

# Estimation of vitamin d concentration in children's thyroid disorders

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# Abstract

Serum vitamin D levels were measured in 60 patients with hyperthyroidism and hypothyroidism and 30 healthy subjects using Liaison chemiluminescent immunoassays (DiaSorin, Italy). Vitamin D deficiency has been defined as less than 10 ng/ml. Patients were evaluated for thyroid antibodies, thyroid function, and demographic parameters. Laboratory measurements of Concentration of thyroid stimulation, Concentration of Thyroxine, Concentration of triiodo thyronin ( $T_3$ ), and Concentration of Vitamin D<sub>3</sub> were taken.

**Keywords:** Vitamin D<sub>3</sub>, Triiodothyronine (T<sub>3</sub>), thyroid disorders

## Introduction

Vitamin D<sub>3</sub> is a fat-soluble vitamin and has a vital role in bodily functions <sup>[1]</sup>. Vitamin D<sub>3</sub> has two main forms:1-D<sub>2</sub> ergocalciferol 2- D<sub>3</sub> cholecalcifero <sup>[2]</sup>. 80% percent of vitamin D<sub>3</sub> is formed as a result of skin exposure to sunlight, which is ultraviolet radiation of type B (B-UV), while the main food sources of vitamin D<sub>3</sub> are fats, fortified foods, vegetables, and grains as secondary sources for its formation in the body  $^{[3]}$ . D<sub>3</sub> is formed in the skin when 7- dehydrocholesterol interacts with radiation. Ultraviolet type B in the bloodstream <sup>[4]</sup> and then it travels from the blood to the liver to bind to a hydroxyl group to form a compound (D(OH)25), then it binds to a second hydroxyl group in the kidneys to form a compound (D3 vitamin dihydroxy-25,1) which is the most effective form of vitamin D<sub>3</sub> <sup>[5]</sup>. Vitamin D<sub>3</sub> works to balance calcium and phosphate in the bloodstream <sup>[6]</sup>. Vitamin D<sub>3</sub> deficiency in the growth stage causes rickets, but in adulthood it causes osteomalacia<sup>[7, 8]</sup>. Thyroid is an endocrine gland situated on the either side of trachea at the root of the neck. It is a butterfly shaped organ responsible for metabolism, growth, internal temperature and many other body functions [9]. Thyroid hormones are stored in the thyroid gland and released when needed in the body <sup>[10]</sup>. Thyroid hormones have a key role in the human metabolism <sup>[11]</sup>. The two essential hormones synthesized in the body by thyroid gland is Triiodothyronine (T<sub>3</sub>) and Thyroxine(T<sub>4</sub>). The biosynthetic pathways of the thyroid hormone starts with the iodine metabolism<sup>[12]</sup>. In the production of thyroid hormones, triiodothyronine  $(T_3)$  is synthesized to a lesser degree than the thyroxine  $(T_4)^{[13]}$ . But triiodothyronine  $(T_3)$  is more active than thyroxine  $(T_4)$  <sup>[14]</sup>. Thyroxine  $(T_4)$  covers about 90% of the secretion and Triiodothyronine (T<sub>3</sub>) is about 9 % of the secretion from the gland. Reverse  $T_3$  (rT<sub>3</sub>) is the third minor hormone secreted by the thyroid gland that covers 1% of the secretion which is not biologically active <sup>[15]</sup>.

#### Material and methods

The study included collecting (90) samples (1/9/2022) to

(1/5/2023) for healthy children and those with thyroid disease, Information was recorded on each of the patients and healthy people, according to an approved questionnaire for this purpose, the scientific part was conducted in the laboratory. Chemistry for the College of Education for Pure Sciences, in addition to several important laboratories in the city connector all devices and consumables are manufactured by international and reputable companies licensed by the Ministry of Health, where a ready-made kit analysis kit from a reputable French company was used to measure clinical variables.

#### Stimation of vitamin D in blood serum

Serum vitamin D was determined by using a loading kit (kit) Biomerieux <sup>[16]</sup> (France).

## **Basic principle**

The quantitative vitamin D (Vitamin D total VITD) in blood serum is estimated by using the enzyme immunoassay competition method, which is done by competition between the vitamin D antigens present in the blood serum sample and the vitamin antigens present in (Solid phase receptacle SPR) due to the association between The anti-vitamin D antibody sites that are bound to the enzyme anti-vitamin D antibody ALP Conjugate, after that the washing process is done with a washing buffer to remove the antibody. The unbound antagonist, after which the impregnated base material is added (methylumbelliferyl phosphate-4), which will bind to the enzyme alkaline phosphatase to give a phosphorescent flash. The intensity of this flash will be directly proportional to the concentration of vitamin D antigens present in the blood serum, whose intensity is measured at a wavelength (450nm). After that, the concentration of vitamin D was determined through the standard curve, through which the vitamin concentration of the standard solutions is directly proportional to the absorbance automatically in the device.

Table 1:	Content	of the	kit (	60	tests)
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60 Vit. D Strips (a)	STR	Ready-to-use. Stabilizer of human origin*	
60 Vit. D Solid phase receptacles 2x30	SPR	Ready-to-use. Interior of SPR device coated with Vitamin D	
-	-	Ready-to-use. 25-(OH) Vitamin D diluted in human serum*+	
Vit D Calibrator 1x1.2 ml (Liquid)	<b>S</b> 1	Preservative. MLE data indicate the calibrator concentration in ng/ml ("Calibrator(S1) Dose	
		Value") and the confidence interval in "Relactive Fluorescence Value")	
-	-	(Calibrator (S1) RFV Range)	
-	-	Ready-to-use	
Vit D Control 1x1.1 ml (Liquid)	C1	25-(OH) Vitamin D diluted in human serum*+preservative. MLE data indicate the confidence	
		interval in ng/ml ("Control C1 Dose Value Range")	

#### Table 2: Reagents

Well	Reagents				
1	Sample well				
2	Conjugate: TRIS, NaCL + anti-vitamin D antibody conjugated with alkaline phosphatase +stabilizer of human origin* + preservative				
3	Pre-treatment solution: TRIS, NaCL + dissociation agent + surfactant +methanol				
4-5-6	Empty wells				
7-8-9	Wash buffer: TRIS, NaCL +Preservative + surfactant				
	Reading cuvette with substrate :4-Methylumbelliferyl phosphate				
10	(0.6mmol/L) + diethanolamine (DEA) (0.62mol/L or 6.6%, PH +) 2.9 1g/L sodium azide				

# Procedure

The blood serum was applied with a strip that contains 10 holes A small conical tube using a pipetting device is used. It is called (Solid Phase Receptacle) (SPR) and it contains the antiantibody Vit. D, which acts on suctioning the serum present in the first fossa, resulting in a reaction between the vitamin antigen with the antibody present in the second fossa, which is bound to the enzyme. Calibrator, one VITD SPR and one VITD Strip were used for each of the following models. Control, Sample by 100µL each, then mix the samples well using the. Vortex-type mixer and make sure there are no bubbles. The VITD Strip and VITD SPR were inserted into the device and a code mark was placed on each of them after the sample was placed in the Strip. Then the SPR works by suctioning the wash solution from the wells <sup>[7-9]</sup> and then returns to one of the empty wells <sup>[4-6]</sup> after all the unbound antibodies have been removed so that only the antigen-antibody-enzyme remains attached to the SPR and this will be repeated The process went through several times, and in this way the unreacted conjugate was disposed of using a scouring pad by suctioning it from one of the pits, and then it was returned to the empty pits. The components of the SPR (antigen-antibody-enzyme) will react with the matrix primer in the last hole to form the enzyme complex-the substrate whose light intensity is measured and whose intensity is proportional to the vitamin D concentration present in the blood serum sample. Each of the wash solution, as well as the Conjucate solution, contain other catalysts that enter and exit from the reaction without effect, but they are used in the bonds and reactions that all occur in the device automatically for a period of 40 minutes.

## **Result and discussion**

#### **Concentration of thyroid stimulation**

There was a significant increase ( $p \le 0.001$ ) in the average concentration of TSH in patients with hypothyroidism (5.6\*\* + 2.6)) compared to the average concentration in the control

group (2.43 + 1.3). The reason for this increase may be that the thyroid gland occurs when it occurs. Acute or chronic disorder that does not produce enough of its hormones in the case of hypothyroidism, and this leads to stimulation of the secretion of thyroid-stimulating hormone. TSH <sup>[17, 18]</sup>.

The reason for the decrease in TSH in patients with hyperthyroidism may be due to the formation of immunoglobulin-Immunoglobulin (IgG-G), which is associated with the membrane receptors psychologically with which the thyroid-stimulating hormone binds in the follicular cells, causing these cells to stimulate and thus increase the secretion of thyroid hormones, while inhibition of the hormone occurs. Thyroid stimulator <sup>[19]</sup>.

## **Concentration of thyroxine**

And the presence of a significant decrease at the level of probability ( $p \le 0.001$ ) in patients with hypothyroidism 3.003 \*\* + 1.26 compared to the control group 6.74 + 1.44 was shown in the table (and this is consistent with what was found by <sup>[19]</sup>, and the reason may be due To a lack of iodine in the diet or due to metabolic causes that go deeper into a disorder of iodide transport that leads to a peak secretion of thyroid hormones <sup>[20]</sup> under normal conditions, TSH secretion is stimulated by the pituitary gland and the hypothalamus, When the thyroid hormones are low, which stimulates their formation and vice versa, when the percentage of hormones is high in the body, the levels of TSH are low or within the normal limit, and this is consistent with what was found by <sup>[21]</sup>.

# Concentration of triiodo thyronin (T<sub>3</sub>)

There was a significant increase at the probability level  $(p \le 0.001)$  in patients with hyperthyroidism (L/IU 3.21\*\*+0.8) compared to the control group (0.91 + 0.45 IU/L), and this is consistent with what was found <sup>[19]</sup> al (The presence of excess amounts of the T<sub>3</sub> hormone than the normal limit in the blood is called thyrotoxicosis, which occurs as a result of excessive

activity of the thyroid gland in various cases such as thyroiditis, Graves' disease, or the conversion of more  $T_4$  hormone to  $T_3$  by the deiodinase enzyme, or eating more Supplements <sup>[21]</sup>.

# Concentration of Vitamin D<sub>3</sub>

The main role of vitamin D is to maintain the balance of calcium and phosphorus, thus maintaining healthy bones. Recent evidence has demonstrated that vitamin D may also have a role in a variety of non-structural disorders such as endocrine diseases and in particular type 1 diabetes, type 2 diabetes, adrenal disease and polycystic ovary syndrome. Low levels of vitamin D have also been associated with thyroid diseases, such as Hashimoto's thyroiditis.

Similarly, patients with new-onset Graves' disease were found to have decreased 25-hydroxyvitamin D concentration. Impaired vitamin D signaling has been reported to promote the formation of thyroid tumors. This review will focus on the role of vitamin d in thyroid diseases, both autoimmune diseases and thyroid cancer, and will summarize the results of vitamin d supplementation studies conducted in patients with thyroid disorders. Although observational studies support a beneficial role for vitamin D in the management of thyroid disease, randomized controlled trials are required to provide insight into the efficacy and safety of vitamin D as a therapeutic tool for this dysfunction.

A significant decrease was found at the level of probability ( $p \le 0.001$ ) in the rate of vitamin D<sub>3</sub> concentration in patients with hypothyroidism dl/ng (18.41\*\*+10.6) compared to the healthy group 41.4 + 11.96, and this is consistent with what <sup>[22]</sup> found. There is a decrease in vitamin D<sub>3</sub> in patients with thyroid autoimmune diseases and that it was lower in patients with hypothyroidism, and the results of the studies were not clear whether treatment with vitamin D<sub>3</sub> had an effect on improving thyroid autoimmune diseases or not and this is consistent with what he found <sup>[23]</sup>.

 Table 3: Shows the rise and fall of the measured hormones

 compared to a control sample

<b>Clinical parameter</b>	Control	Heperthyroidism	Heporthyroidism
PON1 (IU/L)	36 <u>+</u> 21	32.5 <u>+</u> 18	31.4 <u>+</u> 19
T3 (Iu/L)	$0.91 \pm 0.45$	3.21** <u>+</u> 0.8	$0.52 \pm 0.42$
T4 (Iu/L)	6.74 <u>+</u> 1.44	8.57 <u>+</u> 2.9	3.003** <u>+</u> 1.26
TSH (Iu/L)	2.43 <u>+</u> 1.3	0.43 <u>+</u> 0.79	5.6** <u>+</u> 2.6
ViT. D (ng/dl)	41.4 <u>+</u> 11.96	29.11** <u>+</u> 12.5	18.41** <u>+</u> 10.6
Glucose (mg /dl)	82.2 <u>+</u> 9.0	123.48** <u>+</u> 53.6	18.41** <u>+</u> 10.6

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