



## The Vitamin D Deficiency Arises Anemia in Hypothyroidism Iraqi Patients

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### Abstract

The thyroid gland is an endocrine gland that produces two hormones controlling the body metabolism process several disorders can arise when this thyroid produces excessive hormone (hyperthyroidism) which is considered one of the most important thyroid disorders and is associated with various biochemical imbalances such as a reduction in vitamin D which is considered a health problem, this study refers to a significant role for D vitamin in decreasing the frequency of these diseases and demonstrated the association between low vitamin D levels and thyroid diseases, by observed the correlation between hypothyroid patients and vitamin D insufficiency along with its binding protein (DBP) in patients compared to healthy individuals. A recent study indicated a relationship between circulating levels of D vitamin and elevated levels and a decrease in the levels of free thyroxine FT4 and free triiodothyronine FT3 additionally, this study suggests that D vitamin deficiency could linked to erythropoiesis, and this deficiency undoubtedly increase the risk of anaemia, The current study aim to evaluated the concentration of serum vitamin D 25(OH) patient diagnosed with hypothyroidism and associate these levels with thyroid hormones (TF3, TF4 and TSH) as well as to examine the correlation between reduce of vitamin D and blood cell count in these patients.

**Keywords:** Thyroid gland, Vitamin D deficiency, D- binding protein (DBP), RBC.

### نقص فيتامين د يزيد من فقر الدم لدى المرضى العراقيين الذين يعانون من مرض قصور الغدة الدرقية

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### الخلاصة

الغدة الدرقية هي غدة صماء تنتج هرمونين يتحكمان في عملية التمثيل الغذائي في الجسم ويمكن أن تنشأ عدة اضطرابات عندما تنتج هذه الغدة الدرقية هرمونًا زائدًا (فرط نشاط الغدة الدرقية) والذي يعتبر من أهم اضطرابات الغدة الدرقية ويرتبط بالعديد من الاختلالات البيوكيميائية مثل انخفاض في فيتامين د الذي يعتبر مشكلة صحية، تشير هذه الدراسة إلى الدور الكبير لفيتامين د في تقليل تكرار هذه الأمراض، كما بينت العلاقة بين انخفاض مستويات فيتامين د وأمراض الغدة الدرقية، من خلال ملاحظة العلاقة بين مرضى قصور الغدة الدرقية وفيتامين د. القصور مع البروتين المرتبط به (DBP) في المرضى مقارنة بالأفراد الأصحاء. أشارت دراسة حديثة إلى وجود علاقة بين مستويات فيتامين د في الدورة الدموية والمستويات المرتفعة وانخفاض مستويات هرمون الغدة الدرقية الحر FT4 وثلاثي يودوثيرونين حر FT3 بالإضافة إلى ذلك، تشير هذه الدراسة إلى أن نقص فيتامين د يمكن أن يرتبط بتكوين الكريات الحمر، وهذا النقص بلا شك يزيد من خطر الإصابة به. لفقر الدم، تهدف الدراسة الحالية إلى تقييم تركيز فيتامين د 25 (OH) في مصل الدم لدى المرضى المصاب بقصور الغدة الدرقية وربط هذه المستويات مع هرمونات الغدة الدرقية (TSH, TF4, TF3) وكذلك فحص العلاقة بين انخفاض فيتامين د وعدد خلايا الدم لدى هؤلاء المرضى.

**كلمات المفتاحية:** الغدة الدرقية، نقص فيتامين د، بروتين ربط د (DBP).



## 1. Introduction

Hypothyroidism is a common endocrine disorder, affecting populations worldwide resulting from reduced functioning of the thyroid gland, it can be the major (disease of thyroid gland) or less commonly the minor (hypothalamic or pituitary disease) [1]. D Vitamin: recognized as an essential for bone metabolism and skeletal health [2]; therefor, previous studies have indicated a deficiency in D vitamin in autoimmune thyroids and many result have shown that this deficiency could be a risk factor for diabetes, cancers, multiple sclerosis and other autoimmune disease atherosclerosis, and infection diseases [3]. Vitamin D is a steroidal hormone, with food and skin exposure to ultraviolet light considered the major source of D vitamin . the liver can converts D vitamin to 25 (OH) D and the kidneys metabolized it through enzyme 25-hydroxyvitamin D-1 $\alpha$ -hydroxylase (CYP27B1) to its active form 1,25- dihydroxyvitamin D[4]. D Vitamin plays a crucial role in protection against various inflammatory diseases, such as infections, and autoimmune disorder and it affected many organs in the body therefor the thyroid gland is one of the organs that has a receptor for D vitamin. The D vitamin receptor (VDR), is a nuclear receptor, so it that bind to its receptor present in many cell types including thyroid gland [5]. This vitamin has numerous functions, including the regulation of metabolism for calcium and phosphorus homeostasis, cell growth, cell differentiation, and cellular immunity [6], therefore of Vitamin D deficiency considered universal problem with a large insufficiency vitamin D [7,4,8,7] Therefore; a low level of vitamin D is likely to exacerbate the systemic abnormalities associated with hypothyroidism [9,10] .Since hypothyroidism considered deficiency of thyroid activity [11], because the reduced secretion of both T4 and T3 which leading to hyper secretion of pituitary TSH and an significant increase in serum TSH levels [12]. It has been observed that vitamin metabolites affect the thyroid gland by acting on vitamin D receptors in the blood, so an increase in individuals with hypothyroidism [13] .The vitamin D circulates in the blood associated with a specific vitamin D binding protein (DBP) which considered an acute-phase reactant is primarily synthesized in parenchymal cells of hepatic [14]. Its consider a multifunctional glycoprotein that plays an independent role in vitamin D transport such as activated macrophages, increasing chemotaxis of C5-derived peptides and associated with the cellular surface of neutrophils ,monocytes, B cells and T cells [13,14]. additionally vitamin D insufficiency can increase the risk of anemia due to decrease in the haemoglobin and increase in agents that stimulate the erythrocytes production [1] .

## 2. Materials and Methods

Eighty blood samples were collected from Hypothyroidism patients attending .clinic consultation at the following hospitals: Al-Imamian Al- Kadhimyain Medical City in Baghdad, from both the samples were obtained from both sexes (33 males and 47females) aged between 27-45, years during the period between June 2020 to September 2020 .patients with parathyroid disease, a prior history likely to interfere with vitamin-D metabolism and those with vitamin D supplementation in the last one year, and did not give the consent were excluded. For comparison 80 healthy controls from both sexes (33 males and 47females) between the ages 27-45, were included in this study.



### 2.1 Laboratory Analysis

Venous blood (5ml) was obtained from from each patient, (2 ml) used to estimate the red blood cells and hemoglobin levels using hematological analyzer . The remaining collected blood was placed in a test tube free of any preservative material at room temperature for 30 minutes to prepare the serum after bloodclotting the tubes were centrifuged for 5 minutes at 3000 RPM, the serum was stored at -20 °C, then the (TSH), (FT3 ) , DBP and (FT4) was measured by using the Elisa kits [14] .The serum levels for vitamin D was measured by Elisa kit , about (50 µl) of Standard was added in the plate, then ( 50 µl ) of Reagent Detection (A) was placed to all well, also it was covered with the Plate sealer and incubated in 37°C for 1 hour. The well were washed with Wash Buffer (approximately 400 µl). During washing, any remaining wash buffer was removed. About (90 µl) of Substrate Solution was added to each well and incubate within 15-30 minutes at 37°C ,finally (50 µl ) of stop Solution was added to each well and the optical density of each well was read, using a micro plate reader set to 450 nm .

### 3. Results and Discussion

The results of statistical analysis using the program (SPSS) (for the study shown in Table 1, Figure 1, and Figure 2) showed that there was a significant increase ( $P < 0.05$ ) in the level of free triiodothyronine in blood serum (FT3) compared to healthy people. The level was ( FT3 in the serum of patients was  $(14.19 \pm 3.530)$  pmol/L, while the serum level was  $(6.21 \pm 165)$  pmol/L. The results also showed a significant increase in the level of free thyroid hormone (FT4) and thyrotropin (TSH). The levels of FT4 in the blood serum of patients were  $(59.81 \pm 41.175)$  pmol/L compared to the level of serum of healthy people  $(13.06 \pm 1.228)$  pmol/L, while the level of TSH in the serum of patients was  $(1.2200)$  mIU/L compared to serum In healthy people  $(0.32 \pm 0.554)$  mIU/L, the levels of red blood cells in patients were  $(4.7 \pm 9.6)$  ml/microliter, while in the control group  $(6.50 \pm 4.8)$  mL/microliter was associated with a low hemoglobin level in patients of  $12.3 \pm 3.6$  g. /dL compared with the control group  $14.8 \pm 4.6$  g/dL.

**Table 1-** Show the average concentration of thyroid hormones FT3, FT4, TSH for patients with Hypothyroidism and control by mg/ml.

parameter	Hypothyroidism	Healthy controls	P-value
	patients N=80	N=80	
Ft3(pmol/L)	14.19±3.530	6.21±165	*0.00
Ft4(pmol/L)	59.81±41.175	13.06±1.228	*0.00
TSH (mIU/L)	1.220±0.030	0.32±0.554	*0.00
RBC (mil/µL)	4.7±9.6	6.50±4.8	*0.00
Hb (g/dl)	12.3±3.6	14.8±4.6	*0.00

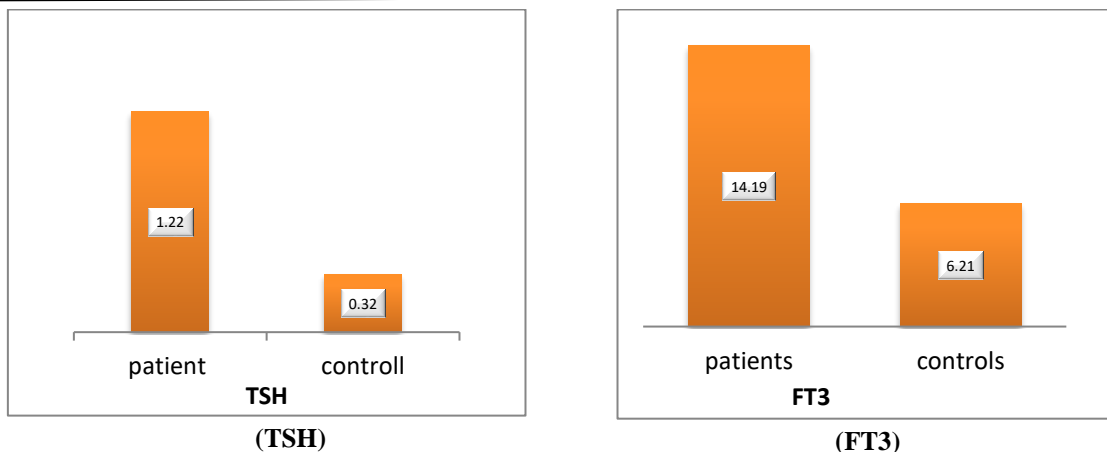


Figure -1 Show the average concentration of thyroid hormones FT3, FT4, TSH and RBC for patients with Hypothyroidism and controls. (a) TSH (b) FT3

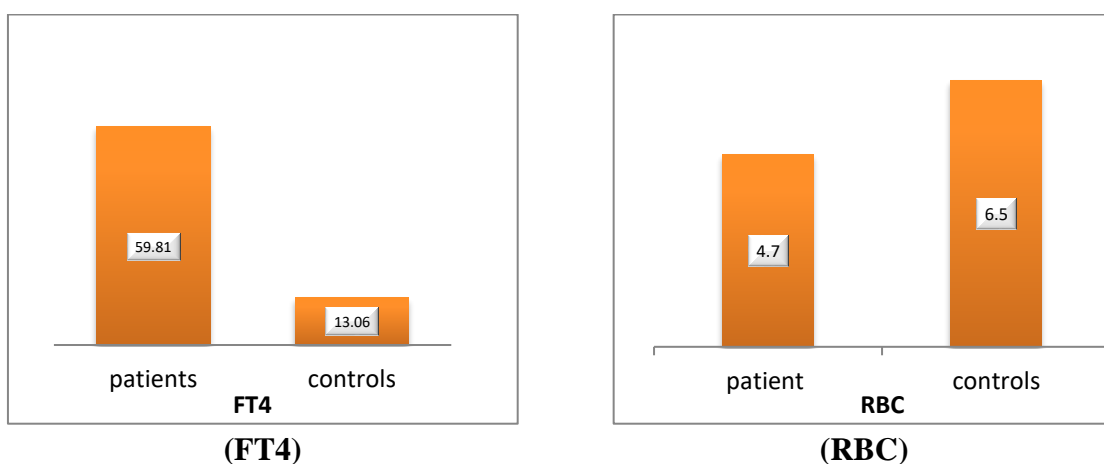


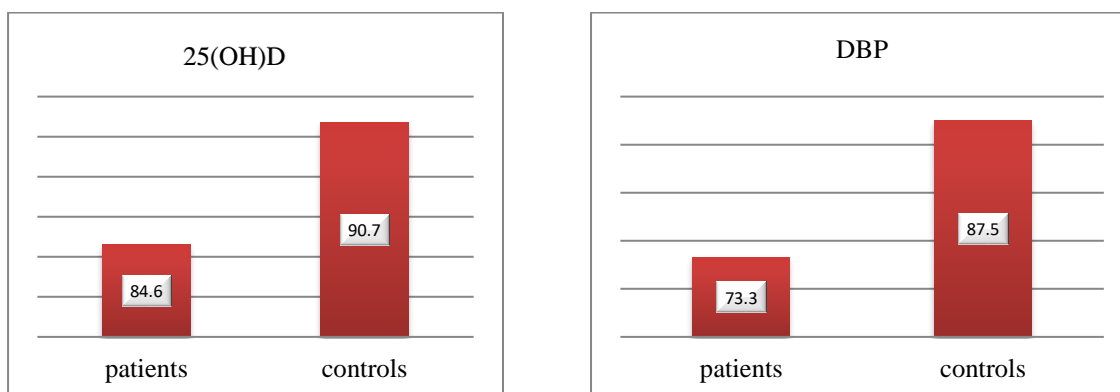
Figure -2 Show the average concentration of thyroid hormones FT3, FT4, TSH and RBC for patients with Hypothyroidism and controls. (a) FT4 (b) RBC

These results of the present study consistent with Amal Mackway et al.,2013 (15), for the TSH ,FT3 and FT4 level and our results for the FT4 level in our result disagree with Musa et al.,2017 (16) who recorded low level, Moreover, the elevated levels of TSH in hypothyroidism could cause a change in metabolism and the functions of specific organs which leads to bone deficiency and other endocrine disorders [13].The results of the present study as shown in Table 2 and Figure 3 indicate that patients that with hypothyroid had significantly decreased levels of 25(OH) vitamin D in the patient group ( $84.6 \pm 16.4$ ) nmol/L compared with healthy controls with level of serum at ( $90.7 \pm 41.8$ ) nmol/L. The results to the serum of healthy individuals ( $87.5 \pm 8.4$ ) ng/ml.



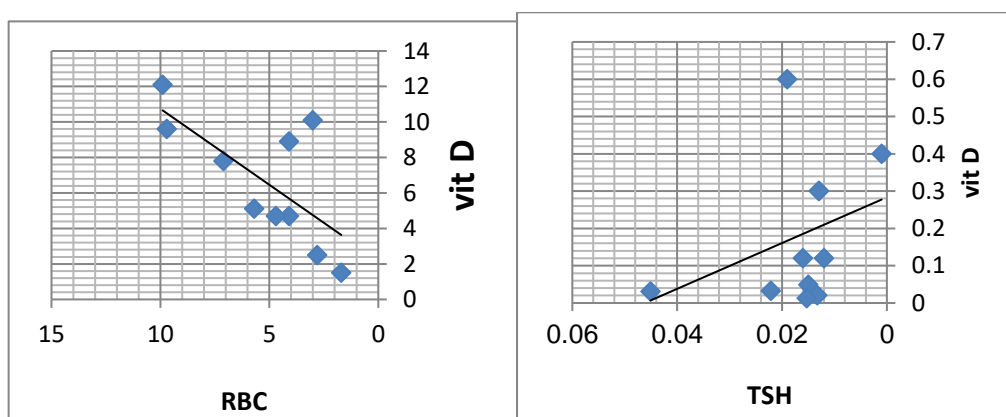
**Table 2-** Show the average concentration of 25 (OH) D vitamin level and DBP level for Hypothyroidism patients and control.

parameter	Hypothyroidism		P-value
	patients N=80	Healthy controls N=80	
25(OH)D (nmol/L)	84.6 ± 16.4	90.7 ± 41.8	*0.00
DBP ng/ml	73.3±8.1	87.5±8.4	*0.00



**Figure -3** Show the average concentration of 25 (OH) D vitamin level and DBP level for Hypothyroidism patients and control. (a) 25(OH)D (b) DBP

This study was agree with, Lohokare et al., 2016 (14) and Amal Mackway et al.,2013(15), and Ahi et al ., 2020(17) who reported a significant discovery of the relationship between non-autonomous hypothyroidism and vitamin deficiency as publishing in the Journal of Cellular and Molecular Immunology (2011) .This study shows low levels of vitamin D and found a negative correlation with TSH levels significant ( $r = 0.0361$ ,  $p < 0.05$ ), also this study suggested a positive correlation between D Vitamin levels of and RBC ( $r = 0.0251$ ,  $p < 0.05$ ), as shown in "figure 3".



**Figure -4** (a) shows the positive correlation between D vitamin and RBC, (b) shows the negative correlation between vitamin D and TSH.



The Vitamin D deficiency is more common in patients with autoimmune thyroid disorders, but the deficiency is also observed in non-immune hypothyroid patients, [17]. The deficiency of Vitamin levels is linked to the degree and severity of hypothyroidism [18]. Causes for low levels of D vitamin may be related to hypothyroidism disorder, and can be explained by one of two mechanisms leading to decreased vitamin D levels in hypothyroidism patients. The first cause is due to poor absorption of vitamin D from the intestine. The second cause could be related to the body's failure to utilize vitamin D regularly [19]. Byron Richards (2008) experimental study showed the effect of D deficiency on the thyroid, reporting that the decrease of vitamin D levels contributed to the possibility of low thyroid hormones [20,21]. We also found a significant increase in DBP levels in the serum of patients with hypothyroidism compared with healthy individuals, since the thyroid gland synthesizes and secretes two major hormones, namely triiodothyronine (T3) and thyroxine, which can be referred to tetraiodothyronine (T4). These hormones are essential for metabolic processes. These molecules have also important roles in early brain maturation, the growth of somatic, bone development, the synthesis of protein and the regulation of red blood cell production [15]. Vitamin D deficiency is associated with mortality, cardiovascular disease, fractures, poor physical function, malignancy, infection and diabetes [22]. Therefore, irregular concentration of thyroid hormones can have adverse effects on different metabolic systems, leading to various inflammatory diseases, such as hypothyroidism. Inflammatory diseases are initiated by inflammation, prompting a protective response in which leukocytes migrate from the vasculature into damaged tissues to destroy the agents that potentially could cause tissue damage [23]. Hypothyroidism is associated with red blood cell defects, the hormones of the thyroid often reduce erythropoiesis by an increase in the proliferation of red blood cells. Thus, hypothyroidism is associated with an increased prevalence of atherosclerosis [16]. This study suggested a positive relationship between vitamin D levels and (RBC) in patients. It is speculated that vitamin D regulates the level of systemic cytokine production, thereby reducing inflammation that causes anemia, which is a chronic disease or inflammation [24]. For instance, vitamin D has been found to impair cytokine release and possibly exerts a direct stimulatory effect on erythroid precursors, since its receptors are also found in several non-renal target sites, such as the bone marrow [25]. (1,25-hydroxyvitamin D) could stimulate erythrocyte precursor cell receptors, promoting maturation and proliferation [26]. Vitamin D deficiency leads to a decrease in the production of calcitriol in the bone marrow, which may limit erythropoiesis [27]. Moreover, the deficiency of vitamin D has a strong effect in reducing the proliferation and the activation of RBCs, as well as it has a major effect in decreasing the synthesis and metabolism of iron and haemoglobin. [28]

#### 4. Conclusion

We can conclude that vitamin D deficiency is associated with anemia in Iraqi patients. These findings could have potentially broad public health implications of vitamin D deficiency in Iraq. Ultimately, if these results can be replicated by others and extended, they could lead to patients' clinical trials to evaluate vitamin D supplementation as therapy for patients with anemia.



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