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Study on Association between Vitamin D Deficiency and Autoimmune Hypothyroidism

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Abstract
Background: *Vitamin D deficiency is a global health problem with evidence being increasingly pointed towards vitamin D as a significant role in reducing the incidence of autoimmune diseases. Research on its role in autoimmune thyroid disease gives contradictory results. We aimed to study the association between vitamin D deficiency and autoimmune hypothyroidism by comparing vitamin D deficiency between anti TPO positive and negative hypothyroid patients and to assess the relation between vitamin D levels and anti TPO levels in anti TPO positive hypothyroidism.*
Methods: *A total of 100 patients, 50 of them anti-TPO positive and 50 anti-TPO negative were included in the study. Serum anti-TPO, serum 25(OH)D, serum fT4 and TSH was measured using immunoassay.*
Results: *The mean serum 25(OH)D level was 25.5ng/ml with a higher prevalence of vitamin D insufficiency (40%). A significant association could be demonstrated between vitamin D insufficiency and autoimmune hypothyroidism both in younger and older females of the reproductive age group but not in males and postmenopausal females We observed only a weak negative correlation between 25(OH)D and anti-TPO titres. TSH was found to be significantly higher in autoimmune hypothyroid patients than in non-autoimmune counterparts (t= 2.15, p =0.034) and a strong positive correlation noticed between anti-TPO titres and TSH levels (p = 0.016; r = 0.24*).*
Conclusion: *Autoimmunity is closely related to thyroid function and increasing autoimmunity directly related to worsening thyroid function. Noticeable association between vitamin D insufficiency and autoimmune hypothyroidism encourages vitamin D supplementation for them*
Keywords: *Vit D deficiency, hypothyroidism, autoimmunity.*

Introduction

Hypothyroidism is defined as a deficiency of thyroid activity resulting from reduced secretion of T4 and T3, and hypersecretion of pituitary TSH. Autoimmune hypothyroidism is the most prevalent organ-specific autoimmune diseases and affect 2 - 5% of the population with great variability between genders (i.e., women 5–15% and men 1–5%)[1]. In India, autoimmune hypothyroidism has been estimated to be one of the most frequent endocrine disorder affecting 42 million people in India. Thyroid autoimmunity presents with increased thyroid autoantibody levels like circulating anti-thyroid peroxidase antibodies (anti-TPO). Autoimmunity may be attributed to environmental

triggers and susceptible genes which are involved in immune regulation.

Many tissues and cells in the body express the vitamin D receptor and so a role of vitamin D in extra-skeletal conditions such as common cancers, cardiovascular disease, and autoimmune diseases including diabetes mellitus and autoimmune hypothyroidism has gained interest over the past few years.[2]

The reported prevalence of Vitamin D insufficiency in India varies from 50-90 %.[3]. Despite enough sunshine, this unexpected insufficiency of Vitamin D levels among Indians has become a matter of concern. Also it could be one of the environmental triggers precipitating autoimmunity in susceptible population.[2, 3]

25-hydroxyvitamin D [25(OH)D] with a half-life of 15 days is considered to be the better indicator of vitamin D status than 1,25-dihydroxy vitamin D [1,25$(OH)_2$D] (15 hours) as in[3]. The available data from studies on occurrence of vitamin D deficiency in relation to thyroid diseases give conflicting results. It is still unclear if any association exists between hypothyroidism and Vitamin D insufficiency.

Currently, the vitamin D status of hypothyroid patients in South India is unknown and considering the potential role of vitamin D in autoimmune diseases, the aim of our study was to evaluate 25(OH)D levels in newly diagnosed hypothyroid patients and to determine if there was any association between vitamin D deficiency and autoimmune hypothyroidism by comparing vitamin D deficiency between anti-TPO positive and negative hypothyroid patients in a south Indian population.

Materials and Methods

This study was approved by the Institutional review board and Institutional ethics committee and was conducted between June 2017and August 2018 at a tertiary care medical college catering for nearly 1000 new patients and referrals on a daily basis. It was a cross-sectional observational analytical study done in newly detected hypothyroid patients above 18 years of age with serum TSH above 10mU/Land attending Thyroid Clinic OPD of the hospital. Unwilling patients, patients with proven liver disorders, renal disorders, primary hyperparathyroidism, post radio –iodine hypothyroidism, or on drugs like anti-epileptics, oral contraceptives, glucocorticoids, vitamin D, Calcium were excluded from the study. Sample size of 50 anti-TPO positive subjects and 50 anti-TPO negative subjects was calculated assuming a power of 80%, significance value 5% from a study by Kivity et al where the prevalence of autoimmune hypothyroidism and non-autoimmune hypothyroidism was found to be 79% and 52% respectively. Informed written consent was obtained from each participant and all subjects underwent the same protocol.

Laboratory Measurements

Serum anti-TPO, serum 25(OH)D, serum fT4 and TSH was measured based on competitive principle using chemiluminescent immunoassays on a Beckman Coulter Access2 automated analyzer. (CLIA; Beckman Coulter, Brea, CA, USA). Sensitivity of serum anti-TPO assay was 0.25 IU/mL. We defined serum 25(OH)D levels as vitamin D deficient (<20 ng/mL), insufficient (21–29 ng/mL), or sufficient (≥30 ng/mL)[5]. A anti-TPO level exceeding 70 IU/mL was considered to constitute a positive anti-TPO reading[6].

Statistical Analysis

Continuous variables were expressed as means (±standard deviation) and categorical variables were presented as numbers (percentage). Continuous variables were compared using the Student's unpaired *t*-test. Comparisons between each group according to categorical variables were done using a chi-square test (two-sided). Karl Pearson correlation coefficient (r) was used to assess the relation between linear related variables. Statistical analysis was performed using the software SPSS for Windows version 18.0 (IBM SPSS Statistics for Windows, Version 18.0. Armonk, NY: IBM Corp was used for all analyses). $p<0.05$ was considered to indicate statistical significance.

Results

A total of 100 patients were enrolled in the study, 50 of them anti-TPO positive and 50 anti-TPO negative. The mean age was 33.9 ± 13.3 years (mean + SD) and 93 % of the patients were females. Most of the people affected were in the range of 20 to 50 years. The overall mean serum 25(OH)D level was 25.5ng/ml. The prevalence of vitamin D deficiency, insufficiency and sufficiency in both the groups was 28% (14% Vs 14%), 40% (14% Vs 26%) and 32% (22% Vs 10%) respectively. The baseline characteristics of both groups were comparable with respect to age and sex.

Vitamin D levels and autoimmune hypothyroidism

On comparing vitamin D prevalence in both the groups, although the prevalence of vitamin D deficiency was comparable in both groups, the prevalence of vitamin D insufficiency was significantly higher in autoimmune hypothyroid patients when compared to non autoimmune hypothyroid patients ($p = 0.017$; $\chi^2 = 8.1$). This association was significant only in females of the reproductive age group (18 – 49 years) ($p = 0.001$; $\chi^2 = 13.36$) and not in postmenopausal females ($p = 0.239$; $\chi^2 = 2.86$) or in males ($p = 0.459$; $\chi^2 = 1.56$). Further analysis revealed that the association was present among both the younger and older females in the reproductive age group ($p \leq 0.01$; $\chi^2 = 15.8$ and $p = 0.037$; $\chi^2 = 6.61$). Mean 25(OH)D levels was also non significantly lower in anti-TPO positive patients (24.6ng/ml) than in anti-TPO negative patients (28.3ng/ml). Moreover, a weak negative correlation could be established between vitamin D levels and anti-TPO levels in anti TPO positive patients ($r = -0.180$; $p = 0.07$). These findings have been detailed in tables 2, 3, 4 and figure 1.

Correlation between Thyroid function (TSH) and vitamin D levels

Vitamin D levels did not show any significant correlation with TSH levels in both groups ($r = -0.08$: $p = 0.589$ in anti-TPO positives and $r = -0.01$: $p = 0.937$ in anti-TPO negatives) as can be seen in table 5 and figure 2

Thyroid function (TSH) and autoimmunity

Next we studied if there was any association between TSH levels and anti-TPO titres. TSH was found to be significantly higher in autoimmune hypothyroid patients than in non autoimmune hypothyroid patients ($t = 2.15$, $p = 0.034$) by using students unpaired t-test to compare between the two groups. On finding a strong association between TSH and anti TPO titres, we also studied if there was any relation between the two variables. We also found a strong positive correlation between anti-TPO titres and TSH levels ($p = 0.016$; $r = 0.24$*).This positive association and correlation has been given in detail in table 6 and figure 3.

Risk factors associated with autoimmunity

Some factors were analyzed to see if anti-TPO positivity was more common in a particular group of hypothyroid population. In our study, when comparing autoimmunity among females in the reproductive age group, females in the age group of 18-30 years had a significant association with autoimmunity. But no association could be seen with gender, diet, family history, other autoimmune disorders, smoking, alcoholism, etc. This has been tabulated in tables 7 and 8

Table 1: Baseline characteristics of thyroid peroxidase antibody-positive and thyroid peroxidase antibody-negative hypothyroid patients in study

S.No	Characteristics	Anti-TPO negative patients n= 50	Anti-TPO positive patients n=50	p value
1.	Age	35	28	0.108
2.	Female gender	46 (92.0%)	47 (94.0%)	1.000
3.	25(OH)D	28.3ng/ml	24.6ng/ml	0.105
4.	TSH	32.1	46.7	0.034
5.	Free T4	0.61	0.52	0.011

Table 2: Association between vitamin D deficiency/vitamin D insufficiency and autoimmune hypothyroidism

Vitamin D	Anti-TPO				χ^2	P
	No		Yes			
	Count	Percent	Count	Percent		
Deficiency	14	50.0	14	50.0	8.1*	0.017
Insufficient	14	35.0	26	65.0		
Normal	22	68.8	10	31.3		

*Significant at 0.05 level

Table 3: Association between vitamin D deficiency/vitamin D insufficiency and autoimmune hypothyroidism based on gender

Sex	Vitamin D	Anti-TPO				χ^2	P
		No		Yes			
		Count	Percent	Count	Percent		
Female in Reproductive age	Deficiency	11	50.0	11	50.0	13.36**	0.001
	Insufficient	9	26.5	25	73.5		
	Normal	18	75.0	6	25.0		
Female not in Reproductive age	Deficiency	3	60.0	2	40.0	2.86	0.239
	Insufficient	3	100.0	0	0.0		
	Normal	2	40.0	3	60.0		
Male	Deficiency	0	0.0	1	100.0	1.56	0.459
	Insufficient	2	66.7	1	33.3		
	Normal	2	66.7	1	33.3		

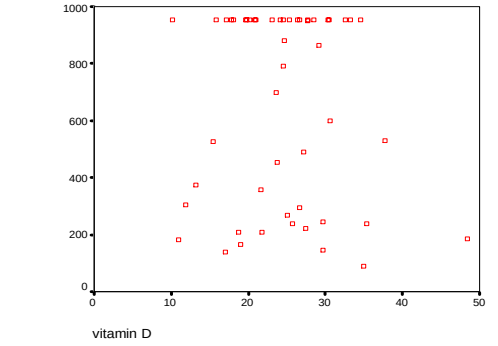
**: - Significant at 0.01 level

Table 4: Association between vitamin D deficiency/vitamin D insufficiency and autoimmune hypothyroidism based on age among women in reproductive age group

Age	Vitamin D	Anti TPO				χ^2	P
		No		Yes			
		Count	Percent	Count	Percent		
18 – 30	Deficiency	5	31.3	11	68.8	15.8	p<0.01
	Insufficient	3	15.8	16	84.2		
	Normal	9	90.0	1	10.0		
31 – 49	Deficiency	6	100.0	0	0.0	6.61*	0.037
	Insufficient	6	40.0	9	60.0		
	Normal	9	64.3	5	35.7		

*Significant at 0.05 level

Fig.1: Scatter diagram of relation between vitamin D levels and anti-TPO levels in anti TPO positive



r=-0.180, p=0.07

Table 5: Correlation of vitamin D levels with TSH levels in both the groups

Anti TPO	R	P
No	-0.01	0.937
Yes	-0.08	0.589

Fig.2: Scatter diagram of correlation of vitamin D levels with TSH levels in both the groups

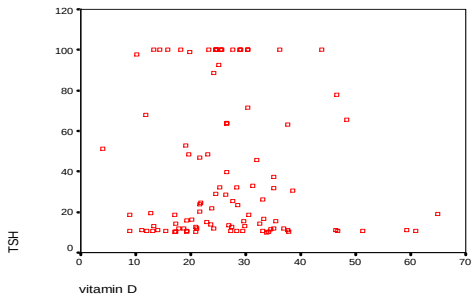
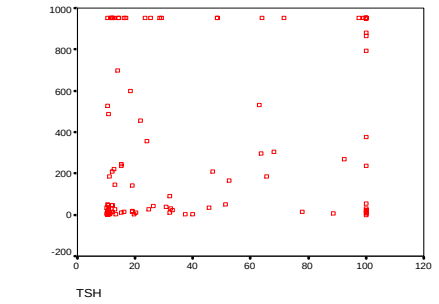


Table 6: Comparison of TSH based on Anti TPO

Anti-TPO	Mean	SD	N	T	P
No	32.1	32.1	50	2.15*	0.034
Yes	46.7	35.9	50		

*: - Significant at 0.05 level

Fig. 3: Scatter diagram of correlation of anti-TPO with TSH levels



r = 0.24* p = 0.016

Table 7: Association of anti-TPO with selected factors

		Anti-TPO				χ^2	P
		No		Yes			
		Count	Percent	Count	Percent		
Age	18 – 30	18	38.3	29	61.7	4.97	0.083
	30 – 49	23	59.0	16	41.0		
	≥ 50	9	64.3	5	35.7		
Sex	Female	46	49.5	47	50.5	0.15	0.695
	Male	4	57.1	3	42.9		
Diet based on	Rice based	42	47.2	47	52.8	4.88	0.087
	Wheat based	8	80.0	2	20.0		
	Soy based	0	0.0	1	100.0		
Family history	No	27	56.3	21	43.8	1.44	0.230
	Yes	23	44.2	29	55.8		
Other Autoimmune diseases	No	50	51.0	48	49.0	2.04	0.153
	Yes	0	0.0	2	100.0		
Smoking	No	50	50.0	50	50.0	-	-
	Yes	0	0.0	0	0.0		
Alcoholism	No	49	49.5	50	50.5	1.01	0.315
	Yes	1	100.0	0	0.0		

Table 8: Association of age and Anti TPO among women in the reproductive age group

Age	Anti-TPO				χ^2	P
	No		Yes			
	Count	Percent	Count	Percent		
18 - 30	17	37.778	28	62.2	3.9*	0.048
31 – 49	21	60	14	40.0		
*Significant at 0.05 level						

Discussion

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 in the pathogenesis of autoimmune diseases[7,8]. As an immune modulator, vitamin D reduces activation of the acquired immune system. Active forms of vitamin D suppresses autoimmune disease pathology 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 such as type 1diabetes, rheumatoid arthritis, multiple sclerosis and Graves disease [2, 7, 8].

Although there is a lot of literature regarding the implication of vitamin D deficiency in the causation of cancer, autoimmune disorders, the physiological role of thyroid hormones in the regulation of vitamin D metabolism is still unclear. Reports from the Americas, Australia, Africa and Asia covering extensive latitudes all indicated that the world's current state of vitamin D inadequacy is discouraging.[9, 10] Recently, it has been shown that even in tropical climates, the population is at high risk of vitamin D deficiency, which may be attributed to a changed lifestyle. The rapid economic development accompanied by a change in life style behaviours like less exposure to sunlight, changing food habits, decreased physical activity among the people and increasing pollution hampering the synthesis of vitamin D could be some of the reasons for the increased incidence and prevalence of vitamin D deficiency in India and other countries[11,16]. The available data from studies on occurrence of vitamin D deficiency in relation to thyroid diseases mostly prove the association of vitamin D deficiency with higher incidence of autoimmune thyroiditis[4,12,13]. Consistent with some studies in literature, we also observed a high prevalence of 25(OH)D insufficiency (40%) in our study[14] and a mean 25(OH)D of 25.5 ng/ml. The prevalence of vitamin D deficiency was low and similar in both the autoimmune and non-autoimmune groups (14%).The majority of the hypothyroid population had sub-optimal vitamin D levels i.e., 68% of hypothyroid population had 25(OH)D levels less than 30ng/ml. Most studies that have evaluated the association of 25(OH)D and thyroid autoimmunity have used serum anti-TPO levels as a marker of thyroid autoimmunity [12,15,16]. We observed a significant association between vitamin D insufficiency and autoimmune hypothyroidism. This association could be demonstrated in younger and older females of the reproductive age group and was not seen in males and in postmenopausal females. This finding is similar to the study done by Choi et al [12]. We also observed a weak negative correlation between 25(OH)D levels and anti-TPO titres in anti-TPO positive patients. This finding is consistent with the Ravinder Goswami et al study[2] done in Indian adults which also showed a weak inverse correlation between serum 25(OH)D and anti-TPO titres[15]. In other studies like Kivity et al[4] and Mac kawy et al [14], a strong negative correlation was established between serum 25(OH)D and anti-TPO titres.

Although both the groups had a mean 25(OH)D insufficiency, autoimmune hypothyroid patients had a lower mean serum 25(OH)D concentration than non autoimmune hypothyroid patients (24.6 Vs 28.3) but the difference was not statistically significant p=0.105; Table 1

This study has not shown a difference in 25(OH)D level related to thyroid function in both autoimmune and non autoimmune hypothyroid patients and no correlation could be established in both the groups. This study is in contrast to studies by Kivity et al[4] and Mac Kawy et al[14] where a negative correlation could be established between 25(OH)D levels and TSH. As stated earlier, we found a weak negative correlation between 25(OH)D and anti-TPO antibody in autoimmune hypothyroid patients. Both these findings suggest that vitamin D deficiency is more closely related to autoimmune thyroid antibody status rather than thyroid function itself in humans. But from our study we 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.

Several environmental and non-genetic triggers have been implicated in the aetiology of autoimmune disorders and in autoimmune hypothyroidism. Some of the risk factors like age, female gender, diet and family history, presence of other autoimmune disorders, smoking and alcoholism were studied. Although autoimmune hypothyroidism is generally associated with middle aged females, in our study, significant association was seen in younger females of the reproductive age

group. The reason for increasing autoimmunity in younger females could be due to change in lifestyle habits, spending less time in outdoor activities. Also no significant association could be established between autoimmune hypothyroidism and other factors.

Our study had some limitations. The strength of the study would have been higher if a group of healthy controls were included to know the vitamin D adequacy of our population. Secondly, a follow up our subjects could have provided more information about worsening of vitamin D insufficiency to deficiency or its persistence over time. It could also have provided some insight into the role of vitamin D as the cause or effect of auto immune hypothyroidism.

Conclusion

Based on our experience, we can say that autoimmunity is definitely associated with worsening thyroid function. But taking the case of vitamin D deficiency, it is more closely related to autoimmune thyroid antibody status rather than thyroid function itself in humans. What we need to decide is how to react to this situation. It might be better to give vitamin D supplements in patients with vitamin D insufficiency to prevent the worsening of their autoimmune status. It also calls for lifestyle modifying factors to improve the overall status of vitamin D in our population

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