

## **Vitamin D Deficiency and Its Association with Thyroid Disease**

**Dr. Amal Mohammed Husein Mackawy,<sup>(1)</sup> Bushra Mohammed Al-ayed,<sup>(2)</sup> Bashayer Mater Al-rashidi<sup>(3)</sup>**

Assistant Professor of Medical Biochemistry, Medical Laboratory Department, Applied Medical Science College, Qassim University,<sup>(1)</sup> Students in the Medical Laboratory Department, Applied Medical Science College, Qassim University, Saudi Arabia.<sup>(2,3)</sup>

### **Abstract**

**Objectives:** Vitamin D deficiency is a global health problem, its role as an immune modulator has been recently emphasized. The evidence is increasingly pointing towards vitamin D significant role in reducing the incidence of autoimmune diseases. However, at this time the research on its role in autoimmune and thyroid disease is not conclusive.

We aimed to examine the relationship between hypothyroidism and vitamin D deficiency and to clarify the relation between serum calcium levels with hypothyroid disease.

**Subjects and Methods:** Serum vitamin D (25-OH) levels were measured in 30 patients with hypothyroidism and 30 healthy subjects, utilizing the spectrophotometric method. Vitamin D deficiency was designated at levels lower than 20 ng/ml. Thyroid hormones (TSH, T3 and T4) and calcium levels were evaluated in all participants.

**Results:** Serum 25(OH) vit D was significantly lower in hypothyroid patients than in controls ( $t=-11.128$ ,  $P=0.000$ ). Its level was insignificantly decreased in females than male patients ( $t= 1.32$ ,  $P >0.05$ ). Moreover, serum calcium levels recorded a significant decrease in hypothyroid patients when compared to controls ( $t= -5.69$ ,  $P = 0.000$ ).

**Conclusion:** Our results indicated that patients with hypothyroidism suffered from hypovitaminosis D with hypocalcaemia that is significantly associated with the degree and severity of the hypothyroidism. That encourages the advisability of vit D supplementation and recommends the screening for Vitamin D deficiency and serum calcium levels for all hypothyroid patients.

**Keywords:** Vit D deficiency, Blood calcium levels, Thyroid diseases.

### **Correspondence:**

**Dr. Amal Mackawy**

Assist Prof. of Medical Biochemistry and Molecular Biology,

AMS College, Qassim University

Tel.: +966163800050

P.O. Box 6666 Buraydah, Qassim, KSA

Mobile: +966508129407

E-mail: [amalmackawy@yahoo.com](mailto:amalmackawy@yahoo.com)

## Introduction

Vitamin D deficiency is a global health problem. <sup>(1)</sup> Over a billion people worldwide are vitamin D deficient or insufficient. <sup>(1)</sup> Yet no international health organization or governmental body has declared a health emergency to warn the public about the urgent need of achieving sufficient vitamin D blood levels. <sup>(2)</sup> Understanding of the role of vitamin D has been evolving since its discovery in the early 20th century from being a simple vitamin to a steroid pro-hormone. <sup>(3)</sup> It has been recognized to be involved in various immune functions as well as bone and muscle development. <sup>(3)</sup> Vitamin D deficiency has been shown to be associated with autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), multiple sclerosis (MS) and type 1 diabetes (T1DM), and that vitamin D supplementation prevents the onset and/or development of these autoimmune diseases. <sup>(4)</sup> Furthermore, it was reported that patients with Hashimoto's thyroiditis, an autoimmune thyroid disease had lower vitamin D levels. <sup>(5)</sup> Vitamin D plays an essential role in calcium homeostasis and the development and maintenance of the skeleton. <sup>(6)</sup> It is recognized as the sunshine fat soluble vitamin. Exposure to ultraviolet B light (290–320 nm) are the main source of vitamin D. <sup>(7)</sup> In the classical endocrine pathway, vitamin D enters the circulation attached to a D-binding protein, is first hydroxylated in the liver to 25(OH) D and then in the kidney to form the active metabolite, 1, 25 dihydroxy vitamin D (1, 25-(OH)<sub>2</sub> D) or calcitriol. <sup>(8)</sup> Serum 25(OH) D, the most abundant circulating precursor of active vitamin D, is the most widely accepted indicator of vitamin D status and reflects combined contributions from cutaneous synthesis. <sup>(9)</sup> Serum 25(OH)D has a half-life of approximately two to three weeks, in contrast, 1,25-(OH)<sub>2</sub>D has a short circulating half-life and is tightly regulated over a narrow range by parathyroid hormone, calcium and phosphate. <sup>(9)</sup> Serum 1,25-(OH)<sub>2</sub>D is not a good measure of vitamin D status since a decrease may not occur until vitamin D deficiency is severe. <sup>(10)</sup> Levels of 25(OH)D 30 to 32 ng/ml is considered to be sufficient, but levels of 20 to 29 ng/mL is insufficient and if it is less than 12 ng/ml is considered an evidence of severe vitamin D deficiency. <sup>(11)</sup> Importantly, both vitamin D and

thyroid hormone bind to similar receptors called steroid hormone receptors. A different gene in the Vitamin D receptor was shown to predispose people to autoimmune thyroid disease including Graves' disease and Hashimoto's thyroiditis. For these reasons, it is important for patients with thyroid problems to understand how the vitamin D system works. <sup>(12)</sup> Vitamin D mediates its effect through binding to vitamin D receptor (VDR), and activation of VDR-responsive genes. While VDR gene polymorphism was found to associate with autoimmune thyroid diseases (AITDs). <sup>(12)</sup>

The purpose of this study was to examine the relationship between hypothyroidism and vitamin D deficiency and to clarify the relation between serum calcium levels with hypothyroid disease.

## Subjects and Methods

Sixty subjects were included in this study. They were living in Qassim region and recruiting to Outpatient Clinics of Buraidah Central Hospital, King Fahd Specialist Hospital and Qassim University Clinics during the period from Sep 2011 to May 2012. Written consent was taken from all participants in this study.

### They were classified into two main groups:

**Group I "control group":** included 30 apparently healthy individuals [13 Male (43%) and 17 Female (57%)], their mean ages  $\pm$  S.D are  $46.1 \pm 6.29$  years. They were not complaining from any chronic medical diseases with normal clinical examinations, no history of thyroid diseases or any chronic illness may interfere with our results. They were not on vitamin D supplements.

**Group II "Hypothyroid patients":** It included 30 patients [12 Male (40%) and 18 Female (60%)], their mean ages  $\pm$  S.D  $46.66 \pm 5.22$  year. They were diagnosed as hypothyroid patients if TSH level was higher than 5.0 mU/L with lower levels of T<sub>3</sub> and T<sub>4</sub> than normal value

### All cases included in this study were subjected to the followings:

- 1- Complete history taking.
- 2- Complete clinical examination.
- 3- Laboratory investigations, including:
  - i. Routine investigations
    - a) Serum T<sub>3</sub>, T<sub>4</sub> and TSH for thyroid dysfunction patients with

reference range (1.2 – 4.4 pg/ml for T3 ) , (0.8 – 2.0 ng/dl for T4 ) and (0.5 – 5.0 mU/l for TSH ) .<sup>(13)</sup>

- ii. Research investigations:
  - Estimation of serum 25 (OH) D levels using spectrophotometric method. This method is based on that vit D forms with antimony trichloride in chloroform a pink color that can be read at 500 nm wavelength.<sup>(14)</sup>
  - Vitamin D deficiency was defined as a serum level of 25OHD of  $< =20$  ng/ml and insufficiency as a serum level between  $>20$  ng/ml and  $<30$  ng/ml and normal  $>or=30$  ng/ml.<sup>(15)</sup>
  - Determination of serum Calcium levels using spectrophotometric method. This method is based on formation of  $Ca^{+}$  ions violet complex with o-cresol-phthalein complex in alkaline medium. The intensity of the color is

measured at 578 nm wavelength. (Crescent Diagnostics, Cat No. CE 500). The test is linear up to Calcium value of 15 mg /dl. Samples with higher value should be diluted 1+1with distilled water and the calcium levels then estimated and the results are multiplied by 2.<sup>(16)</sup>

**Statistical Analysis**

Results were statistically analyzed by SPSS 11.5 for Windows. The mean and the standard deviation (SD) for all the variables were calculated. Analysis of variance F test (ANOVA) was used to compare the results of all examined cases in all studied groups. The differences between mean values for each tested variable have been tested by student’s “t” test. The correlations between serum VitD, calcium and TSH were presented by correlation coefficient ( $r^2$ ). Results considered significant or non-significant when  $P >$  or  $<$  0.05, respectively.

**Results**

The mean values  $\pm$  S.D of all studied parameters, age and sex distribution in all studied groups are shown in table (1).There were no statistical difference ( $P > 0.05$ ) between groups regarding age and sex. Statistical analysis and results of serum 25(OH) vit D and serum calcium levels in the studied groups are given in table (1) and Fig (1).

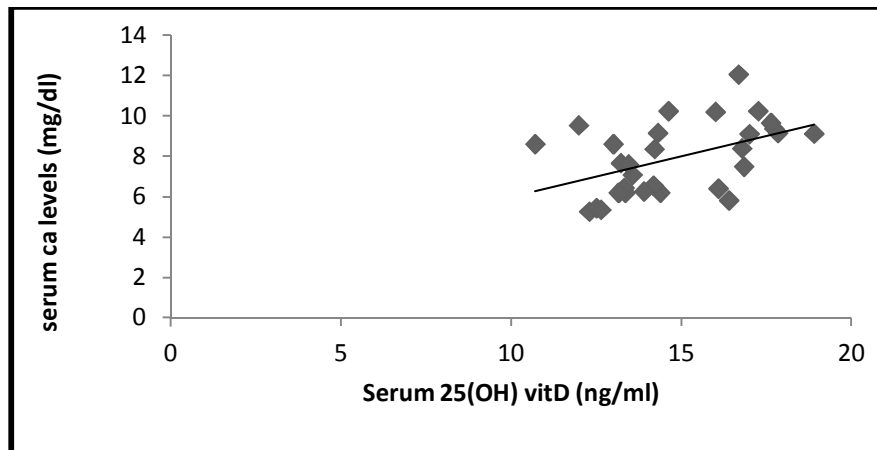


Figure (1): Correlations between serum Calcium and 25(OH)vit D Levels in hypothyroid patients.

**Table (1): Dermatological and clinical characteristics of the participants used in the present study.**

Parameters : Mean $\pm$ SD	Group I	Group II	t- value , p- value
<b>Sex</b>	13 M (43%) 17 F (57%)	12 M (40%) 18 F (60%)	<b>p&gt;0.05</b>
<b>Age (years)</b>	46.1 $\pm$ 6.29	46.66 $\pm$ 5.22	<b>t= - 0.379, p&gt;0.05</b>
<b>Serum 25(OH)Vit D (ng/ml)</b>	44.53 $\pm$ 14.91	14.79 $\pm$ 2.11	<b>t= 11.13, p = 0.000</b>
<b>Serum Calcium (mg/dl)</b>	10.37 $\pm$ 1.55	7.92 $\pm$ 1.77	<b>t= 5.69, p = 0.000</b>
<b>SerumTSH (mU/L)</b>	3.66 $\pm$ 0.91	6.92 $\pm$ 0.97	<b>t= -13.38, p = 0.000</b>
<b>Serum T3 (pg/ml)</b>	2.94 $\pm$ 1.01	1.08 $\pm$ 1.02	<b>t= 4.78, p = 0.000</b>
<b>Serum T4 (ng/dl)</b>	1.59 $\pm$ 0.30	0.64 $\pm$ 0.46	<b>t= 5.48 , p = 0.000</b>

By using t-test to compare between the two groups, serum 25(OH) vit D level was significantly lower in hypothyroid patients than in controls ( $t= -11.128$ ,  $P =0.000$ ) as illustrated in table 1. On comparing serum 25 (OH) vit D levels according to the sex distribution, they were insignificantly decreased in females than those of male in controls and hypothyroid

patients ( $t= - 0.160$ , and  $t= -1.32$ ,  $P >0.05$ ) respectively, table (2).

Serum calcium levels recorded a significant difference between the studied groups ( $t= 5.69$ ,  $P = 0.000$ ) as shown in table (1). In hypothyroid patients, serum calcium levels were insignificantly decreased in females than male patients ( $t= - 0.016$ ,  $P >0.05$ ) table (2).

**Table (2):Mean $\pm$  SD of serum 25(OH) vit D, Calcium and TSH levels in hypothyroid patients according to sex.**

Parameters Mean $\pm$ SD	Male =12	Female=18	t-test /p- value
<b>25(OH) vit D ng/ml</b>	15.58 $\pm$ 2.27	14.27 $\pm$ 1.89	<b>t= -1.32/p=0.213</b>
<b>Calcium levels (mg/dl)</b>	7.92 $\pm$ 2.13	7.89 $\pm$ 1.55	<b>t= 0.016/p= 0.988</b>
<b>TSH (mU/L)</b>	6.80 $\pm$ 1.02	7.05 $\pm$ 0.96	<b>t=- 0.535/p=0.597</b>

On comparing the two groups, serum TSH level was significantly higher in hypothyroid patients than that of controls ( $t= 13.38, P= 0.000$ ).When serum TSH levels in hypothyroid patients were compared regarding to the sex, we noticed a non-significant difference between males and females patients ( $t= - 0.535, P >0.05$ ), table (2). Serum T<sub>3</sub> and T<sub>4</sub> were significantly higher in controls than those of hypothyroidism ( $t= 4.78, t= 5.48, P = 0.000$ ) respectively.

Regarding the control group, significant positive correlations were recorded between serum 25 (OH) vit D and each of serum calcium levels ( $r =0.762, P < 0.05$ ), T<sub>3</sub> ( $r = 0.598, P < 0.05$ ). On the other hand, there were significant negative

correlations between serum 25 (OH) vit D and TSH ( $r = -0.589, P < 0.05$ ), with non-significant correlation with T<sub>4</sub> ( $r = 0.045, P > 0.05$ ). Serum calcium levels had a negative significant correlation with serum TSH ( $r = - 0.40, P =0.029$ ). Otherwise it was non-significantly correlated with either T<sub>3</sub>and T<sub>4</sub>. There were significant positive correlations between serum 25 (OH) vit D and each of serum calcium levels ( $r =0.477, P =0.008$ ) (Fig.1), T<sub>3</sub> ( $r = 0.564, P =0.001$ ) (Fig.3), with significant negative correlation with TSH ( $r = - 0.489, P =0.000$ ) in hypothyroid patients (Fig.2).Concerning serum calcium levels , it was noticed to have a negative significant correlation with serum TSH ( $r= - 0.461, P =0.010$ ) with a significant positive correlations with T<sub>3</sub> and T<sub>4</sub> ( $r= 0.475 , P= 0.008$ ).

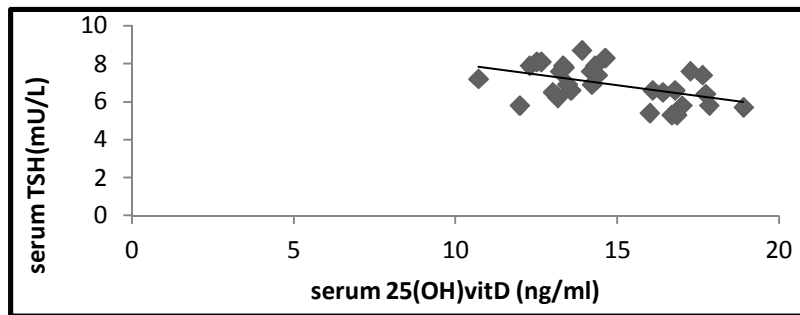


Figure (2): Correlations between 25(OH)vit D and serum TSH levels in hypothyroid patients.

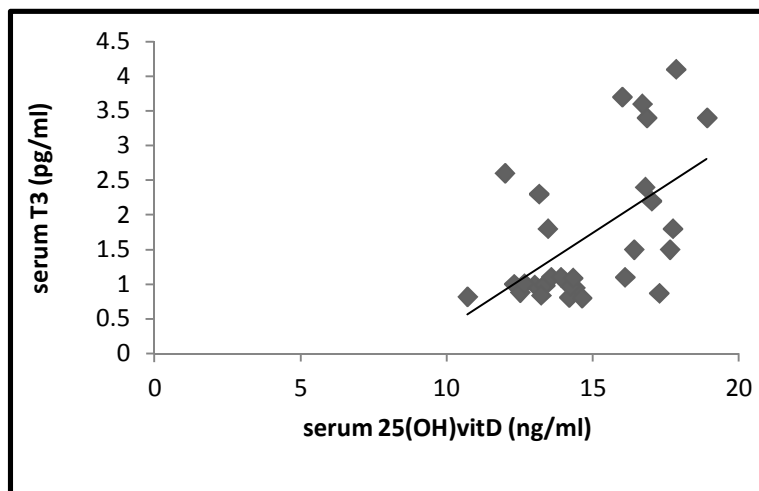


Figure (3): Correlations between 25(OH) vit D and serum T3 levels in hypothyroid patients.

## Discussion

Vitamin D is known for its primary role in bone and mineral homeostasis, and it has been shown recently that its deficiency is associated with various diseases such as cardiovascular disease, cancer, infection, and adiposity as well as osteoporosis.<sup>(17)</sup> Interestingly, it has been shown recently that vitamin D has potent immunomodulatory effects and plays important roles in the pathogenesis of autoimmune diseases.<sup>(12)</sup> Serum concentration of 25(OH)D is the best indicator of vitamin D status. It reflects vitamin D produced cutaneously and that obtained from food and supplements<sup>(18)</sup> and has a fairly long circulating half-life of 15 days.<sup>(19)</sup> In contrast to 25(OH)D, circulating 1,25(OH)<sub>2</sub>D is generally not a good indicator of vitamin D status because it has a short half-life of 15 hours and serum concentrations are closely regulated by parathyroid hormone, calcium, and phosphate<sup>(19)</sup>. Levels of 1, 25(OH)<sub>2</sub>D do not typically decrease until vitamin D deficiency is severe.<sup>(20-21)</sup> Therefore, in the present study we measured serum 25(OH)D rather than 1,25(OH)<sub>2</sub>D to ensure we are getting more accurate results. Few studies have been conducted in order to find any significant association between the levels of vitamin D and hypothyroidism and to determine whether vitamin D deficiency involves in the pathogenesis of hypothyroidism or rather a consequence of the disease and those that yielded conflicting results.

To our knowledge, there are some researchers examined the prevalence of vit D deficiency in Saudi populations but our study was one from few studies aimed to examine the association between Vit D and calcium levels with hypothyroidism in Saudi Arabia mainly Qassim region. We therefore undertook this study to evaluate the levels of vitamin D and calcium among patients with hypothyroidism compared to healthy controls who did not complain from hypothyroidism or any thyroid diseases.

Our results revealed decreased serum 25 (OH) vit D levels in females than those of male controls and patients, otherwise this decrease was non-significant but we can refer this non-significant decrease to the small sample size of our study.

In concordance to our results, previous studies have observed that serum 25(OH)D

levels did not differ significantly between males and females.<sup>(22) (23)</sup>

Moreover, **Hashemipour et al**<sup>(24)</sup> studied the prevalence of Vit D in Tehran and found non-significant differences between males and females without association between Vit D and sunlight exposure. In contrast to our results, **Sedrani**,<sup>(25)</sup> **Al-Jurayyan et al**,<sup>(26)</sup> **Fida**,<sup>(27)</sup> **Naeem et al**,<sup>(28)</sup> stated that vit D serum levels are significantly more decreased in females than males. Although several authors have reported higher serum levels of 25(OH)D in normal men than in normal women,<sup>(29-30)</sup> data has not been available for patients with hypothyroidism. In Saudi Arabia, the prevalence of vitamin D deficiency was significantly lower in the elderly persons than in young students of both sexes, and was significantly higher in females than in males.<sup>(31)</sup>

However, a study from Japan including 200 euthyrotic patients with Graves' disease found vitamin D deficiency in 40% of women and around 20% of men ( $p < 0.005$ ).<sup>(32)</sup> The discrepancies between these studies can be explained by differences in the selection of patients, dietary vitamin D intake, exposure to sunlight, and seasonal variations.

Furthermore, the present study showed that vitamin D and calcium serum levels were significantly lower in hypothyroid patients compared to the controls. We recorded a significant positive association between Vit D and calcium levels in both groups.

Vit D and calcium serum levels had negative correlation when compared to TSH levels. These results suggested that there may be a significant association between vitamin D deficiency and hypothyroidism. Our results were in harmony with the previous studies that showed the prevalence of vitamin D insufficiency in Hashimoto's cases (92%) was significantly higher than that observed in healthy controls (63%,  $p < 0.0001$ ).<sup>(33-34)</sup>

**Byron Richards (2008)**<sup>(35)</sup> studied the effect of vit D deficiency on thyroid gland in experimental study, he reported that a lack of vitamin D contributed to the possibility of low thyroid hormones.

One of two mechanisms may explain the low levels of vitamin D in patients with hypothyroidism. First, the low levels of vitamin D may be due to poor absorption of vitamin D

from the intestine. Second, the body may not activate vitamin D properly.<sup>(12)</sup> Other articles have demonstrated that patients with Graves's disease also have low levels of Vitamin D.<sup>(36)</sup> Importantly, both vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. A different gene in the Vitamin D receptor was shown to predispose people to autoimmune thyroid disease including Graves' disease and Hashimoto's thyroiditis.<sup>(12)</sup>

Vitamin D inhibits the production of Th1 polarizing cytokine (IL-12), thereby indirectly shifting the polarization of T cells from a Th1 toward a Th2 phenotype. In the CD4+ T cell response, vitamin D directly inhibits the production of Th1 cytokines (IL2 and IFN- $\gamma$ ), and enhances Th2 cytokine (IL-4) production.<sup>(37)</sup> In addition, recent numerous studies have shown the relation of vitamin D and various autoimmune diseases. Vitamin D receptor (VDR) gene polymorphisms and vitamin D status are associated with different autoimmune diseases.<sup>(38-39)</sup> Furthermore, vitamin D supplementation prevented the onset and/or development of several kinds of autoimmune diseases in humans and animal models.<sup>(37)</sup> These results suggested that vitamin D deficiency might cause the onset and/or development of several kinds of autoimmune diseases.

Recent studies have demonstrated a role of vitamin D in Graves Disease (GD). First, Vitamin D related gene polymorphisms such as VDR gene and vitamin D binding protein gene are associated with GD. Second, Vitamin D deficiency modulates Graves' hyperthyroidism induced by thyrotropin receptor immunization in BALB/c mice. Third, Vitamin D analog inhibits inflammatory responses in human thyroid cells and T cells.<sup>(40-41)</sup>

On the other hand, study had been conducted in Netherlands showed that Vitamin D deficiency is not associated with early stages of thyroid autoimmunity.<sup>(42)</sup>

We also observed a significant difference in serum calcium levels between the studied groups with lower level seen in hypothyroid patients were it insignificantly decreased in females than male patients. A study conducted in showed significant changes in ionized Ca, but not total Ca means that the physiologically active form of Ca is affected, while the overall concentration of Ca is still significantly

unchanged.<sup>(43)</sup> Calcium has an effect on the hypothyroid patients; there is even no correlation with thyroid hormones parameters.

### Conclusion

Our results indicated that patients with hypothyroidism suffered from hypovitaminosis D with hypocalcaemia. Moreover, the positive significant correlation between each of serum vit D and calcium with thyroid hormones and that negative significant correlation with TSH levels, suggested that deficiency of serum vit D and calcium levels were significantly associated with degree and severity of the hypothyroidism which encourage the advisability of vit D supplementation. Screening for Vitamin D deficiency and serum calcium levels recommended for all hypothyroid patients.

### Limitations and Recommendations

The limitations of this study could be summarized in three points; first, the small number of subjects, second, limited in its ability to conclude that vitamin D status is directly related to the pathogenesis of hypothyroidism; third, we have to measure the parathyroid hormone (PTH) and clarify its effect on vit D and hypothyroidism disease.

Therefore, the direct role of vitamin D in those patients with thyroid problem should be examined by further prospective clinical studies and examine the effect of the treatment of vitamin D and PTH on hypothyroidism.

Further studies with a larger number of subjects are needed to determine whether vitamin D deficiency is a casual factor in the pathogenesis of hypothyroidism or rather a consequence of the disease. Moreover, supplementary Vit D and calcium are recommended to patients with hypothyroidism.

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