Original Article

A Prospective Study to Evaluate the Possible Role of Cholecalciferol Supplementation on Autoimmunity in Hashimoto's Thyroiditis

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Introduction : Several studies have reported a low Vitamin D status in Autoimmune Thyroid Diseases (AITD), indicating association between Vitamin D deficiency (<20 ng/ml) and thyroid autoimmunity. If supplementation of Vitamin D decreases anti-TPO antibody titres, in future it may become a part of AITDs' treatment, especially in those with Vitamin D insufficiency (21-29 ng/ml) or deficiency.

Objectives : Our study aims to assess any potential therapeutic role of Vitamin D in the management of HT.

Study Design : It is a randomised, double blind, single centre, placebo-controlled study.

Results : Significant negative correlation between Serum anti TPO Antibody and Vitamin D level; statistically significant reduction of anti TPO Antibody titre in intervention group compared to Placebo group.

Conclusions : Vitamin D can be a therapeutic option in Hashimoto's Thyroiditis.

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Key words **: Randomised Controlled Trial, Negative Correlation, Hashimoto, Cholecalciferol.**

Hashimoto Thyroiditis (HT), an autoimmune disease in which thyroid cells are destroyed by antibody and call-mediated immune processes. Hashimoto Thyroiditis is the commonest cause of Goitre in iodine-sufficient regions[1].

The aetiology of Hashimoto disease is not clearly understood. Many patients form antibodies to different types of thyroid antigens, most commonly it is anti-thyroid peroxidase (anti-TPO). Some patients also develop antibodies to Thyroglobulin and TSH receptor (TBII). These antibodies damage the thyroid tissue resulting in diminished thyroid hormone production. Serum antibody may be negative in 10-15% of patients with clinically evident disease[2,3].

Vitamin D is linked with autoimmunity probably by virtue of its anti-inflammatory and immunomodulatory properties. The dendritic cells are antigen-presenting cells originating from bone marrow and also a primary target for the immunomodulatory activity of Vitamin D. 1,25[OH]2D has direct immunomodulatory effects at the level of the T cell Vitamin D receptor. Together, these immunomodulatory effects can lead to the protection of tissues, for example thyroid cells in autoimmune Thyroiditis. Considering that in HT, a T cell-mediated immune disorder, immunologic damage occurs when MHC class II HLA-DR antigens is expressed on the surface of thyrocytes, induced by the production of Th1 type inflammatory cytokines (eg, IFN-γ). Moreover, at another stage, after being activated by T cells, B cells' ongoing proliferation might be inhibited and apoptosis might be induced by 1,25[OH]2D. Thus, 1,25[OH]2D might decrease antibodies that react with thyroid antigens. The appropriate levels of Vitamin D that are sufficient to improve the immune regulatory function and lead to an effective immune response, should be determined.

Low Vitamin D status in Autoimmune Thyroid Diseases (AITD) or HT has been reported in several clinical studies. This indicates an association between Vitamin D deficiency and autoimmune thyroid disorder. If thyroid antibody titres is decreased by supplementing Vitamin D, It may become a part of the therapeutic regimens of AITD, especially in Vitamin D deficienct or insufficient patients[4]. So, our study tries to assess any potential therapeutic role of Vitamin D

Editor's Comment :

- Vitamin D has an important role to play in the pathogenesis of autoimmune disorder.
- Deficiency of Vitamin D is one of the important risk factor of Autoimmune Thyroid Diseases (AITD).
- Underlying pathogenesis of AITD can be reversed by supplementing Vitamin D.
- It could be a great therapeutic option in the management of Hashimoto's Thyroiditis as there is marked improvement of antibody titre and TSH level.

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in the treatment of Hashimoto's Thyroiditis.

Aims and Objectives

Low Vitamin D status has been linked with the pathogenesis of Autoimmune Thyroid Disorder, especially HT. However, there are only few preliminary interventional studies for HT. Vitamin D supplementation is beneficial or not for AITD or HT, should be evaluated. Treatment of HT focussed on mainly thyroid hormone supplementation, so if a therapeutic advantage of Vitamin D supplementation is identified/confirmed, it will be helpful in the treatment of HT and may be a part of treatment of HT patients.

So, the objective of our study is evaluating the role of Vitamin D on an excessive thyroid immune response.

Materials and Methods

(1) Study area : NRS Medical College and Hospital, Kolkata (Department of General Medicine).

(2) Study period : 1 year (January, 2019 to December, 2019)

(3) Sample size : 100 patients both Male and female.

(4) Sample Design : Patients attending outpatient Department in NRS Medical College and Hospital.

(5) Study Design : Prospective, Hospital based, Single centre study.

(6) Inclusion criteria :

Newly diagnosed patients (age >18 years and of both sexes) with HT and Vitamin D deficiency.

(7) Exclusion criteria: Patients suffering from:

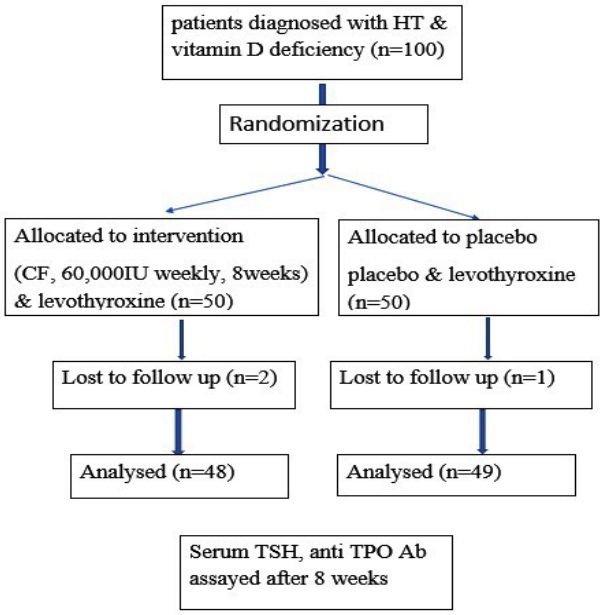
- Other Autoimmune Diseases
- Chronic illnesses like Diabetes Mellitus, Chronic Kidney Disease, Chronic Liver Disease, Malignancy.
- Pregnancy

(8) Study tools : Estimation from serum:

- TSH
- Free thyroxine (FT4)
- 25 hydroxy vitamin D
- Anti-thyroid Peroxidase (anti-TPO) antibody

(9) Study techniques :

This is a prospective study conducted in NRS Medical College and Hospital, Kolkata, India. Total 100 adult patients of both sexes diagnosed with HT and vitamin D deficiency (Vitamin D<30 ng/ml)[12], having none of the exclusion criteria and getting treatment on out-patient department basis, who gave informed consent were included in our study. Blood samples drawn for anti TPO antibody and 25hydroxy Vitamin D from all the participants. The correlations between serum Vitamin D and anti TPO antibody were measured and presented by correlation coefficient (r2). Study participants are randomly assigned into two groups by random permuted block. Cholecalciferol supplement given in the dose of 60,000 IU weekly for 8 weeks in one group (n=50). Another group (n=50) were given placebo (empty soft gelatine capsule). At the onset of the study, patients were requested to keep their habitual diet and routine level of physical activity throughout the study period and not to take any medication that might affect their reproductive physiology. Compliance to the consumption of supplement and Placebo was examined by empty blister packets. However, 2 patients from Cholecalciferol group and 1 patient from control group lost to follow up. After 8 weeks blood anti-TPO antibody level measured in both the groups (n=48 & 49 in 2 group). The change in the mean value of anti TPO antibody measured and statistical significance of the change checked. Results considered significant or non-significant when P> or <0.05, respectively.



Flowchart showing the Methodology of our Study

TSH, T4 measurement Performed with chemiluminescence using ADVIA Centaur XP Immunoassay System.

(10) Work plan : Study was done over 12 months. Data collected and compilation done and then statistical analysis done by standard statistical method.

(11) Statistical analysis : For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. p-value = 0.05 was considered for statistically significant.

Observations

Laboratory parameters before intervention :

	Cholecalciferol group	Placebo group
TSH (mU/L), mean±S.D	50.8±21.18	43.7±25.99
Free T4 (ng/dl), mean±S.D	0.55±0.10	0.55±0.12
25 hydroxy Vitamin D (ng/ml)mean±S.D	11.53±2.05	11.69±3.85
Anti-TPO antibody (IU/ml)mean±S.D	545.06±230.82	686.97±290.19

Laboratory parameters after intervention :

	Cholecalciferol group	Placebo group
TSH (mU/L), mean±S.D	3.70±0.37	5.36±2.62
Anti-TPO antibody (IU/ml)mean±S.D	378.60±160.49	571.10±254.09

Correlation between Serum 25 hydroxy Vitamin D and Serum anti TPO antibody :

		Serum anti TPO antibody (IU/ml)
Serum 25 hydroxy Vitamin D (ng/ml)	Pearson Correlation Coefficient (r)	-0.775**
	p-value	<0.0001

Negative Correlation was found between Serum 25 hydroxy Vitamin D (ng/ml) *versus* Serum anti TPO antibody (IU/ml) which was statistically significant.

Correlation between Serum TSH and Serum 25 hydroxy Vitamin D :

		Serum 25 hydroxy Vitamin D (ng/ml)
Serum TSH (mU/L)	Pearson Correlation Coefficient (r)	-0.301**
	p-value	0.003

Negative Correlation was found between Serum 25 hydroxy vitamin D (ng/ml) *versus* Serum TSH (mU/L) which was statistically significant.

Distribution of mean serum anti-TPO antibody level (IU/ml) [mean±SD] in both groups before and after intervention (Fig 1) :

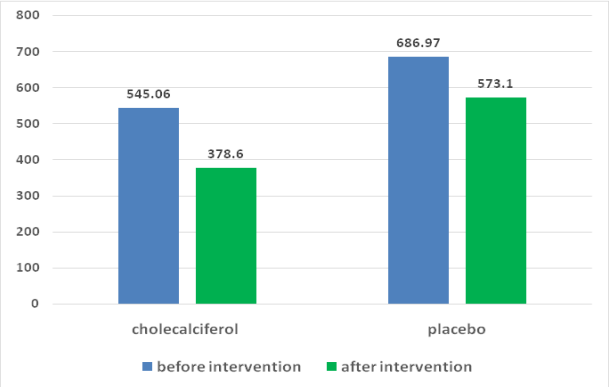


Fig 1 — Flowchart showing the methodology of our study

Reduction of serum anti -TPO antibody level in Cholecalciferol group is 30.5% and reduction of serum anti -TPO antibody level in Placebo group is 16.5%

Discussion

This study is carried out with the total number of 100 outdoor based patients of diagnosed Hashimoto's Thyroiditis (elevated Anti-thyroid peroxidase antibody) and Vitamin D deficiency (Vitamin D<30 ng/ml)[12] in Nil Ratan Sircar Medical College and Hospital within the mentioned study period. The study focussed on evaluating the role of vitamin D on an excessive thyroid immune response ie, the effect of supplementing Vitamin D on thyroid autoimmunity and that Vitamin D deficiency and the risk of HT are closely associated and the potential application of Vitamin D in the therapy of AITD.

The result demonstrates a negative Correlation between Serum 25 hydroxy Vitamin D (ng/ml) *versus* anti TPO antibody (IU/ml) (statistically significant). Pearson Correlation Coefficient (r)= -0.775, p value= 0.0001. Goswami *et al* conducted a community-based survey on 642 adults to investigate the relationship of serum Vitamin D concentrations and thyroid autoimmunity. Their results highlighted a inverse association (statistically significant) between Vitamin D and anti TPO Ab[40]. This inverse correlation was substantiated in the following studies[5-8].

Mackawy and co-workers demonstrated a strong negative association between serum vitamin D and TSH levels, leading to the speculation that Vitamin D deficiency in HT patients lead to progression into hypothyroidism ie, TSH > 5.0 m UI/l [45]. Our study also demonstrates negative Correlation between Serum 25 hydroxy Vitamin D (ng/ml) *versus* Serum TSH (mU/L) and the result was statistically significant. Pearson Correlation Coefficient (r)= -0.301, p value=0.003.

So, the results clearly indicates that Vitamin D deficiency is one of the important risk factor of Hashimoto's Thyroiditis.

Mean (mean± SD) Serum anti TPO antibody (IU/ml) before intervention was 545.06± 230.82 and after Cholecalciferol supplementation the mean value decreased to 378.6±160.49. So, there is a 30.5% reduction in the mean value of anti TPO antibody level. Difference of mean Serum anti TPO antibody (IU/ml) was statistically significant (p<0.0001).

In the Placebo group the mean Serum anti TPO antibody (IU/ml) (mean±SD) of patients was 686.97± 290.19 and after 8 weeks of Placebo the mean value was 573.1±254.09. So, in the Placebo group the reduction is only 16.5%. Difference of mean Serum anti TPO antibody (IU/ml) was statistically significant (p<0.0001).

Therefore, in line with the hypothesis the data contributes clearer understanding that supplementing Vitamin D helps in reduction of underlying autoimmune injury to thyroid gland. This result also supports the previous research. Simsek *et al* prospectively evaluated 82 patients with HT randomized in two groups: the first group treated with cholecalciferol for one month and the second group without vitamin D replacement. Their results indicated that TPO Ab and Tg Ab levels were significantly diminished by the vitamin D supplementation therapy in the first group [46]. These findings were also confirmed by other prospective studies and randomized controlled trials[9-11].

So, the result of our study clearly indicates that vitamin D supplementation could render a positive effect on thyroid function and thyroid autoimmunity.

Limitations :

(1) Vitamin D status could not be measured at the end of 8 weeks because of economic constraints. So, it is difficult to determine the optimal level of Vitamin D needed for improving the evolution of this immunological disorder.

(2) Cholecalciferol is used in HT patients in our study, although active form calcitriol might be more beneficial as Vitamin D binding protein level may affect the conversion of inactive Vitamin D form and thus alters its function on immune cells.

(3) HT patients with normal Vitamin D level have been excluded from the study, so from our study we cannot comment on beneficial effect of Vitamin D supplementation in HT patient with normal Vitamin D level.

(4) As we used empiric dose of levothyroxine in both the groups instead of a fixed dose, we could not analyze any role of Vitamin D supplementation in reducing the levels of serum TSH in HT

(5) There is still a gap in the knowledge regarding the effects of Vitamin D supplementation in the HT patients- whether it will help in decreasing the replacement dose of levothyroxine or whether it will stop the need of levothyroxine replacement if used in pre-clinical stages of HT.

Conclusions

- The 8 weeks randomized; double-blind, placebo-controlled clinical trial demonstrates a negative correlation between Serum 25 hydroxy Vitamin D *versus* anti TPO antibody level.
- Treatment with 60,000 IU cholecalciferol weekly for 8 weeks, is associated with significant decrease in antithyroid antibody titers. It also improved serum TSH level compared with the placebo, ie, supplementary treatment with cholecalciferol seems to have beneficial effects on AITD.
- However, large multicentre studies are needed to investigate further the impact of vitamin D supplementation on meaningful long-term clinical end points in AITD.

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