Patients with a raised TSH and a normal thyroxine level are described as having "subclinical hypothyroidism" and "mild thyroid failure," respectively. *(Thvilum M, et al,. 2012*)

MATERIALS AND METHODS

Sample collection

Venous blood samples were obtained in test tubes using aseptic techniques. A blood sample was centrifuged for 5 minutes at 3000 rpm 2 hours later. The acquired serum was collected in a polythene tube with a stopper and kept at -20°C until it was tested. Separated serum or plasma should not be kept in a stored condition for longer than 8 hours in order to estimate TSH. Serum or plasma should be kept at +2 to 8°C if assays are not finished within 8 hours. The divided samples should be stored at -15°C to -20°C if assays are not finished within 48 hours. In order to avoid analyte degradation, frozen samples should only be defrosted once. For the TSH, 0.3 mL of serum is the absolute minimum. A 55 µl sample volume is required for each test.

Determination of T3

Principle

Within this test, the sample is introduced to a reaction vessel along with a stripping agent to liberate the T3 bound to proteins. To create an anti-T3 alkaline phosphate conjugate, the T3 in the sample competes with the T3 counterpart attached to biotin. The T3 analogue: antibody complexes are bonded to the streptavidin-coated solid phase of the resultant antigen: antibody complexes. The sample T3: antibody complexes and other substances not attached to the solid phase are removed by washing after these complexes are separated in a magnetic field. Following these divisions, the vessel is loaded with

the chemiluminescent substrate Lumi-Phos* 530, and the amount of light produced by the reaction is measured using a luminometer. The amount of total T3 in the sample, which is ascertained using a stored, multi-point calibration curve, is inversely proportional to the light output.

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Procedure

A functioning tracer reagent solution is added to the serum after pipetting it into the designated wells, and the mixture is then swirled. It is then combined with a biotinylated tT3-specific antibody conjugate solution. Working reagent solution is added to each well and incubated after swirling and incubation. Wash buffer is then added and decanted many times. After applying the substrate solution, the relative light in each well is measured after 30 minutes.

Determination of T4

Principle

A specific quantity of anti-T4 antibody is coated on microtiter wells for the T4 EIA. This is then combined with a set quantity of T4 conjugated and a measured amount of serum sample. T4 and conjugated T4 compete for the few binding sites on the anti-T4 antibody during incubation. The unbound T4 conjugate is removed from the wells by washing them five times with wash buffer after a 15-minute incubation at 37–40°C. Substrate A/B mixed reagent is added, and the mixture is then incubated for five minutes. Using a Chimiluminescence microplate reader, read the light units.

Procedure

The working conjugate reagent is added, the standards, sample, and controls are pipetted into the correct wells, properly mixed, and then incubated. Add the working substrate solution to each well after working wash buffer solution has been used to rinse and flick the microtiter wells. Following incubation, a chemiluminiscence microtiter is present in each well for the light units. Results are read 30 minutes after substrate addition.

Determination of TSH

Principle

Thyrotropin and human thyroid-stimulating hormone (hTSH) concentrations in human serum and plasma can be determined quantitatively using a chemiluminescent immunoassay. It is a two-site immunoenzymatic assay called the Access Hypersensitive hTSH assay. A sample was added to a reaction vessel together with buffered protein solution, goat anti-hTSH-alkaline phosphatase conjugate, and paramagnetic particles coated with immobilized mouse monoclonal anti-hTSH antibody. The goat anti-hTSH-alkaline phosphatase conjugate interacts with a distinct antigenic location on the hTSH whereas the immobilized monoclonal anti-hTSH on the solid phase binds to the hTSH. After incubation, the chemiluminescent substrate Lumi-Phos* 530 is applied, holding components that are bound to the solid phase in a magnetic field. A luminometer is used to measure the light produced. When the sample's complete concentration of human thyroid-stimulating hormone was introduced to a reaction vessel, light was created in a proportional manner.

Results

T3 marker investigation on people of various ages and sexes. Normative Interval: 0.87-1.78 ng/ml

Table 1: Sample groups with different age and sex.

Sample groups	Age group	T3 levels 0.87 - 1.78 (ng/ml)	Average
1	25-30	1.18 - 1.7	1.44
2	31-35	0.97 - 1.64	1.305
3	36-40	0.92 - 1.37	1.145
4	41-45	1.45	1.45
5	46-50	1.2	1.2
6	51-55	2.75	2.75
7	55-60	2.46	2.46

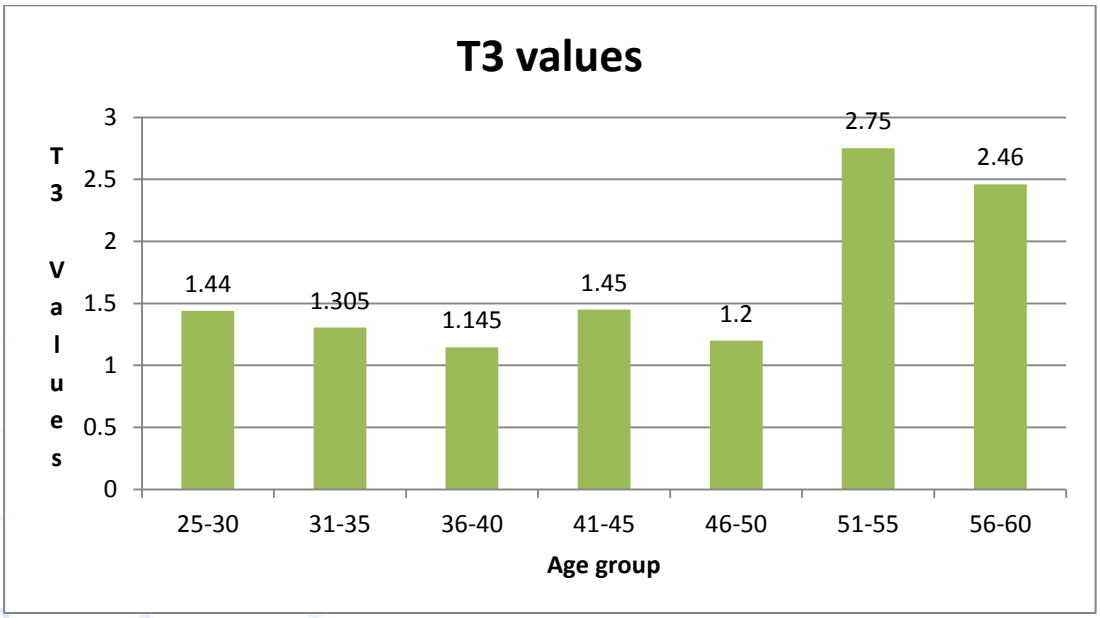

Figure 3: T3 values (ng/ml) of age groups samples.

Table 2 : Statistical analysis of table 1

t-Test: Paired Two Sample for Means		
	Variable1	Variable 2
Mean	4	1.6785714
Variance	4.6666667	0.4201726
Observations	7	7
Pearson Correlation	0.7147341	
Hypothesized Mean Difference	0	
df	6	
t Stat	3.4967502	
P(T<=t) one-tail	0.0064386	
t Critical one-tail	1.9431803	
P(T<=t) two-tail	0.0128771	
t Critical two-tail	2.4469118	

T4 marker investigation on participants of various ages and sexes.

6.09 to 12.23 ng/ml as a reference range.

Table 3: Sample groups with different age and sex.

Sample groups	Age group	T3 levels (ng/ml)	Average
1	25-30	6.34 - 7.04	6.69
2	31-35	6.5 - 7.63	7.065
3	36-40	6.16 - 7.01	6.585
4	41-45	6.74	6.74
5	46-50	6.23	6.23
6	51-55	12.77	12.77
7	56-60	13.21	13.21

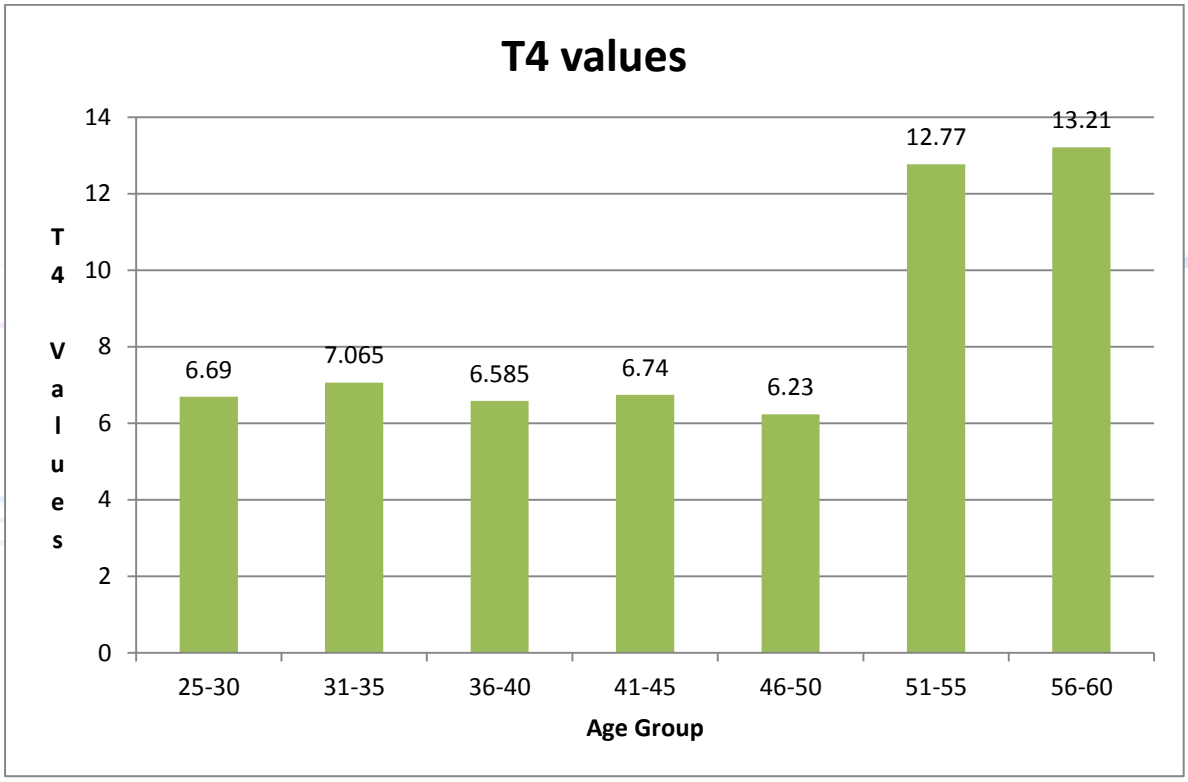

Figure 4: T4 values (ng/ml) **of different age groups samples.**

Table 4: Statistical analysis of table 3

t-Test: Paired Two Sample for Means		
	Variable 1	**Variable 2**
Mean	4	8.47
Variance	4.6666667	9.610625
Observations	7	7
Pearson Correlation	0.7619102	
Hypothesized Mean Difference	0	
df	6	
t Stat	-5.860533	

P(T<=t) one-tail	0.0005454	
t Critical one-tail	1.9431803	
P(T<=t) two-tail	0.0010908	
t Critical two-tail	2.4469118	

TSH marker investigation on participants of various ages and sexes. 0.34 to 5.6 ng/ml is the reference range.

Table 5: Sample groups with different age and sex.

Sample groups	Age group	TSH levels (ng/ml)	Average
1	25-30	1.17- 0.68	0.925
2	31-35	1.04- 2.78	1.91
3	36-40	2.4- 1.56	1.98
4	41-45	0.87	0.87
5	46-50	2.24	2.24
6	51-55	0.27	0.27
7	56-60	0.12	0.12

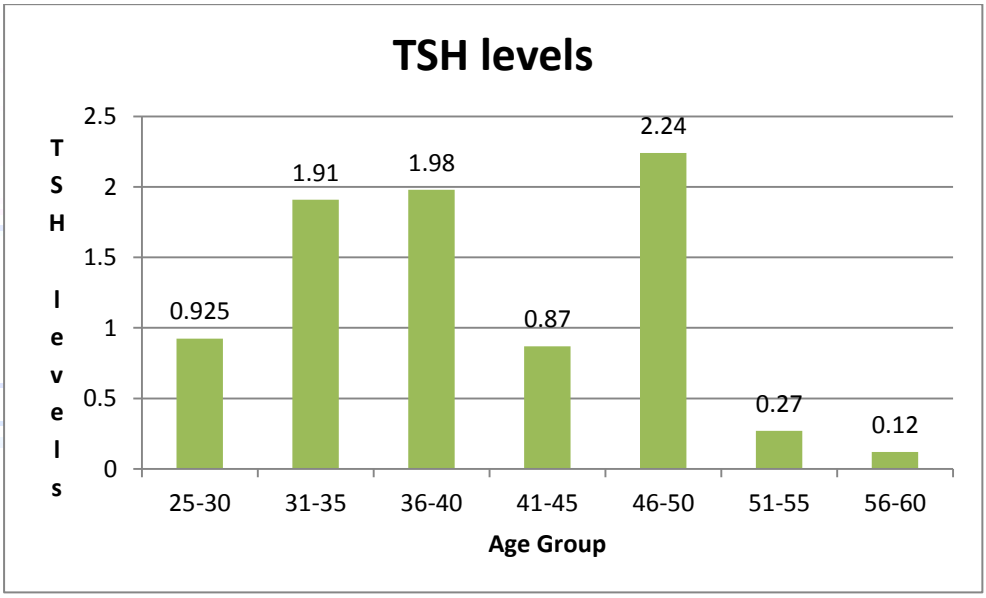

Figure 5: TSH values (ng/ml) of different age groups samples.

Table 6: Statistical analysis of table 5

t-Test: Paired Two Sample for Means		
	Variable1	**Variable 2**
Mean	4	1.1878571
Variance	4.6666667	0.7348155
Observations	7	7
Pearson Correlation	-0.489165	
Hypothesized Mean Difference	0	
df	6	
t Stat	2.7702818	

P(T<=t) one-tail	0.0162052	
t Critical one-tail	1.9431803	
P(T<=t) two-tail	0.0324104	
t Critical two-tail	2.4469118	

Discussion

Table (1) lists the serum T3 concentrations from the patients. According to the findings, age groups 51 to 55 and 56 to 60 have significantly greater levels of T3 markers than other age groups. Table (3) lists the serum T4 levels from the patient's samples. According to the findings, age groups 51 to 55 and 56 to 60 have significantly greater levels of T4 markers than other age groups.

In Table (5), serum TSH values are presented according to age groups. The TSH levels were found to be considerably higher in the age groups of 31 to 35 and 46 to 50, but lower in the older age groups of 51 to 60, table (6). Due to the pituitary's decreased TSH release with advancing age, serum TSH values fall in elderly healthy persons.

The exact process causing the decrease in TSH secretion is yet unclear. According to our research, serum TSH and T3 levels diminish with aging. The greater sensitivity of the thyrotrophesto negative feedback by T4 may be the cause of the decreased level of TSH secretion, but other factors, such as a decreased level of hypothalamic TRH secretion, should also be taken into account *(Balachandar S, et al, 2016).*

Although decreased TSH levels cause a decrease in thyroidal T4 production in older subjects, serum levels of total and free T4 (FT4) don't alter. This is due to a decrease in T4 degradation caused by outer ring deiodination with age. The level of this inactive metabolite, rT3, appears to rise with age. Thyroid stimulating hormone (TSH) and free T3 levels in the blood clearly fall with age, whereas serum (free) T4 levels do not vary. With age, the inactive metabolite rT3 appears to rise *(Baumgarten HD, et al,. 2019).*

In addition to disease and hunger, pharmaceutical use can affect thyroid function testing in geriatric age groups. The strong positive correlation between age and medication use is well-known. Without having a direct impact on thyroid function, 34 medications, including lithium, heparin, glucocorticoids, amiodarone, and propanolol, can result in hypothyroidism, hyperthyroidism, or abnormal thyroid function tests by affecting TBG status, suppressing TSH secretion, or inhibiting T4 to T3 conversion *(Brose MS, et al,. 2014).*

As a result, while interpreting changed thyroid function tests in geriatric age groups, these aspects must be taken into account. Additionally, in the older age groups, variables including genetic variation and psychosocial factors may affect thyroid function testing. It has been increasingly clear in recent years that genetic variables, namely genetic variability in thyroid hormone pathway genes, play a significant role in the difference in thyroid function tests between different people. Thyroid hormone pathway gene polymorphisms have been linked to insulin, body composition, and blood T3 levels in older individuals *(Baumgarten HD, et al,. 2019).*

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