Vitamin D Evaluation in Autoimmune Thyroid Diseases

Indu Prasad[1], Renu Kumari[2], Anand Saran[3]

ABSTRACT

Introduction: 25-Hydroxy vitamin D (25(OH)D) deficiency is linked with predisposition to various autoimmune diseases e.g. type 1 diabetes and multiple sclerosis. The present study aims to explore the relationship between vitamin D status and circulating TSH levels with thyroid autoimmunity and thyroid hormone levels.

Material and Methods: Serum levels of 25(OH)D, TSH, FT_3, FT_4, and thyroid autoantibodies (Anti TPO Antibodies), Vitamin D and calcium levels were measured in 52 patients with hypothyroidism and 52 healthy subjects. Vitamin D deficiency was defined as a serum level of 25OHD of ≤20 ng/ml and insufficiency as a serum level between >20 ng/ml and <30 ng/ml and normal ≥30 ng/ml.

Result: Mean age of the hypothyroid subjects was 39.05±6.83 years while that of the euthyroids was 39.07±6.05 years,(p> 0.05). Serum TSH was significantly raised in group I with p <0.0001.Serum Anti TPO was also significantly raised,(p = 0.002) in hypothyroids (50.55±98.95 µIU/ml) when compared with euthyroids (6.86 ± 9.26 µIU/ml). Serum Vit. D was also significantly decreased (p<0.0001) in group I. Serum calcium in group I patients was found to be 7.67 ± 8.34 mg/dl which is significantly lower (p=0.0344) than that of the group II (10.16 ± 0.74 mg/dl).

Conclusion: Our results indicated that patients with AI hypothyroidism suffered from hypovitaminosis D with hypocalcaemia that is significantly associated. This encourages the advisability of 25 (OH) vit D supplementation and recommends the screening for Vitamin D deficiency and serum calcium levels for all hypothyroid patients.

Keywords: 25(OH)D, Anti TPO Antibodies, Vitamin D, AI hypothyroidism, Euthyroids, hypovitaminosis D

INTRODUCTION

Deficiency of Vitamin D has become a common health problem in the general population. Vitamin D insufficiency has been linked to various morbidities e.g. cardiovascular disease, insulin resistance, fatty liver disease, type-2 diabetes and its complications, infections, and cancer.[1] Apart from a role in skeletal metabolism, vitamin D has been recognized as both an exogenous and an endogenous player in endocrinopathies such as type 1 and type 2 diabetes mellitus, adrenal diseases, and polycystic ovary syndrome.[2,3] It has also been linked to several autoimmune disorders including autoimmune thyroid disorders (AITD).[4,5]

Autoimmune thyroid diseases (AITD) are the most common organ specific autoimmune disorder.[6–8] Out of them Graves' disease (GD) and Hashimoto's thyroiditis (HT) are the main clinical presentations, characterized by lymphocytic infiltration of the thyroid parenchyma.[2,9] Despite of the advancements in understanding the pathophysiology of AITD, its primary underlying cause is still unknown.[9,10] Like other autoimmune diseases, genetically susceptible factors and various environmental factors contribute to the occurrence of AITD.[9–11]

Few researchers have examined the impact of vitamin D deficiency on AITDs. A Vitamin D receptor gene was found to show the predisposition of people to autoimmune thyroid diseases. So, its become important for patients with thyroid problems to understand the mechanism how vitamin D system works in our body.

Both vitamin D and thyroid hormone bind to the steroid hormone receptors. Moreover, Vitamin D mediates its effect by binding to vitamin D receptor (VDR), and activation of VDR-responsive genes.VDR gene polymorphism was found in association with autoimmune thyroid diseases (AITDs).[12] Vitamin D has also been shown to influence the thyroid follicular cells of rat by directly inhibiting thyrotropin-stimulated iodide uptake in a dose dependent manner.[13] In a study by Orbach H, Vitamin D levels were found to be lower in patients with AITDs than in healthy volunteers.[14] In contrast to it, a recent Indian study has found only a weak association between low vitamin D levels and AITDs.[15]

So, the present study was aimed to evaluate the association between serum vitamin D levels and AITDs.

MATERIAL AND METHODS

The present case control study included a total of 104 subjects of age and sex matched attending the Department of Endocrinology during the period from November 2015 to March 2015, having one or more clinical manifestations of hypothyroidism, e.g., fatigue, weakness, loss of strength, loss of stamina, weight gain, coarse dry hair, dry, rough and pale skin, hair loss, cold intolerance, muscle cramps, frequent muscle aches, constipation, depression, irritability, memory loss, and in women abnormal menstrual cycle were recruited for the study. They were further divided into two groups.

Group I -"Hypothyroid patients" comprised of 52 diagnosed cases of Autoimmune hypothyroidism.

Group II -"Control group". It included of 52 apparently healthy individuals with normal clinical examinations and were not complaining of either any chronic medical diseases, or history of thyroid diseases or any chronic illness that may interfere with results to be obtained. Also, these volunteers were not on any sort of vitamin D supplementations.

Exclusion criteria

- Subjects with a history of thyroid disease.
- Medications that may affect the thyroid function, such as oral contraceptives, oestrogen and iodine.

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- Individuals taking immunosuppressive drugs like-glucocorticoids.
- History of chronic kidney, liver or cardiac disease, malignancy, epilepsy.
- Other autoimmune diseases tuberculosis, immunodeficiency.
- Pregnancy during the study period for female subjects.
- Any medication history that involved taking calcium or vitamin D supplements 3 months before blood sampling.

Laboratory Tests

Blood samples of 5 ml were collected from all 104 patients in the morning after an overnight fasting of 8-12 hours. The serum separated after centrifugation of samples were used to measure TSH, fT4, fT3, Anti TPO and 25(OH)D levels by Beckman Coulter Access 2 Chemiluminiscense.

Determination of serum Calcium level was done by O-Cresolphthalein Complexone (OCPC) method in fully automated auto-analyzer AU 400.

Vitamin D deficiency was defined as a serum level of 25OHD of ≤20 ng/ml and insufficiency as a serum level between >20 ng/ml and <30 ng/ml and normal ≥30 ng/ml.[15]

STATISTICAL ANALYSIS

The data obtained were expressed as means and standard deviations. Statistical analysis was performed with Graph pad Prism 5.0 statistical software. Student's unpaired t test was applied to the result data of both the groups for comparison. Correlation of TSH and Anti TPO with each of Vitamin D was done by Pearson's correlation coefficient.

Results of the study were discussed at 95% confidence interval; interpretation of the test results was done according to p value ($P < 0.05$ - significant and $P \geq 0.05$ - not significant).

RESULTS

The present case control study consisted of 52 patients of hypothyroidism, that were compared with 52 euthyroid patients of the same age group and sex. Hypothyroid group consisted of 57.6 % of females as the majority while euthyroid group includes 53.81% males as majority (Figure 1).

Table 1 shows comparison of parameters between group I and group II. The mean age of the hypothyroid subjects was 39.05±6.83 years while that of the euthyroids was 39.07±6.05 years,(p> 0.05). Serum TSH was significantly elevated in group I with p <0.0001. Serum Anti TPO was also significantly raised, (p = 0.002) in hypothyroids (50.55±98.95 µIU/ml) when compared with euthyroids (6.86 ± 9.26 µIU/ml). Serum Vit. D was also significantly decreased (p<0.0001) in group I. Serum calcium in group I patients was found to be 7.67 ± 8.34 mg/dl which is significantly lower (p=0.0344) than that of the group II (10.16 ± 0.74 mg/dl).

Table 2 shows that mean age of hypothyroid females was 38.86±6.30 years which was not significant with the hypothyroid males. Though the mean±SD of TSH and Anti-TPO of both the sexes were increased but were not significant (p>0.005). Mean±SD of Vit. D was found to be significantly lower (p<0.005) in hypothyroid females (13.73±5.20 ng/ml). Similarly, serum Calcium was also found to be significantly lower (p<0.005) in hypothyroid females (5.96±1.04 mg/dl) when compared with that of the hypothyroid males (7.28±1.40 mg/dl).

Figure-2 shows significant negative correlation between Anti-TPO and serum 25 (OH) vit D (r = -0.3089, p < 0.0259).

Figure-3 also shows significant negative correlation between TSH and serum 25 (OH) vit D (r = -0.4060, p < 0.0028).

DISCUSSION

Majority of the patients in our study were females (57.6%). This finding was similar to that of Mackawy Amal Mohammed Husein et al.[16] Fida[17], Naeem et al.[18] They stated that serum Vit D levels were significantly more decreased in females than males. This was in accordance with our finding. Although several authors have reported that Vit D levels did not differ

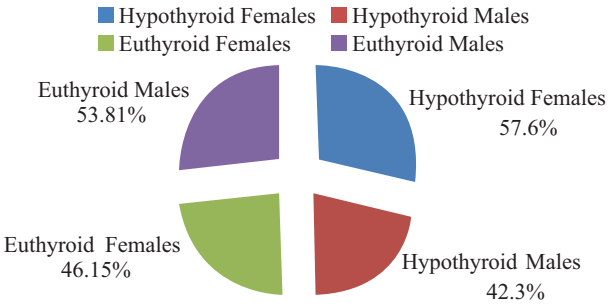

Figure-1: Sex wise distribution of Patients

Parameters	Group I	Group II	t- value, p- value
Age(in years)	39.05 ± 6.83	39.07 ± 6.05	p > 0.05
S. TSH (µIU/ml)	12.89 ± 11.13	2.22 ± 1.21	t = 6.86, p <0.0001
S. fT3 (pg/ml)	2.31 ± 1.13	3.02 ± 0.43	t =4.14, p <0.0001
S. Anti TPO (µIU/ml)	50.55 ± 98.95	6.86 ± 9.26	t =3.17, p = 0.002
S. Vit. D(ng/ml)	15.87 ± 5.61	31.39 ± 4.63	t = 15.37, p <0.0001
S. Calcium (mg/dl)	7.67 ± 8.34	10.16 ± 0.74	t = 2.14, p = 0.0344

Table-1: Showing comparison of parameters in both the groups

Parameters	Hypothyroid Females	Hypothyroid Males	p value
Age (years)	38.86 ± 6.30	39.63 ± 7.61	p>0.005
S. TSH (µIU/ml)	14.06 ± 12.5	11.28 ± 8.87	p>0.005
S. Anti TPO (µIU/ml)	54.67 ± 122.4	44.95 ± 55.09	p>0.005
S. Vit. D (ng/ml)	13.73 ± 5.20	18.80 ± 4.85	p<0.005
S. Calcium (mg/dl)	5.96 ± 1.04	7.28 ± 1.40	p<0.005

Table-2: Comparision of serum 25(OH) 25 (OH) vit D, Calcium and TSH levels in hypothyroid patients according to sex

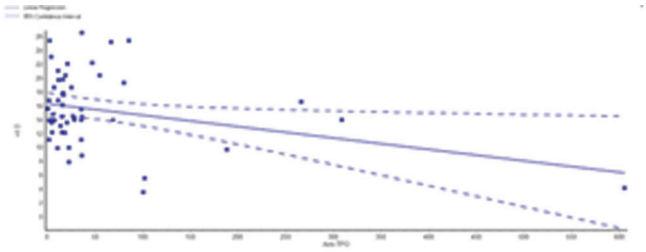

Figure-2: Correlation of Anti- TPO with Vit. D

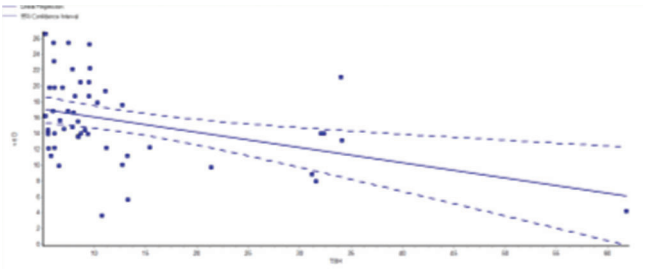

Figure-3: Correlation of TSH with Vit. D

significantly between males and females.[16,19,20]

Furthermore, the present study showed that vitamin D and calcium serum levels were significantly lower in hypothyroid patients when compared to the controls. A significant positive association was recorded between Vit D and calcium levels in both groups. Husein et al[16] found the similar finding in their study.

We have noticed a significant negative correlation of serum 25 (OH) vit D with Anti- TPO and TSH showing a significant association between vitamin D deficiency and hypothyroidism. This was in harmony with some of the previous studies.[16,21-23]

A significant difference was seen in the serum calcium levels between the studied groups with lower level seen in hypothyroid patients. It was significantly decreased in females than male patients. This was in contrast to that of Husein et al.[16]

Vitamin D deficiency has been recognized as a global health problem. Because of its role in homeostasis of blood calcium level, as well as in decreasing the risk of rickets fractures in children, osteoporosis, and osteomalacia in old age, vitamin D is of immense importance in our body. Besides its classical role in skeletomuscular functions, vitamin D has been recently identified as a factor that is deeply involved in both innate and adaptive immunity.[24] 'Secosteroid Hormone', the biologically active form of vitamin D, essential for bone and mineral homeostasis, has also been shown to have immunoregulatory and anti-inflammatory effects. So, low level of vitamin D in blood either due to less absorption or deficient intake was found to be associated with several autoimmune conditions, such as type 1 diabetes mellitus, Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis.[25,26] Recently, it has been shown that the population in tropical areas are even at high risk of vitamin D deficiency. This may be attributed to a lifestyle changing behaviour.[27]

The best indicator of vitamin D status is the serum concentration of 25(OH)D which reflects vitamin D produced cutaneously and that obtained from food and other supplements.[24] This 25(OH)D has a half-life of about 15 days in the circulation.

Low levels of vitamin D in patients with hypothyroidism could be attributed to either poor absorption of vitamin D from the intestine. or inability of body to activate vitamin D properly.[28]

Importantly, both vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. Also a different gene has been found in the Vitamin D receptor which shows predisposition of people to autoimmune thyroid disease including Graves' disease and Hashimoto's thyroiditis.[28] Besides this, vitamin D also inhibits the production of Th1 polarizing cytokine (IL-12), thereby indirectly shifts the polarization of T cells from a Th1 toward a Th2. Vitamin D directly inhibits the production of Th1 cytokines (IL2 and IFN-c), in the CD4+ responsive T-cells, and inturn enhances Th2 cytokine (IL-4) production.[29] Vitamin D receptor (VDR) gene polymorphisms and vitamin D status are also associated with different autoimmune diseases.[30-31] This can be regulated by the vitamin D-binding protein[32] and the CYP27B1 hydroxylase.[33]

Furthermore, some of the studies have shown that vitamin D supplementation prevents the onset and/or retards the development of several kinds of autoimmune diseases in humans and animal models.[29] These results have suggested that vitamin D deficiency might be a of cause of onset and/or development of several kinds of autoimmune diseases.

Nevertheless, further research is still required in this field to answer some of the questions like- Would vitamin D supplementation be beneficial to AITD patients? or lower serum vitamin D level is associated with higher risk of this disease? Supplementation of vitamin D could whether prevent the onset of AITD in susceptible populations, or decrease thyroid autoantibody titre in blood?

Few limitations of the present study were as follows. We couldn't measure the TSH receptor-stimulating antibodies. Second, it was focused on middle-aged and elderly individuals only. So, the relationship between vitamin D status and serum TSH levels in younger individuals remained unknown.

CONCLUSION

Our results thus indicated that deficiency and insufficiency of Vitamin D is significantly common amongst the AITD patients. So, screening for Vitamin D deficiency and serum calcium levels could be helpful and thus should be recommended for all the hypothyroid patients. Also, Vitamin D supplementation in these patients could have a beneficial effect on reduction of circulating TPO-Ab titres.

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