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# Predicting positive anti-thyroid peroxidase antibody chance in vitamin D deficient patients

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## Abstract

**Introduction:** The relationship between vitamin D deficiency and thyroid gland disorders is controversial.
**Objectives:** Given the prevalence of vitamin D deficiency and the significance of thyroid disorders, the purpose of the study was to examine the relationship between anti-thyroid peroxidase (anti-TPO) antibody and vitamin D deficiency.
**Patients and Methods:** This cross-sectional study was conducted on 35 patients with vitamin D deficiency and 35 people as a control group with normal vitamin D considered more than 20 ng/mL. Serum TSH and anti-TPO antibody tests were assessed for all subjects in both groups. The level of 25 [OH] D3 less than 20 ng/mL was considered as the deficiency of vitamin D and the serum level of anti-TPO antibody more than 40 IU/mL as positive level.
**Results:** Mean age of patients with vitamin D deficiency was 37.00 ± 13.8 years and 46.3 ± 15.9 years in the control group. Mean age in the two groups was different ($P=0.010$). Around 51.4% of patients had vitamin D deficiency. There were no significant differences in TSH level among the groups ($P=0.436$). Anti-TPO antibody (anti-TPO Ab) level in 31.4% of the patients with vitamin D deficiency and in 11.4% of patients in the control group was positive with a significant difference ($P=0.041$). Logistic regression analysis showed the chance of positive anti-TPO Ab in people with vitamin D deficiency was 3.55% of the subjects without vitamin D deficiency (OR = 3.55, 95%, CI: 1.01-12.55, $P=0.049$).
**Conclusion:** Considering the greater chance (three-fold) of positive anti-TPO Ab titers in patients with vitamin D deficiency in this study, more interventional studies are suggested concerning the effect of vitamin D deficiency on anti-TPO Ab.

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## Introduction

Vitamin D is a fat-soluble vitamin, most of which is gained by exposure to sunlight and a small part through nutrition (1). The serum level of 25 [OH] D3(25 hydroxy-vitamin D3) is commonly used as a true measurement of total vitamin D. The fluctuations in the levels of 25 [OH] D3 depend more on sunlight than the absorption of vitamin D precursors through nutrition (2).

According to the results of the epidemiological studies, levels of 25 [OH] D3 more than 20 ng/mL are suitable to maintain bone health (3). Numerous factors affect serum levels of vitamin D, such as exposure to sunlight, skin pigmentation, age, obesity, physical activity, medications and nutrition (1, 3).

In the past two decades, the vitamin D receptor (VDR) has been seen not only in the bones, kidneys and intestines but also in the immune system (T-cell, B-cell, macrophages and monocytes), the genital system, the endocrine system, the muscles, the brain, the skin and the liver, indicating that the role of vitamin D is not limited to the skeletal system (4). Vitamin D suppresses the activity of lymphocytes including Th1, Th2, Th17 and the reduction of CD4 and other related cytokines by VDR and inhibits pro-inflammatory processes (5,6).

Numerous studies have shown that vitamin D plays an important role in reducing the risk of chronic diseases, autoimmune disease, infections and cardiovascular disease (6-9). Thyroid diseases are the most common

### Key point

The relationship between vitamin D deficiency and thyroid gland disorders is controversial. In the present study, we examine the relationship between anti-thyroid peroxidase (anti-TPO) antibody and vitamin D deficiency. Our study showed a greater chance (three-fold) of positive anti-TPO Ab titers in patients with vitamin D deficiency; however, more interventional studies are suggested concerning the effect of vitamin D deficiency on anti-TPO Ab.

endocrine disorders. Here, autoimmune thyroid disease (AITD) including Hashimoto's thyroiditis and Grave's disease are accounting for five percent of the world population. In AITD, autoimmunity plays a fundamental role with T-cell and B-cell infiltration in the thyroid gland and the production of specific auto-antibodies to respond to thyroid antigens, including anti-TSH receptor (TRAb), peroxidase anti-thyroid peroxidase antibody (ant-TPO Ab) and antithyroglobulin (10).

Hashimoto's thyroiditis and Graves' disease are AITDs (11). Hashimoto's thyroiditis is associated with other chronic lymphocytic names or autoimmune thyroiditis with the enlargement of the thyroid gland and the production of autoantibody of thyroid and lymphocytic infiltration and various degrees of thyroid function declining (1). It is more common among women (8), with an annual incidence of autoimmune thyroiditis – 4 in 1000 women and 1 in 1000 men (3).

Graves' disease is also an AITD with common manifestations such as thyrotoxicosis and goiter. As one of the most common autoimmune diseases in the world, Graves' disease has a worldwide prevalence of 14 per 100 000. Graves' disease is a multi-factorial disease that causes the loss of immunity to thyroid antigens and triggers an immune response by interfering with genetic factors and environmental factors. For example, VDR gene polymorphism is associated with the risk of Graves's disease (12).

Various studies on the relationship between the level of vitamin D and positive antibody titer and the level of the thyroid-stimulating hormone have shown controversial results (6-9).

In a study by Choi et al, 6685 subjects (58% males and 42% females) were selected. AITD cases were diagnosed according to anti-TPO Ab and thyroid ultrasonography results. Then, the level of 25OHD was measured since in their study the level of vitamin D more than 30 ng/mL was considered normal. It was observed that vitamin D level in women with positive anti-TPO Ab (anti-TPO Ab more than 60 IU/mL was considered positive in their study) was lower compared to the control group. This decrease was more pronounced in women before menopause; however, there were no differences between the level of vitamin D in those with positive anti-TPO Ab and the control group in men. It seems that despite the relationship between the level of vitamin D and anti-TPO Ab in non-postmenopausal women, there was no clear correlation between the decrease in vitamin D levels and positive anti-TPO Ab in men, since the results were different in the two genders (13).

## Objectives

Given the prevalence of vitamin D deficiency, the prevalence of autoimmune thyroid disorders, and the different results in the relationship between these two disorders in various population and previous studies (6-9,13), we aimed to study the chance of positive TPO-Ab titer (as a thyroid disorder antibody) in patients with vitamin D deficiency.

## Patients and Methods

### *Study design*

This cross-sectional study was conducted on 35 patients with vitamin D deficiency and 35 control subjects admitted to the endocrine clinic in Semnan between 2016 to 2017. According to a study by Arslan et al, 24.2% of their control group and 67.5% of their subjects with different degrees of vitamin D deficiency had also positive anti-TPO Ab (7) and also considering 99% confidence level and 80% power, our sample size was obtained from the comparison of the ratio in two independent groups for each group as 35 participants.

Subjects with vitamin D level ≤20 ng/mL were included in the case group (vitamin D deficiency) and the control group was those considered healthy by the vitamin D levels more than 20 ng/mL.

People under 20 years of age, pregnant women, patients with other autoimmune disorders, history of any thyroid disorder, known cardiovascular disease, renal or hepatic diseases, underlying malignancy, previous malabsorption of metabolic diseases, and or using calcium and vitamin D supplements, and glucocorticoid and thyroid drugs for the last three months were excluded.

The goals of the study were explained to the subjects and entered if they accepted; then, participants were divided into two groups of vitamin D deficiency and normal levels of vitamin D(3). The levels of TSH and anti-TPO Ab were measured in each group by ELISA method.

### *Statistical analysis*

Data were analyzed by SPSS 18. The chi-square, Mann–Whitney U and Shapiro–Wilk tests were used to compare two related groups, and logistic regression was used to estimate the simultaneous effects of age and gender on the levels of anti-TPO Ab. $P<0.05$ was considered to indicate significance.

## Results

In this study, 35 patients with vitamin D deficiency and 35 controls were enrolled. Around 27 patients with vitamin D deficiency and 30 controls were female. The two groups were matched for gender ($P = 0.356$). Mean age of patients with vitamin D deficiency was 37.00 ± 13.8 years and 46.3 ± 15.9 years in the control group. Mean age in the two groups was different ($P = 0.010$; Table 1).

Moreover, 51.4% of patients with vitamin D deficiency (case group) and 62.9% of patients with normal vitamin D level (control group) had normal TSH (0.4 to 4 µIU/L). The distribution of TSH levels in the two groups did not differ significantly ($P = 0.436$; Table 2). There were no significant differences in TSH levels in subjects less than 40 years of age ($P = 0.419$) and 40 years or older ($P = 0.385$).

In 31.4% of patients with vitamin D deficiency and 11.4% of the control group, the serum anti-TPO Ab level was >40 IU/mL. Frequency of positive level for serum anti-TPO Ab (more than 40 IU/mL) in patients with vitamin D deficiency was significantly more than the control group ($P = 0.041$; Table 2).

Logistic regression analysis was performed to study the simultaneous effects of age and gender on the levels of anti-TPO Ab. Logistic regression analysis showed the chance of vitamin D deficiency in individuals with anti-TPO Ab ≥40 IUmL that was 3.55 times more than the individuals with anti-TPO Ab<40 IU/mL (OR = 3.55, 95% CI: 1.12-1.55, $P$=0.049).

## Discussion

Although there was no significant correlation between vitamin D deficiency and TSH in the two groups, the chances of vitamin D deficiency in people with anti-TPO Ab levels ≥40 IU/mL were 3.55 times higher than those who had anti-TPO Ab levels less than 40 IU/mL.

The results of this study showed that 51.4% of the patients with vitamin D deficiency and 62.9% of control group had normal TSH levels (between 0.4 to 4 units). In people less than 40 years old also 40 years or older, the distribution of TSH levels in the two groups did not differ significantly. In a study in Turkey in 2012, 155 subjects with similar age and body mass index were divided into three groups with severe and moderate vitamin D deficiency and normal levels of vitamin D (the level of vitamin D more than 20 ng/mL was considered normal). The levels of TSH and anti-TPO were measured in them. The results showed a positive correlation between vitamin D and TSH levels and a negative correlation between vitamin D and anti-TPO levels (7), which was consistent with the results of our study.

In a cross-sectional study in fall 2012 by Mansournia et al in Tehran, 86 people including 41 individuals with Hashimoto's thyroiditis and 45 healthy people were selected as controls. Then the levels of 25OHD and TSH were measured in each group, showing that 25OHD level had a negative correlation with TSH (14). In a cross-sectional analytical study in 2015 by Dehghan-Manshadi et al in Yazd, 1093 people, including 251 men and 842 women were selected. Accordingly levels of 25 OHD and TSH (with normal TSH levels ranging from 0.3 to 3.6 μIU/L) were measured since the relationship between these factors was examined during summer and winter. In this study, vitamin D levels of more than 30 units were considered normal. Ultimately, a statistically significant relationship was found between TSH and 25 OHD. They concluded that the diet and lifestyle associated with vitamin D levels may have a significant effect on thyroid hormones (15), which was contrary to the findings of our study. One of the important reasons for the difference between the results can be explained by the time of sampling and testing, which can be justified according to the season (winter and autumn or summer) and take into account the direct effect of sunlight on the level of vitamin D. In a study by Effraimidis et al, 78 euthyroid patients with genetic susceptibility for AITDs and 78 healthy individuals were selected. In the second phase (longitudinal study), 67 patients with positive TPO Ab and 67 healthy people were enrolled. TSH levels (normal range considered between 0.4 to 5.7 μIU/L) and 25OHD were measured in both groups (9). In line with the results of our study, it was found that the level of TSH hormone is not related to the decrease in vitamin D levels.

The results of the study by Shin et al reported a significant relationship between 25 (OH) D3 and anti-TPO Ab. In this study, anti-TPO Ab levels ranged from zero to 13.7

**Table 1.** Distribution of the age and gender of the subjects in the two groups

| Variables | | Vitamin D deficiency (n = 35) | | Control (n = 35) | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| Age (years) | <30 | 11 | 31.4 | 4 | 11.4 |
| | 30-39 | 12 | 34.4 | 10 | 28.6 |
| | 40-49 | 3 | 8.6 | 6 | 17.1 |
| | ≥50 | 9 | 25.7 | 15 | 42.9 |
| Gender | Female | 27 | 77.1 | 30 | 85.7 |
| | Male | 8 | 22.9 | 5 | 14.3 |

**Table 2.** Distribution of TSH and anti-TPO-Ab levels in patients with vitamin D deficiency and control group

| | | Vitamin D deficiency (n = 35) | | Control (n = 35) | | *P* value |
|---|---|---|---|---|---|---|
| | | No. | % | No. | % | |
| TSH (μIU/L) level | <0.4 | 1 | 2.9 | 2 | 5.7 | 0.436 |
| | 0.4-4 | 18 | 51.4 | 22 | 62.9 | |
| | >4 | 16 | 45.7 | 11 | 31.4 | |
| Anti-TPO Ab (IU/mL) level | <40 | 24 | 68.6 | 31 | 88.6 | 0.041 |
| | ≥40 | 11 | 31.4 | 4 | 11.4 | |

IU/mL and the vitamin D level more than 30 ng/mL was considered normal levels. It was found that 25(OH)$D_3$ level is an independent factor affecting the presence of TPOAb in AITDs (8). In the study by Kivity et al, three groups including 50 AITDs, 42 non-AITDs and 98 healthy subjects were selected and serum vitamin D levels were measured (normal range of vitamin D was considered higher than 10 ng/mL). Anti-thyroid antibodies were significantly associated with the prevalence of vitamin D deficiency in their patients, which was consistent with our findings. Regarding the role of vitamin D in the pathogenesis of AITDs, using vitamin D supplements recommended in these patients (6). Another study indicated that vitamin D deficiency is associated with autoimmune diseases, and is linked to the effects of autoantibodies associated with these diseases (16). The cause of vitamin D deficiency in hypothyroidism may be due to low-absorption of this vitamin from the intestines or to the inactivation of vitamin D (17). Both thyroid hormones and vitamin D bind to similar receptors called steroid hormone receptors. Several polymorphisms have been identified in the genes of VDRs that predispose individuals to thyroid diseases such as Graves' disease and Hashimoto's. Moreover, some antibodies including anti-TPO Ab can interact with receptors similar to vitamins D, which may have devastating effects (18-21). Other studies have stated that vitamin D deficiency is associated with AITD, thereby vitamin D supplementation can be used to treat thyroid-dependent autoimmune diseases by reducing anti-TPO Ab (12,22). In addition, the active form of vitamin D inhibits the production of inflammatory cytokines via binding to this vitamin receptor and production of interleukine-6 by monocytes, macrophages and T lymphocytes (23).

Our study showed no statistically significant relationship between vitamin D and anti-TPO Ab levels. However, two studies in India and South Korea showed that people with Hashimoto's as thyroid autoimmune disease had lower 25-hydroxyvitamin D serum levels than healthy subjects (13,24).

## Conclusion

Our study did not examine other factors affecting thyroid function and vitamin D levels. However, attempts were made to matching of two groups hence confounding effect of the underlying variables minimized as possible, while some factors also affected TSH secretion including sex hormones, genetic predisposition, or environmental factors (25).

## Limitations of the study

Thyroid function and vitamin D levels are influenced by some factors such as season and gender that were not considered in our study and this is a limitation of our study, therefore further studies are recommended to consider these factors.


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## Authors' contribution

SD provided technical assistance, collection and preparation of the manuscript. RG acted as a biostatics consultant. MD designed, supervised the study and prepared the final draft of the article.

## Conflicts of interest

The authors declare no conflict of interest.

## Ethical issues

The research followed the tenets of the Declaration of Helsinki. This paper was extracted from research project, at the department of internal medicine, Semnan University of Medical Sciences (Grant#1140). The study was approved by the ethics committee of the Semnan University of Medical Sciences (#IR.SEMUMS.REC.1395.146). Accordingly, written informed consent was taken from all participants before any intervention. The source of data used in this paper was from the MD thesis of Sepideh Mahmoodifar, the student of general practitioner, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran. Besides, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.


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