Publisher
MEDRESEARCH
www.medresearch.in

International Journal of Medical Research and Review

2020 Volume 8 Number 4 July-August

E-ISSN:2320-8686
P-ISSN:2321-127X

Research Article
Hypothyroidism

Is there any association between vitamin D deficiency and anti-thyroid peroxidase positive hypothyroidism: A cross-sectional study

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DOI: https://doi.org/10.17511/ijmrr.2020.i04.02

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Background: Vitamin D deficiency is prevalent worldwide and it is believed to have a role as an immune modulator. However, the association between vitamin D levels and anti-thyroid peroxidase positive (TPOAb) hypothyroidism is still controversial. **Aim:** To elucidate the association between vitamin D levels and anti-thyroid peroxidase (TPOAb) positive hypothyroidism. **Materials and Methods:** Serum Vitamin D, thyroid peroxidase antibody, and thyroid function test were measured in 105 patients, who were sub-grouped into the TPOAb positive and TPOAb negative hypothyroidism category. **Results:** Vitamin D level, was found significantly lower in patients with TPOAb positive hypothyroidism as compared to patients TPOAb negative hypothyroidism (13.27±5.18vs. 17.74±6.03ng/ml, respectively, $P<0.05$), as well as between patients with TPOAb positive hypothyroidism and control group (13.27±5.18vs. 29.66±9.41 ng/ml, respectively, $P<0.05$). Within the patients' group, there was a significant negative correlation between serum 25 (OH) vitamin D and TSH ($r=-0.438$, $P<0.05$), anti-TPO ($r=-0.275$, $P<0.05$). Furthermore, insignificant positive correlations were recorded between serum 25 (OH) vitamin D, and each of T3, T4 ($r=-0.056$, 0.097, $P>0.05$). **Conclusion:** The current study observed significant low levels of 25(OH)D3 in TPOAb positive hypothyroid patients.

Keywords: Vitamin D deficiency, anti-thyroid peroxidase antibody, autoimmune thyroiditis

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How to Cite this Article

Shankar M, Singh M. Is there any association between vitamin D deficiency and anti-thyroid peroxidase positive hypothyroidism: A cross-sectional study. Int J Med Res Rev. 2020;8(4):295-301.
Available From
https://ijmrr.medresearch.in/index.php/ijmrr/article/view/1203

To Browse

Introduction

More than 1 billion people worldwide have been suffering from vitamin D deficiency [1]. Recent studies have shown that vitamin D acts as an immune-modulator and has an important role in reducing the risk of chronic Illnesses including diabetes mellitus, kidney diseases, infectious, cardiovascular diseases, and autoimmune diseases such as autoimmune thyroid diseases (AITD) [2-8].

Vitamin D and thyroid hormone have common receptors and bind to steroid hormone

Manuscript Received	Review Round 1	Review Round 2	Review Round 3	Accepted
2020-07-29	2020-08-11	2020-08-21		2020-08-25

Conflict of Interest	Funding	Ethical Approval	Plagiarism X-checker	Note
No	Nil	Yes	7%	

Receptors. Gene polymorphism in the Vitamin D receptor (VDR) has been shown to predispose people to AITD. [9,10]. In recent studies, Vitamin D has been known as an immunomodulator in autoimmune diseases such as Hashimoto's thyroiditis (HT) and Grave's disease [9-10].

Few studies have examined the association between the levels of Vitamin D and TPOAb positive hypothyroidism but found conflicting results. A study by Goswami et al. found a weak association between 25(OH)D3 levels and thyroid peroxidase antibody (TPOAb) titers [11].

Whereas study by Kivity and colleagues [12] found significantly low levels of 25(OH)D3 with autoimmune thyroid disease, Hence, the current study hypothesized that there may be an association between vitamin D levels and thyroid peroxidase antibody (TPOAb) positive hypothyroidism.

Aim

Hence, the current study aimed to examine the association between vitamin D levels and anti-thyroid peroxidase (TPOAb) positive hypothyroidism.

Materials and Methods

Setting: Tertiary care center located in north India, in the Lab medicine, department of Pathology.

Duration and type of study: Conducted from Fab 2020 to May 2020.

Sampling Methods: Patients were selected consecutively who attended lab medicine.

Sample Size calculation: A total of one hundred and five subjects were included in this study.

Inclusion criteria: (1) Age >18 years with recently diagnosed hypothyroidism not on treatment, (2) not on Vitamin D supplements, and (3) willing to participate in the study.

Exclusion criteria:(1) any chronic illness (2) temporary hypothyroidism such as drug-induced, in pregnant women, and patients who took vitamin or calcium supplements and medications that may interfere with serum levels of 25 (OH) vitamin D (e.g., antiepileptics, steroids, etc) (3) not willing for participation in the study The patients who met the inclusion criteria were included. A complete relevant history and physical examination were done.

Participants were divided into the following groups: group I included 70 patients with hypothyroidism representing the case group, and this group was further subdivided according to retrospective selection into 35 patients with TPOAb positive and TPOAb negative hypothyroidism. Group II included 35 healthy participants as a control group.

Ethical consideration and Permission: Written informed consent was taken from all participants.

Laboratory measurements: After overnight fasting for 10–12 hours, blood samples were collected for the following measurements: 25 (OH) vitamin D, thyroid-stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3) and TPOAb.

After aseptic precaution, a blood sample was collected by venipuncture at the fasting state, the serum was separated by centrifugation and then stored at –20oC for a week until analyzed. Vitamin D status was evaluated by measurement of serum 25(OH)D3 levels, thyroid function test with a chemiluminescent immunoassay method (Seimens Adiva Century CP system).

Serum 25 (OH) vitamin D concentrations of less than 10 ng/ml were defined as vitamin D deficiency and from 10 to 29 ng/ml as insufficiency. A value of 25(OH) vitamin D concentration from 30 to 100 ng/ml was considered as a normal vitamin D level, whereas greater than 100 ng/ml as toxicity. They were diagnosed as hypothyroid patients if the TSH level was higher than 6.2mlU/ml with lower levels of T3 (Ref. range =0.69-2.02ng/ml) and T4 (Ref. range = 4.4-11.6mg/ml) than normal value.

Estimation of anti-thyroid peroxidase antibodies (anti-TPO) was done by (Cobas e 411 analyzer Roche diagnostic, U.S.A.) electrochemiluminescence immunoassay. Values greater than >27.6IU/ml (TPOAb) were considered positive.

Statistical Analysis: Data were statistically analyzed by SPSS version-23 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 studied groups. The differences between mean values for each tested variable have been tested by the Student's "t" test. Correlation between variables of interest was performed using Pearson's correlation. Results are considered significant when the p-value is < 0.05.

Results

This study enrolled 105 participants who were subdivided into two groups: Group I included 70 patients with hypothyroidism representing the case group, and this group was further subdivided according to retrospective selection into 35 patients with TPOAb positive hypothyroidism and TPOA negative hypothyroidism. Group II included 35 healthy participants as a control group.

The clinicopathological characteristics of the study groups are listed in Table 1. Age and sex distribution showed no statistical difference (P>0.05) between the patients and control groups (P>0.05). Serum levels of 25 (OH) vitamin D recorded a significant difference between the studied groups as illustrated in Table 1. Regarding vitamin D sufficiency, it was revealed that 25 (OH) vitamin D was deficient in 28.6% (20/70) and insufficient in 70% (49/70) of the patient group, whereas it was 1.4% (1/70) sufficient in the patients. On the contrary, 25 (OH) vitamin D was deficient in only 8.6% (3/35), insufficient 65.7% (23/35), and sufficient in 25.7% (9/35) of the control group (P<0.01).

On comparing patients with TPOAb positive hypothyroidism with TPOAb negative hypothyroid-ism and each of them with the control group (Table 2), there was an insignificant difference in Age and sex. Moreover, there was an insignificant difference in TSH between patients with TPOAb positive and TPOAb negative hypothyroidism (P>0.05)

Regarding 25 (OH) vitamin D level, there was a significant difference between patients with TPOAb positive and patients with TPOAb negative hypothyroidism (P<0.05), as well as between patients with TPOAb positive hypothyroidism and control group (P<0.05), and between patients TPOAb negative hypothyroidism and control group (P<0.05) [Table 2] (Fig. 1).

Within the patient's group (Table 3), insignificant positive correlations were recorded between serum 25 (OH) vitamin D, and each of T3, T4 (P>0.05). On the contrary, there was a significant negative correlation between serum 25 (OH) vitamin D and TSH (r=−0.438, P<0.05), anti-TPO (r=−0.275, P<0.05).

It was found that no significant association among Serum 25(OH) D3 levels, TPOAb, and TSH levels in TPO Ab positive hypothyroid patients according to sex (Table 4).

Table-1: Comparison between the study groups regarding the clinicopathological characteristics.

Variants	Group I=patients (n=70) (mean ±SD)	Group II=control (n=35) (mean±SD)	t-test t/χ2a	p-value
Age (years)	38.49±12.77	37.74±13.16	0.214	0.832
Sex F/M [n (%)]	57(81%)/13(19%)	27(77.2%)/8(22.9%)	0.000a	1.000
T3 (pg/dl)	0.879±.25	1.2±1.03	-2.139	.040
T4 (ng/dl)	7.5±2.40	8.51±2.1	-1.029	0.311
TSH (mlU/ml)	10.56±6.2	1.9±1.12	7.44	0.000
25(OH)D3 (ng/ml) VitD sufficiency [n (%)] Deficient Insufficient Sufficient	15.51±6.0220(28.57%) 49(70%) 1(1.4%)	29.66±9.413(8.6%) 23(65.7%) 9(25.7%)	-5.455 15.947a	0.0000.000

Table-2: Clinicopathological comparison between patients with TPOAb positive, TPOAb negative hypothyroidism, and control group.

Variants	TPOAb positive hypothyroidism (n=35)(mean±SD)	TPOAb negative hypothyroidism (n=35)(mean±SD)	Control group (n=35) (mean±SD)	One way ANOVA TESTF/χ2a	P-value	P1	P2	P3
Age (years)	38.37±12.35	38.60±13.66	37.74±13.16	4.410	0.202	0.937	0.832	0.771
Sex F/M [n (%)]	28(80%)/7(20%)	29(82.9%)/6(17.1%)	27(77.2%)/8(22.9%)	3.420	0.112	0.06	0.232	0.435
T3 (pg/dl)	0.894±.243	0.863±.27	1.2±1.03	0.908	0.655	0.647	0.040	0.023
T4 (ng/dl)	8.006±2.3	7.04±2.3	8.51±2.1	5.8	0.155	0.057	0.311	0.005
TSH (mlU/ml)	9.7±5.8	11.42±6.55	1.9±1.12	0.752	0.721	0.272	0.000	0.000

25 (OH) vitaminD (ng/ml)	13.27±5.18	17.74±6.03	29.66±9.41	56.89	0.017	0.002	0.000	0.000
Vitamin D sufficiency [n (%)] Deficient Insufficient Sufficient	12(34.3%) 23(65.7%) 0(0%)	8(22.8%) 26(74.3%) 1(2.8%)	3(8.6%) 23(65.7%) 9(25.7%)	19.233a	0.003	0.003	0.001	0.013

ANOVA, analysis of variance; F/M, female/male; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone. χ test. P1, patients with TPOAb positive hypothyroidism versus patients with TPOAb negative hypothyroidism. P2, patients with TPOAb positive hypothyroidism versus the control group. P3, patients TPOAb negative hypothyroidism versus the control group. A P-value of less than 0.05 is significant. A P-value of less than 0.01 is highly significant.

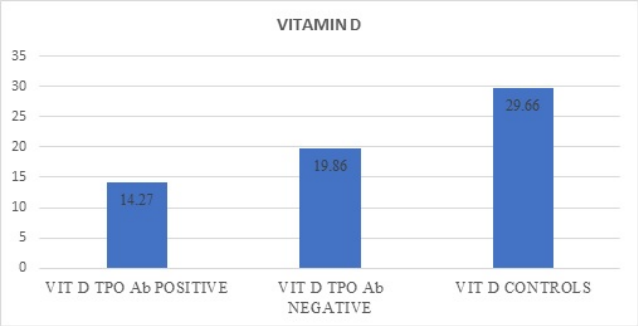

Fig-1: Comparison of 25 (OH) vitamin D levels between TPOAb positive, TPOAb negative hypothyroid patients and control group.

Table-3: Correlation between 25 (OH) vitamin D and other variables in the patient group.

Variants	r (correlation coefficient)	P value
Age (years)	0.343	0.433
T3(pg/dl)	0.056	0.645
T4 (ng/dl)	0.097	0.425
TSH (mlU/ml)	-0.438	0.000
Anti TPO IU/ml	-0.275	0.024

Table-4: Serum 25(OH) D3, TPOAb, and TSH levels in TPO Ab positive hypothyroid patients according to sex.

Parameters	Male n=7 (20%) [Mean ±SD]	Female n=28(80%) [Mean ±SD]	t-test, p-value
Age (years)	43.29±13.16.22	37.14±11.21	t=-0.630, p=0.552
TSH (mlU/ml)	8.75±2.1	11.15±10.44	t= 0.521, p=0.621
TPOAb (IU/ml)	84.71±73.42	365.56±425.94	t=1.577, p=0.166
25(OH) Vit D (ng/ml)	12.70±5.7	15.56±7.4	t=1.343, p=0.228

Discussion

In recent studies, Vitamin D has been known as an immunomodulator in autoimmune diseases such as Hashimoto's thyroiditis (HT) and Grave's disease [9-10]. The goal of the present study was to investigate the vitamin D status in patients with TPOAb positive hypothyroidism. Few studies have shown vit D deficiency is associated with hypothyroidism but its role as a cause of hypothyroidism or consequence of hypothyroidism is yet to be elucidated.

In the present study, vitamin D status was deficient in 28.57% (20/70) and insufficient in 70% (49/70) patients with hypothyroidism, whereas it was deficient in 8.6% (3/35) and insufficient in 65.7% (23/35) in the control group. It was found that 34.3% (12/35) of patients with TPOAb positive hypothyroidism had vitamin D deficiency, whereas only 22.8% (8/35) of patients with TPOAb negative hypothyroidism had vitamin D deficiency (P<0.05). Furthermore, 65.7% (23/35) of patients with TPOAb positive hypothyroidism were vitamin D insufficient in comparison with 74.3% (26/35) of patients with TPOAb negative hypothyroidism. The community-based Indian studies done on apparently healthy controls reported a prevalence of vit d deficiency is ranging from 50% to 94%.13-16 Prevalence of 25 (OH)D3 estimated in urban population was 75% in females and 62% in males, whereas the prevalence of Vitamin D deficiency was slightly lower in a rural area as 70% in females and 44% in males [17]. The prevalence of Vitamin D deficiency was found to be high in hypothyroid patients in the present study when compared to the general Indian population as described in earlier studies.

The levels of vitamin D in patients with hypothyroidism are reduced by two mechanisms. Firstly, it may be the result of poor absorption from the intestine. Secondly, vitamin D may not activate the body properly [18]. Both vitamin D and thyroid hormone bind to common receptors called steroid hormone receptors. Hence, a gene polymorphism in the vitamin D receptor may be a risk factor for the development of AITD [18].

There was a significant difference regarding vitamin D level among the studied groups and (15.98±7.

15ng/ml versus 29.66±9.41ng/ml, p<0.000) control group. Our results are in agreement with Kivity et al who in their study found that the prevalence of vitamin D deficiency [25 (OH) vitamin D level <25 nmol/l] was significantly higher in patients with AITD compared with healthy individuals (P<0.001). They also reported Vitamin D deficiency was correlated with the presence of antithyroid antibodies (P=0.01), hence the vitamin D has a role in the pathogenesis of AITD [12].

However, in the present study serum, TSH level was higher in patients TPOAb negative hypothyroidism (11.42±6.55 mlU/ml) than in patients with TPOAb positive hypothyroidism (9.7±5.8 mlU/ml) but it is not significant (P>0.05). This may suggest that vitamin deficiency is more closely related to thyroid antibodies rather than thyroid function.

It was found within the patient's group (Table 3), insignificant positive correlations were recorded between serum 25 (OH) vitamin D and each of T3, T4 (r=0.056 and 0.097, P>0.05). Moreover, there was a significant negative correlation between serum 25 (OH) vitamin D, TSH, and anti-TPO (r=−0.275, −0.438 respectively p<0.05).

Our results are in concordance with the study by Shin et al. and Khare J et al who reported that patients with elevated anti-thyroid antibodies had lower levels of serum 25 (OH) vitamin D3 than others (P<0.05) [19,20]. Similarly, Tamer et al. concluded that the prevalence of vitamin D insufficiency [25 (OH) vitamin D level <75 nmol/l] in 161 HT cases was significantly higher than in 162 healthy controls (92 vs. 63%, respectively, P<0.0001) [9]. Furthermore, Colak et al. reported that 94.4% of patients with HT had vitamin D deficiency [21].

Mansournia et al in their study also found a significant inverse association between serum 25 (OH) vitamin D levels and TSH in HT [22].In addition, Metwalley et al and Salwa S Hosny et al have shown that vitamin D deficiency has a negative correlation between 25 (OH) vitamin D anti-TPO and TSH (r= −0.533, −0.445 and −0.582, −0.459, respectively [p<0.05]) [23,24]. Their results are in agreement with the present study.

However, in contrast to the present study Yasmeh et al. have found no association with anti-TPO positivity and weak inverse correlation between 25 (OH) vitamin D and anti-TPO levels [25] Study by Goswami et al. found a weak association between 25(OH)D3 levels and thyroid peroxidase antibody (TPOAb) titers [11].

In this study, when compared with Serum 25(OH) D3, TPOAb, and TSH levels in TPO Ab positive hypothyroid patients according to sex no significant correlation was found.

(Table 4) Khare et al in their study when they compared according to sex, found no significant association with TSH and TPOAb levels similar to the present study. But they found a significant association with females and Vit D deficiency [20].

The conflicting and variable results of the studies are partly owing to interlaboratory and inter-assay variability in the measurements of 25 (OH) vitamin D, differences in the selection of patients, exposure to sunlight, seasonal variations, dietary vitamin D intake and the different cutoff levels used to define vitamin D deficiency or insufficiency.

Limitation

The present study sample size was small and antithyroglobulin antibody and thyroglobulin levels were not measured.

Conclusion

This study shows the prevalence of Vitamin D deficiency is higher in hypothyroid patients as compared to the general population. Furthermore, the current study found that 25(OH)D3 was significantly low in TPOAb-positive as compared to TPOAb-negative hypothyroid patients. However, Vitamin D supplementation is helpful to these hypothyroid patients still require future validation by larger studies.

What does the study add to existing knowledge?

The current study found severe hypovitaminosis D is seen among patients with TPOAb-positive hypothyroidism hence routine vitamin D screening is recommended among these patients.

Author's Contributions

Dr. Meenakshi Shankar: Concept, study design, and manuscript preparation.

Dr. Mukul Singh: Concept, study design, and manuscript preparation.

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