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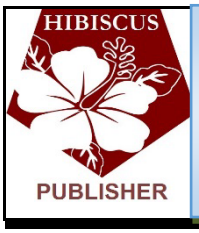

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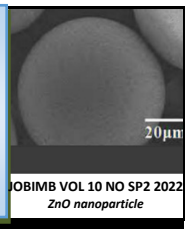

Assessment of Serum 25-Hydroxy Vitamin D Level Among Hypothyroidism with and without Thyroid Peroxidase Antibodies

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ABSTRACT

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 analytical cross-sectional case-control and hospital-based study were conducted at Professor Al-Mahdi M. Ali Center for Diabetes and Endocrinology in Khartoum from February to October 2020. Sixty subjects with hypothyroidism were recruited from the follow-up clinic. Thirty age and sex-matched subjects were used as control. Thyroid Function Tests [Thyroid-stimulating hormone (TSH), Free Thyroxine (FT4), and Free triiodothyronine (FT3)] were measured using TOSOH AIA 360 system analyzer, and serum 25-OH vitamin D level was measured using semiautomatic I-Chroma-II reader and thyroid peroxidase antibodies (TPO antibodies) were measured in both cases and controls was estimated using ELISA. The results were analyzed using SPSS version 21. TSH was significantly increased in both TPO antibodies positive and TPO antibodies negative hypothyroid subjects than in the controls (22.3±4.44), (10.5±2.55) (3.42±0.75) with a *P-value* of 0.000. FT4 has decreased in both TPO antibodies positive and TPO antibodies negative subjects than in the control (0.51±0.21) (0.56±0.19) (2.0±0.55) with *a P-value* of 0.002. FT3 has significantly decreased in both TPO antibodies positive and TPO antibodies negative subjects than in the control (0.73±0.31)(0.73±0.49)(3.67±0.81) with a p-value of 0.0001. 25-OH Vitamin D level was lower among hypothyroid subjects than in the control. Subjects with positive TPO antibodies 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) with *P-value* 0.000. Females 46(77%) were more than males 14(23%) and geographically most subjects were coming from the central part of Khartoum state.

INTRODUCTION

Vitamin D deficiency is common all over the world. The major cause of vitamin D deficiency is the lack of appreciation that sun exposure in moderation is the major vitamin D source 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 conditions associated with 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 [2].

Recent studies have shown that vitamin D has potent immunomodulatory effects and plays an essential role in the pathogenesis of autoimmune diseases [5]. 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 [5,6]. As an immune modulator, vitamin D reduces the 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 favouring less development of self-reactive T cells and autoimmunity. Therefore, vitamin D deficiency could theoretically increase the risk of autoimmune diseases [4,5,7]. 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 [8] and has a relatively long circulating half-life of 15 days [9]. 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].

In this study, the serum levels of 25-OH vitamin D rather than 1, 25(OH) 2 vitamin D levels 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. Other studies yielded conflicting results. In Sudan, some researchers examined the prevalence of vitamin D deficiency among the general Sudanese population. However, this study aimed to assess the vitamin D level in hypothyroid subjects with or without TPO antibodies.

MATERIALS AND METHODS

Study population

This cross-sectional case-control and hospital-based study were conducted at Professor Mahdi M. Ali's Center for Diabetes and Endocrinology from February to October 2020. The Sudan International University research committee and the management of Professor Mahdi M. Ali Center for Diabetes and Endocrinology in Khartoum approved the study. Ninety subjects were recruited for the study. **Inclusive**: Follow-up subjects with hypothyroidism. **Exclusion:** Subjects with acute complications, clinical history of hypertension, renal disease, cancer, liver disease, diabetes, bone disease, pregnancy, on vitamin D supplements, and 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. 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', comprises thirty healthy subjects with ages ranging from 18 to 60 years, who have a normal physical examination, and normal laboratory findings. The second group, designated as the 'Case Group', included sixty subjects known to have hypothyroidism with ages ranging from 18 to 60 years. Cases were diagnosed with hypothyroidism based on TSH levels higher than 4.3 mIU/L, FT4 lower than 0.82 ng/dl, and FT3 lower than 2.17 pg/mL. Cases are further classified into those who are anti-TPO positive when the antibody titre is >1IU/mL and those who are anti-TPO negative when the antibody titre is < 1IU/mL. The 25-OH Vitamin D level in the subjects is diagnosed to be deficient when the result is <10 ng/mL, insufficient when the result ranges from 10-30 ng/mL and sufficient when the result ranges from 30-100 ng/mL. Blood samples were collected in plain containers to estimate the thyroid hormones TSH, FT4, FT3, anti- TPO and 25-OH vitamin D levels. TSH was measured based on a non-competitive immune enzymometric assay, which was performed entirely in the ST AIA-PACK TSH test cups using the TOSOH AIA system analyzer. FT4 and FT3 were measured based on a competitive enzyme immunoassay performed entirely in the test cups of the 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 semiautomatic I-Chroma- II readers. 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 material for;
Anti-TPO ELISA (IgG, human) positive and negative control,
TSH TOSOH ST AIA-PACK three readers Multi-Analyte Control FT4 TOSOH ST AIA – PACK three readers Multi-Analyte Control FT3 TOSOH ST AIA – PACK three reader Multi-Analyte Control Ichroma™ Vitamin D Control.

Statistical analysis

SPSS software version 21 was 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 AND DISCUSSION

A total of 90 subjects were enrolled of which 60 subjects had hypothyroidism and 30 subjects as a control group.
Table 1 shows the mean age of the case versus the control group and the BMI versus the control group. **Table 2** shows the mean of TSH in case versus controls and various cases versus control groups. **Table 3** shows the comparison of the mean of TSH, FT4, FT3 and 25- OH vitamin D levels in anti-TPO in hypothyroidism (positive and negative)patients versus the control group. **In Table 4,** the comparison of TSH, FT4, FT3 and TPO antibodies according to 25-OH vitamin D level is shown.

Fig. 1. Shows the distribution of case groups according to gender: 14 (23%) were males while 46(77%) were females, while **Fig. 2** shows that 24(40%) of the subjects were housewives while 36(60%) were working-class of both genders. **Fig. 3** shows the geographical distribution of the subjects. It indicates that 55% of subjects are 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. **Fig. 4** shows the distribution of the study group according to their 25-OH vitamin D level status.

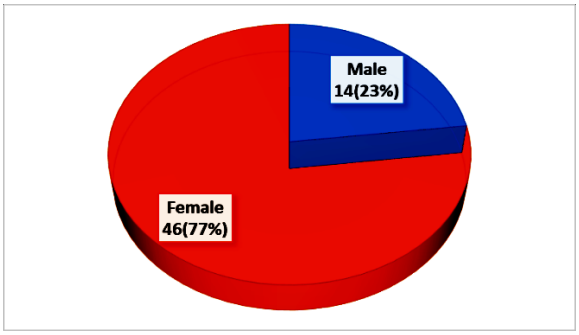

Fig. 1. Distribution of case groups according to their gender

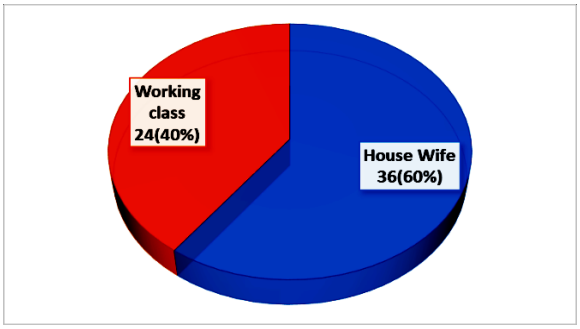

Fig. 4. Distribution of case groups according to their Working status.

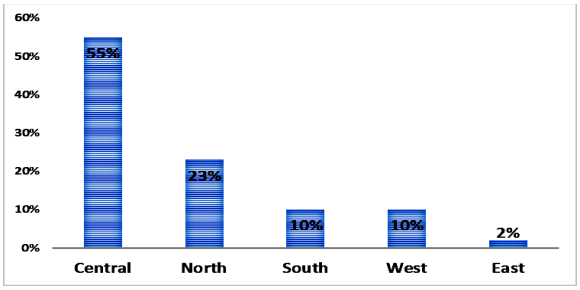

Fig. 3. Distribution of study groups according to their location

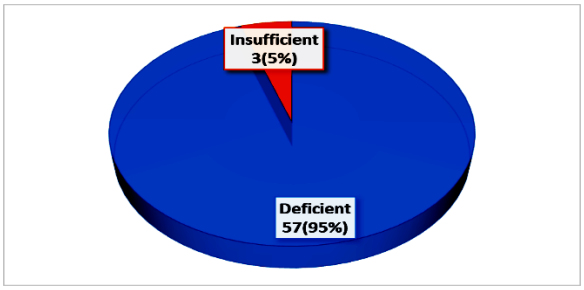

Fig. 4. Distribution of study group according to their vitamin D status.

Table 1. Mean±SD age and BMI among the study groups

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

Note:
The table shows the mean±SD and probability *p-value*
p-value<0.05is considered significant.
A t-test was used for the comparison.

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 VitaminD ng/mL	9.13±6.26	48.7±10.0	0.000
TPO IU/mL	1.43±1.78	0.53±0.21	0.007

Note:
The table shows the mean±SD and probability *p-value*
p-value<0.05 is considered significant.
A t-test was used for the comparison.

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

Note:
The table shows the mean±SD and probability *p-value*
p-value <0.05 is considered significant.
ANOVA was used for the comparison.

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

Note:
The table shows the mean±SD and probability *p-value*
p-value <0.05 is considered significant.
t-test was used for the comparison

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 female than males (**Fig. 1**). This agrees with Anaya J.M *et al.* their study states 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 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 were significantly decreased in TPO antibody-positive subjects than in TPO antibody-negative subjects compared to the 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, the 25- OH vitamin D levels in TPO antibody-positive subjects were significantly decreased than in TPO antibody-negative subjects when compared to the control. This agrees with the study done by Kumari *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]. 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 a subject with insufficient 25-OH vitamin D. This relationship confirms with Răcătăianu (2018) findings in his experimental study 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[16].

The study done by Tamer G *et al*. on 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 [17]. Dong Yeob Shin *et al.,* in their study on low serum vitamin D is associated with anti-thyroid peroxidase antibody in autoimmune thyroiditis [18] Our study recommended that Screening for 25-OH vitamin D deficiency is essential in all hypothyroid subjects. Further studies with a larger sample size could yield more data. A different geographical location in Sudan should be considered. Studying other clinical parameters such as calcium, lipid profile, vitamin D gene-related polymorphism and vitamin D binding protein could also be considered.

CONCLUSION

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 antibodies. The decrease in 25-OH vitamin D level was found to be associated with autoimmune hypothyroidism.

LIMITATIONS

The limitation of this study includes the following:
Most of the participants were females.
The sample size was small.
Inability to fully confirm that vitamin D deficiency was the main trigger of hypothyroidism and autoimmune disease.

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