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**ORIGINAL ARTICLE**

# Assessment of Serum 25-Hydroxy Vitamin D Level among Hypothyroidism Sudanese Patients with and without Thyroid Peroxidase Antibodies

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## ABSTRACT

**Background:** The association between serum 25-Hydroxy vitamin D (25-OH vitamin D) levels in hypothyroid subjects with and without thyroid peroxidase (TPO) antibodies is controversial. There is increasing evidence that 25-OH vitamin D level is associated with autoimmune diseases. The study aimed to assess serum 25-OH vitamin D levels among hypothyroidism Sudanese patients with and without thyroid peroxidase (TPO) antibodies.

**Methods:** analytical cross-sectional conducted at The Center for Diabetes and Endocrinology in Khartoum from February to October 2020. Sixty subjects with hypothyroidism were recruited from the follow-up clinic. Other sixty age and sex-matched subjects were selected as control. Thyroid function tests (TSH, FT4, and FT3) were measured using TOSOH AIA 360 system analyzer, and serum 25-OH vitamin D level was measured using a semiautomatic I-Chroma-II reader and thyroid peroxidase antibodies (TPO antibodies) were measured in both cases and controls using ELISA technique. The results were analyzed using SPSS version 21.

**Results:** TSH was significantly increased in both TPO antibodies positive and TPO antibodies negative hypothyroid subjects than in the control (22.3±4.44), (10.5±2.55) (3.42±0.75), respectively, with *a P-value* of 0.000. The level of 25-OH Vitamin D was lower among hypothyroid subjects than in the control. Subjects with positive TPO antibody had lower 25-OH vitamin D levels than the TPO-antibody negative subjects compared to control (7.45±4.50) (10.5±7.18) (48.8±10.0), respectively, with *a P-value* of 0.000. Females 46 (77%) were more than males 14(23%).

**Conclusions**: 25-OH vitamin D level was low in patients with hypothyroidism. Subjects with positive TPO-antibodies had lower serum 25-OH vitamin D compared to TPO-antibody negative subjects.

**Keywords:** Antithyroid Peroxidase (TPO); Vitamin D deficiency; Hypothyroidism.

## INTRODUCTION

The major cause of vitamin D deficiency is the lack of exposure to sunlight, the major source of the vitamin for most humans [1]. However, 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 [2]. The diseases of the thyroid include the excessive release of thyroid hormone (hyperthyroidism) and those associated with thyroid hormone deficiency (hypothyroidism)[3]. Vitamin D is known for its primary role in bone and mineral homeostasis. It has recently been shown that its deficiency is associated with various diseases such as cardiovascular disease, cancer, infection, adiposity, and osteoporosis [4]. The discovery of vitamin D receptors in most tissues and cells in the human body has provided new insights into vitamin D's function as a unique hormone [5].

Recent studies have shown that vitamin D has potent immune-modulatory effects and plays an essential role in the pathogenesis of autoimmune diseases [6]. The discovery of the vitamin D receptor (VDR) in monocytes, dendritic cells, and activated T cells highlighted the potential involvement of vitamin D in the immune system and the pathogenesis of autoimmune diseases [6,

7]. As an immune modulator, vitamin D reduces activation of the acquired immune system. Active forms of vitamin D suppress autoimmune diseases by regulating differentiation and activity of CD4+ T cells resulting in a more balanced T1/T2 response favoring less development of self-reactive T cells and autoimmunity. Therefore, vitamin D deficiency could theoretically increase the risk of autoimmune diseases [6-8]. The serum concentration of 25(OH) D is the best indicator of vitamin D level. It reflects vitamin D produced cutaneously and obtained from food and supplements [9]. It has a relatively long circulating half-life of 15 days [10]. In contrast to 25(OH)D, circulating 1,25(OH)2D is generally not a good indicator of vitamin D level because it has a short half-life of 15 hours and serum concentrations are closely regulated by parathyroid hormone, calcium, and phosphate [10]. Levels of 1, 25(OH) 2D do not typically decrease until vitamin D deficiency is severe [11, 12].

So in this study, the serum levels of 25-OH vitamin D were measured to attain accuracy. Few studies have demonstrated the association between vitamin D levels and hypothyroidism and determined whether vitamin D deficiency is involved in the pathogenesis of hypothyroidism or rather a consequence of the disease. This study aimed to assess the vitamin D level in hypothyroid subjects with and without TPO antibodies.

## METHODS

***Study population:***

This cross-sectional study was conducted at The Center for Diabetes and Endocrinology from February to October 2020. Al-Neealin University research committee and the Center for Diabetes and Endocrinology approved this study in Khartoum approved the study. One hundred twenty subjects were recruited for the study. Subjects with hypothyroidism under follow-up with an endocrinologist were included in the study, while subjects with acute complications, clinical history of hypertension, renal disease, cancer, liver disease, diabetes, bone disease, pregnancy, on vitamin D supplements, age less than 18 years and more than 60 years were excluded from this study.

***Ethical consideration:***

Written informed consent was obtained from all the participants in the study. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

An interviewer-administered questionnaire was used to obtain demographic and clinical data from each participant. A physician carried out the participants' classification into study and control groups based on clinical history and examination.

***Sampling and Data Collection:***

Participants were divided into two groups based on their thyroid hormone levels. The first group, designated as the 'Control group', comprised sixty healthy subjects with ages ranging from 18 to 60 years, having a normal physical examination, and normal laboratory findings. The second group, designated as 'Case Group', included sixty subjects known with hypothyroidism with ages ranging from 18 to 60 years.

Blood samples were collected in plain containers to estimate the thyroid profile (TSH, FT4, and FT3), anti- TPO, and 25-OH vitamin D levels. TSH was measured based on a non-competitive immunoassay which was performed using a TOSOH AIA system analyzer. FT4 and FT3 were measured based on a competitive enzyme immunoassay performed using a TOSOH AIA system analyzer. The serum levels of 25-OH vitamin D in the samples were measured using fluorescence immunoassay (FIA), which was performed entirely with a semiautomatic I-Chroma- II reader. The anti-TPO was measured based on non-competitive ELISA and was performed manually and read using a STAT FAX microplate reader. The precision and accuracy of the techniques used in this study were checked each time a batch was analyzed using quality control materials.

Cases were diagnosed with hypothyroidism based on TSH levels higher than 4.3mIU/L, FT4 lower than 0.82 ng/dl, and FT3 lower than 2.17pg/ml. Cases are further classified into those who are anti-TPO positive when the antibody titer is >1I U/ml and those who are anti-TPO negative when the antibody titer is < 1I U/ml. The 25-OH Vitamin D levels in the subjects were classified as deficient when the result is <10ng/ml, insufficient when the result ranges from 10-30ng/ml, and sufficient when the result ranges from 30-100ng/ml.

***Statistical analysis***

SPSS software version 21was used to analyze the data and expressed as mean ± SD. Variables were compared between hypothyroid subjects and control groups by students' t-test, and the ANOVA method with a Probability value(*P*-value) of < 0.05 was considered significant

## RESULTS

A total of 120 subjects were enrolled of which 60 subjects had hypothyroidism and 60 subjects as a control group.

Table 1: Shows the mean ±SD age of case (n=60) (37.62±8.00) versus the control group (n=30) (40.77±10.37) (*p-value*=0.115) and the BMI mean±SD for the case (n=60) (27.63±9.17) versus the control group(n=60) (29.64±12.07) (*p-value*= 0.382).

Table 2: The mean ± SD of TSH in case was (15.8±2.53mIU/L) versus control (3.43±0.75mIU/L) (*p-value*=0.001), FT4 in case (0.54±0.20ng/dl) versus control (2.04±0.54ng/dl) (*p-value*=0.000), FT3 in case (0.74±0.41pg/ml) versus control (3.67±0.81pg/ml) (*p-value* =0.000), 25-OH vitamin D was significantly low in case (9.13±6.26 ng/ml) versus control (48.7±10.0 ng/ml) (*P-value*=0.000) and TPO antibodies in case (1.43±1.78) versus control (0.53±0.21) (*P-value* =0.007).

Table 3: The mean±SD of TSH in the TPO antibodies positive group was significantly high (22.3±4.44mIU/L) compared to the TPO antibodies negative group (10.5±2.55), both means are significantly higher than the control (3.42±0.75mIU/L) (*P-value* = 0.000). The mean ±SD FT4 in the TPO antibodies positive group was (0.51±0.21ng/dl) and the mean in the TPO antibodies negative was (0.56±0.19ng/dl) which was significantly lower than the control (2.04±0.55ng/dl) (*p-value*= 0.002). The mean ± SD of 25-OH vitamin D level in the hypothyroidism TPO antibodies positive group was (7.45±4.50ng/ml) and the TPO antibodies negative group was (10.5±7.18ng/ml) compared to the control (48.8±10.0ng/ml) they were significantly lower (*p-value*= 0.000).

Table 4: The mean ± SD of TSH in 25-OH vitamin D deficient patients was significantly higher (16.1±10.5mIU/L) than insufficient (8.73±6.33mIU/L) (*p-value* 0.033). FT4 also more lower in 25-OH vitamin D deficient patients (0.54±0.20ng/dl) compared to insufficient (0.65±0.23ng/dl) (*p-value* 0.011). FT3 in 25-OH vitamin D deficient patients was also lower (0.48±0.03pg/ml) compared to insufficient (0.75±0.42pg/ml).

The mean of TPO antibodies in 25-OH vitamin D deficient patients was (1.49±1.2) higher compared to the insufficient group (0.19±0.15) with (a *p-value* of 0.002).

Figure 1: Shows the distribution of case groups according to gender: 14
(23%) were males while 46 (77%) were females.

Figure 2: Shows that 24(40%) of the subjects were housewives while 36(60%) were working-class of both genders.

Figure 3: Shows the geographical distribution of the subjects. It indicates that 55% of subjects were from the central part of Khartoum, with 23% from the North, 10% from the West, 10% from the South, and 2% from the Eastern part of Khartoum.

Figure 4: Shows the distribution of the study group according to their 25-OH vitamin D level status.

**Table 1:** Mean±SD of Age and BMI among the Study Group

| Variables | Case (Mean±SD) | Control (Mean±SD) | *P-value* |
|---|---|---|---|
| Age years | 37.62±8.00 | 40.77±10.37 | 0.115 |
| BMI kg/m | 27.63±9.17 | 29.64±12.07 | 0.382 |

**Table 2:** Comparison of TSH, FT4, FT3, TPO, and 25-OH Vitamin D levels between case and control group**.**

| Parameters | Case (Mean±SD) | Control (Mean±SD) | *P-value* |
|---|---|---|---|
| TSH (mIU/L) | 15.8±2.53 | 3.43±0.75 | 0.001 |
| FT4 (ng/dl) | 0.54±0.20 | 2.04±0.54 | 0.000 |
| FT3 (pg/ml) | 0.74±0.41 | 3.67±0.81 | 0.000 |
| 25-OH Vitamin D (ng/ml) | 9.13±6.26 | 48.7±10.0 | 0.000 |
| TPO Ab (IU/ml) | 1.43±1.78 | 0.53±0.21 | 0.007 |

**Table 3:** Comparison of the mean ± SD of TSH, FT4, FT3, and 25- OH vitamin D levels in anti-TPO in hypothyroidism (positive and negative) patients versus the control group**.**

| Parameters | Mean±SD | | | *P-value* |
|---|---|---|---|---|
| | Anti-TPO Negative (<1IU/ml) | Anti-TPO positive(>1IU/ml) | Control | |
| TSH mIU/L | 10.5±2.55** | 22.3±4.44** | 3.42±0.75 | 0.000 |
| FT4 ng/dl | 0.56±0.19** | 0.51±0.21** | 2.04±0.55 | 0.002 |
| FT3 pg/ml | 0.73±0.49** | 0.73±0.31** | 3.67±0.81 | 0.000 |
| 25-OH VitaminD ng/ml | 10.5±7.18** | 7.45±4.50** | 48.8±10.0 | 0.000 |

**Table 4:** Comparison of TSH, FT4, FT3, and TPO antibodies according to 25-OH vitamin D level.

| Parameters | Insufficient (Mean±SD) (10-30ng/ml) | Deficient (Mean±SD) (<10ng/ml) | *P-value* |
|---|---|---|---|
| TSH mIU/L | 8.73±6.33 | 16.1±10.5 | 0.033 |
| FT4 ng/dl | 0.65±0.23 | 0.54±0.20 | 0.011 |
| FT3 pg/ml | 0.75±0.42 | 0.48±0.03 | 0.000 |
| TPO | 0.19±0.15 | 1.49±1.2 | 0.002 |

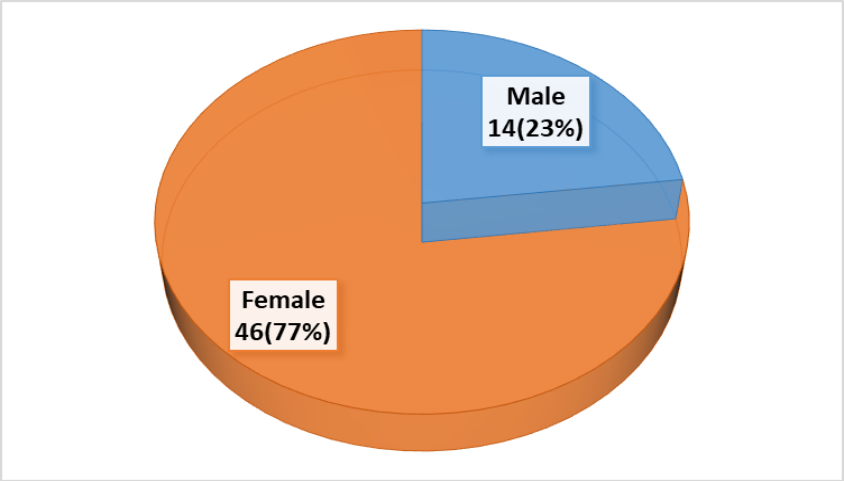


**Figure 1:** Distribution of case groups according to their gender

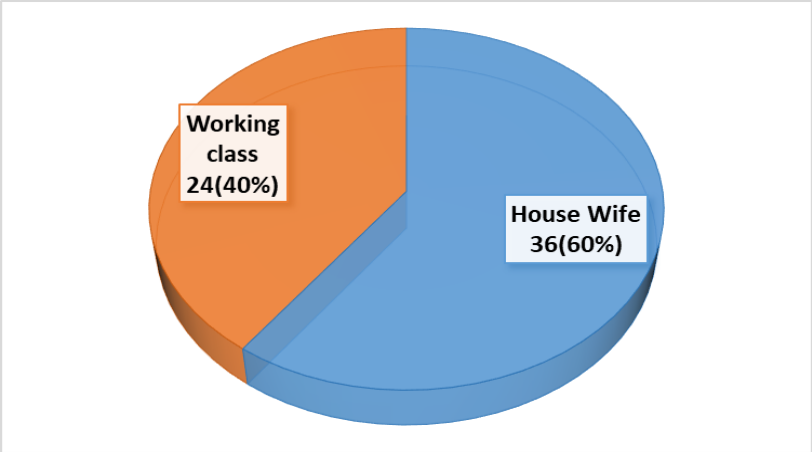


**Figure 2:** Distribution of case groups according to their Working status.

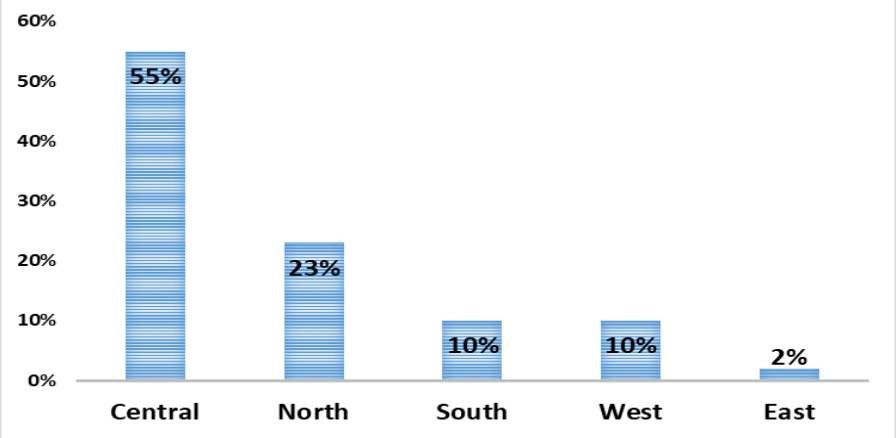


**Figure 3:** Distribution of study groups according to their location

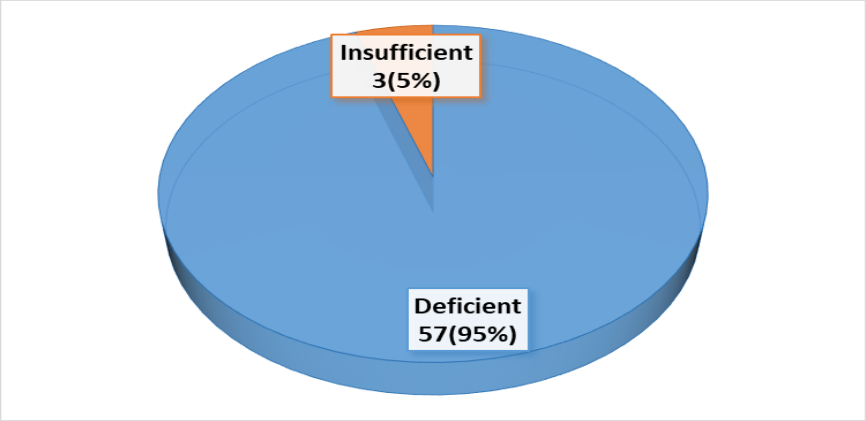


**Figure 4:** Distribution of study group according to their vitamin D status.

## DISCUSSION

In this study, a total of Ninety (90) subjects were recruited. Sixty (60) of these subjects had hypothyroidism. Out of the (60) hypothyroid subjects: 14(23%) were males, while 46(77%) were females. This value indicates that the disease's prevalence is more in females than in males (figure 1). This agrees with Anaya J.M*etal.* Their study stated that hypothyroidism is the most prevalent organ-specific disease and affects 2 - 5% of the population with significant variability between genders (i.e., women 5–15% and men 1–5%) [13]. The same finding was also confirmed by Z Meng et al [14] who reported that the prevalence of hypothyroid is common in females. This may be to the fact that females are more susceptible to autoimmune diseases than males. As had been reported recently, many autoimmune disorders tend to affect women during periods of extensive stress, such as pregnancy, or during a great hormonal change. This is comparable to our findings as the patients were of the same age.

The thyroid function results show that TSH and TPO antibodies were significantly increased, especially in TPO antibody-positive than in TPO antibody-negative subjects compared to the control. FT3 and FT4 have significantly decreased in TPO antibody-positive subjects than in TPO antibody-negative subjects compared to control. This agrees with the study done by Sajan Christopher *et al*., which found that autoimmunity is closely related to thyroid function, and increasing autoimmunity is directly related to the worsening thyroid function as seen by increasing TSH levels in anti-TPO positive patients [14]. In contrast, the25- OH vitamin D levels in TPO antibody-positive subjects were significantly decreased than in TPO antibody-negative subjects when compared to control. This agrees with the study done by Idiculla J. *et al*, that 25-OH vitamin D levels in patients with hypothyroidism were significantly lower than in euthyroid controls and that TPO-Abs positive patients had lower levels of 25- OH vitamin D in comparison to those who are negative to Anti-TPO [15].

Brent et al, reported that radiation is the most risk factor linked to effects on the thyroid. And the most common thyroid manifestation of radiation is hypofunction [16]. This finding agrees with our results, we found that 55% of patients with autoimmune hypothyroidism are from the center of Khartoum where medical radiations are more.

This study compares subjects that are deficient in 25-OH vitamin D and those with insufficiency, showing an increase in TSH and Anti-TPO among those with a deficiency in 25-OH vitamin D. In contrast,

FT4 and FT3 were lower in 25-OH vitamin D deficient subjects than in subjects with insufficient25-OH vitamin D. This relationship confirms Richards Byron's (2008) findings in his experimental study that explored the effect of vitamin D deficiency on the thyroid gland. In this study, he reported that a lack of vitamin D contributed to the possibility of low thyroid hormones [17]. The study done by Tamer *et al*. relative vitamin D insufficiency in Hashimoto's thyroiditis suggests that vitamin D deficiency is more closely related to anti-thyroid antibody titer than thyroid function itself in humans[18]. *Dong Yeob Shin et al.*, in their study on low serum vitamin D, is associated with anti-thyroid peroxidase antibody in autoimmune thyroiditis[19].

## CONCLUSIONS

25-OH Vitamin D levels were significantly decreased in subjects with hypothyroidism. TPO antibody-positive subjects suffered more from hypovitaminosis D than those negative for TPO antibody. The decrease in 25-OH vitamin D level was found to be associated with autoimmune hypothyroidism**.**

***Limitations :***

The limitations of this study include the following; most of the participants were females. Inability to fully confirm that vitamin D deficiency was the main trigger of autoimmune hypothyroidism and it should be screened for other autoimmune endocrine diseases.

***Conflict of Interest:*** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

***Financial Disclosures:*** None

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