Print ISSN: 2321-6379
Online ISSN: 2321-595X

Original Article

Vitamin D Status in Hashimoto's Thyroiditis: A Case–control Study in a Tertiary Care Hospital

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Abstract

Background: *V*itamin D plays a significant role in the modulation of both innate and adaptive immune system. As Vitamin D deficiency was a risk factor for some autoimmune diseases, we aimed to evaluate the serum Vitamin D levels in autoimmune hypothyroidism, i.e., Hashimoto's thyroiditis (HT) and investigated the association between serum Vitamin D levels in autoimmune hypothyroids.

Aims and Objectives: The objective of this study is to measure Vitamin D and to assess the association of levels of Vitamin D and serum calcium with HT.

Materials and Methods: A case–control study was done, in which thirty healthy controls (Group 1) and thirty patients with altered hypothyroidism (Group 2) were included based on thyroid stimulating hormone levels and autoantibody positivity. Serum Vitamin D was estimated by chemiluminiscence immunoassay, serum calcium was estimated using arsenazo III method in all the subjects. Data were analyzed using Microsoft excel 2011 version, Graph Pad Prism software version 8.2.1. and Open Epi Info.

Results: Serum Vitamin D levels were found to be in lower level among cases (mean ± SD15.15 ± 7.05 ng/ml) when compared to controls (mean ± SD35.1 ± 6.89 ng/ml), (P < 0.0001). Serum calcium was decreased in cases when compared to controls. Serum calcium in cases was found to be mean ± SD: 8.43 ± 0.86 mg/dl and in control mean ± SD: 9.78 ± 0.55 mg/dl.

Conclusion: According to the results of the present study, it may be suggested that Vitamin D may play a role in the etiopathogenesis of HT. It can also be considered as a marker of severity of the disease. Hypocalcemia in cases may also be related to the severity of the disease. Thus, monitoring of serum 25(OH)D and calcium levels may be helpful in assessing the severity and also predicting the progression of the disease.

Key words: Autoimmune thyroid disease, Calcium, Hashimoto's thyroiditis, Vitamin D

INTRODUCTION

The biologically active form of Vitamin D, a secosteroid hormone essential for bone and mineral homeostasis, has been shown to have immunoregulatory and anti-inflammatory properties.[1] Most of the known biological effects of Vitamin D are mediated through the Vitamin D3 receptor (VDR),[2] and can be regulated by the vitamin D-binding protein[3] and the CYP27B1 hydroxylase.[4] The immune modulator properties of Vitamin D are ascribed to its effect on cells of the innate and adaptive systems including T- cells, B lymphocytes, macrophages, and dendritic cells (DCs), all of which express VDRs. Primary target for the immunomodulatory activity of Vitamin D are DCs. Vitamin D has an inhibitory role on DC-dependent T-cell activation and promotes tolerogenic properties that favor the induction of regulatory rather that effector T cells. In addition, *in vitro* studies showed that activation of CD4 T cells expressing VDR by Vitamin D promotes a Th2 phenotype (with interleukin [IL]-4 and IL-5 production) while suppressing Th1 activity (with interferon-gamma and IL-2 production).[1]

Both Vitamin D and thyroid hormone compete to similar receptors called steroid hormone receptors. A different

Access this article online

www.ijss-sn.com

Month of Submission : 06-2021
Month of Peer Review : 07-2021
Month of Acceptance : 07-2021
Month of Publishing : 08-2021

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gene in the Vitamin D receptor (VDR) was shown to predispose people to autoimmune Hashimoto's thyroiditis (HT).[5] Vitamin D hampers the production of Th1 polarizing cytokine (IL-12), thereby indirectly shifting the polarization of T cells from a Th1 toward a Th2 phenotype. In the CD4+ T cell response, Vitamin D directly inhibits the production of Th1 cytokines (IL2 and IFN-c) and enhances Th2 cytokine (IL-4) production.[6] VDR gene polymorphisms and Vitamin D status are associated with different autoimmune diseases including autoimmune thyroid disease.[7,8] Furthermore, Vitamin D supplementation prevented the onset and/or development of several kinds of autoimmune diseases in humans and animal models.[6]. These results suggest that Vitamin D deficiency could be the potential cause for the onset and/or development of several kinds of autoimmune diseases.

Vitamin D may be involved in the pathogenesis of Hashimoto's thyroiditis based on its ability to modulate the immune system by suppressing the proliferation of activated T cells and enhancing the phagocytic ability of macrophages. Vitamin D deficiency has been recognized as a global health problem. Because of its role in homeostasis of blood calcium level, Vitamin D is of immense importance in our body.

In autoimmune hypothyroidism (AITD), there is an increased production of calcitonin which promotes the tubular excretion of calcium.[9] In hypothyroid patients, there can be decrease in blood calcium levels because of impaired mobilization of calcium into bones.[10]

MATERIALS AND METHODS

A case–control study was performed in the Department of Biochemistry at Osmania General Hospital, Hyderabad and was approved by the ethical committee. Informed consent was obtained from all individuals. This study was performed in thirty cases and thirty healthy controls during December 2018 to May 2019 as shown in Table 1. 5 ml of venous blood was drawn under aseptic conditions, into serum vacutainers with clot activator. Samples were allowed to clot and then centrifuged. The serum was separated and stored in Eppendorf tubes at −20°C.

Inclusion Criteria

1. Autoimmune hypothyroid patients with thyroid-stimulating hormone (TSH) values >5.5 μIU/ml and positive for both TPOAb and TgAb antibody[11,12]
2. Age: 20–60 years, both male and female
3. Healthy controls were selected, both genders were taken
4. Subjects who gave consent to participate in the study.

Exclusion Criteria

1. Rheumatoid arthritis
2. Systemic lupus erythematosus
3. Inflammatory bowel disease
4. Multiple sclerosis
5. Type I diabetes mellitus.

Parameters Estimated

1. Serum Vitamin-D 25(OH)D levels by chemiluminiscence immunoassay in Siemens Advia Centaur XPT.Serum 25(OH)D have a half-life of approximately 2–3 weeks, in contrast, 1,25-(OH)2D has a short circulating half-life and is tightly regulated over a narrow range by parathyroid hormone, calcium, and phosphate
2. Serum calcium levels by Arsenazo III in Beckmann coulter AU 5800analyzer.

Reference range

8.6–10.3 mg/dl.

Statistical Analysis

The data were analyzed using Microsoft excel 2011 version, GraphPad Prism software version 8.2.1 and Open Epi Info.

RESULTS

Case–control study was conducted among the cases with Hashimoto (n = 30) and controls without Hashimoto (n = 30), with cases and control group ratio of 1:1. There was a significant association of low Vitamin D and calcium among HT compare to the control groups, the data are subjected to unpaired t-test as shown in Table 2.

DISCUSSION

Vitamin D plays a significant role in modulation of the immune system, enhancing the innate immune response while exerting an inhibitory action on the adaptive immune system.[13,14] Almost all immune cells, including B cells, T cells, and antigen-presenting cells (APCs), such as DCs and macrophages, harbor VDR and 1α-hydroxylase.[6,13,15] At the level of the APCs, 1,25(OH)2D inhibits the surface expression of major histocompatibility complex Class II antigens and co-stimulatory molecules and inhibits the maturation and differentiation of DCs as well as their survival and activation, leading to decreased antigen presentation and T cell activation. Moreover, 1,25 (OH)2D also modulates DC-derived cytokine expression by inhibiting the production of IL-12 and IL-23 (major cytokines driving Th1 differentiation) and IL-17 producing T helper (Th17) cell differentiation, respectively) and enhancing the release of IL-10. Thereby, 1,25(OH)2D indirectly shifts the

polarization of T cells from a Th1 and Th17 phenotype toward a Th2 phenotype.[6,16] In addition, 1,25(OH)2D directly leads to a shift from a pro-inflammatory to a more tolerogenic immune status, which includes diverse effects on T cell subtypes. 1,25(OH)2D inhibits Th1 cell proliferation, differentiation, and production of cytokines (IL-2 and interferon-γ), as well as Th17-derived cytokines (IL-17 and IL-21) but also assists the production of anti-inflammatory Th2 cytokines(IL-3, IL-4, IL-5, and IL-10), transfer the balance from a Th1 and Th17 phenotype to a Th2 cell phenotype. 1,25(OH)2D favors regulatory T cell (Treg) cell development through modulation of DCs and by directly targeting T cells, thereby blocking Th1 development. Finally, 1,25(OH)2D inhibits B-cell proliferation and differentiation into plasma cells, immunoglobulin secretion (IgG and IgM), memory B-cell generation, and also induces B-cell apoptosis[6,13-17] The ability of 1,25(OH)2D to suppress the adaptive immune system promotes immune tolerance and appears to be beneficial in autoimmune thyroid disease.[13,15]

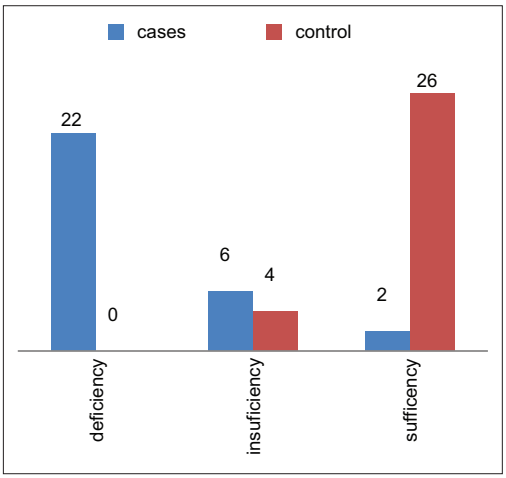

Figure 1: Bar diagram showing distribution of Vitamin-D among cases and controls

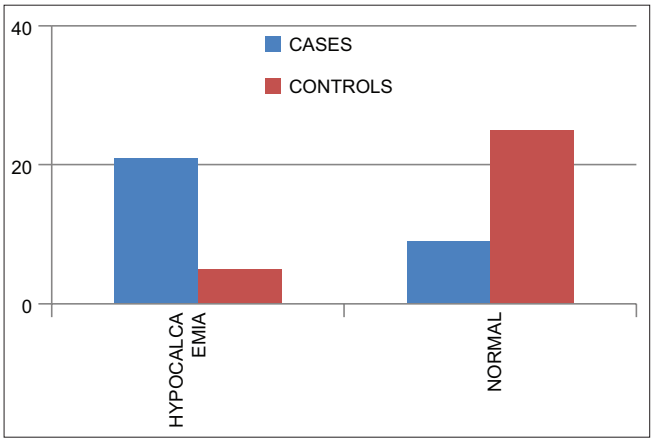

Figure 2: Bar diagram showing distribution calcium among cases and control

In this study, mean ± SD of serum vitamin D in controls was 35.1 ± 6.89 ng/ml and in cases was 15.15 ± 7.06 ng/ml. The difference in mean of control and cases was significant with a $P < 0.0001$ vitamin D levels were categorized as shown in the Table 3. The study shows that deficiency and insufficency of vitamin D are higher in cases than in control as shown in Figure 1. The Mean ± SD of calcium in controls was found to be 9.78 ± 0.55mg/dl and in cases 8.43 ± 0.86 mg/dl. The difference in mean of control and cases was significant with a $P < 0.0001$. Hypocalcemia is more predominant among cases than control group as shown in Figure 2.

The findings in the present study are also in harmony with the following studies.

A study conducted by Mackawy *et al*.[8] 60 subjects were included in this study, Group I included 30 controls and Group II 30 hypothyroids. Serum 25(OH) Vitamin D was significantly lower in autoimmune hypothyroid patients (14.79 ± 2.11) than in controls (44.53 ± 14.91) ($t = -11.128$, $P = 0.000$). Moreover, serum calcium levels recorded a significant

Table 1: Classification of cases and controls

Group	Subjects	Number
Group 1	Healthy subjects (controls)	30
Group 2	Patients with autoimmune hypothyroidism	30

Table 2: Descriptive analysis

Variable	Cases	Control	
Total	30	30	
Age (Mean±SD)	34.7±7.07	37.47±7.017	
Gender			
Male	2	4	
Female	28	26	
Vitamin-D (Mean±SD)	15.15±7.06 ng/ml	35.1±6.89 ng/ml	Unpaired T test
Deficiency	22 (73%)	0	*P*≤0.0001
Insufficiency	06 (20%)	4	T value=11.02
Sufficiency	02 (6.6%)	26	Df=58
Calcium (Mean±SD	8.43±0.86 mg/dl	9.78±0.55 mg/dl	
Hypocalcaemia	21	5	*P*≤0.0001 T value=7.135 Df=58
Normal	9	25	

Table 3: Vitamin D status

Serum 25-hydroxyvitamin D (ng/ml)	Vitamin D status
≤10	Severe deficiency
10–20	Deficiency
21–29	Insufficiency
≥30	Sufficiency
>150	Toxicity

decrease in hypothyroid patients (7.92 ± 1.77) when compared to controls (10.37 ± 1.55) ($t = -5.69$, $P = 0.000$).

A study conducted by Idiculla *et al*.[18] noted mean levels of Vitamin D were significantly lower in the group with hypothyroidism. These point to higher possibility of hypothyroidism in individuals deficient in Vitamin D. Among those with hypothyroidism TPO-Ab positive patients had a lower mean level of Vitamin D, and there was a significantly higher number of patients with severe VDD in this group.[18]

The mean level of Vitamin D in the TPO-Ab-positive hypothyroid group was 10.4 + 7.2 ng/ml in comparison to the TPO-Ab negative group 15.3 + 10.3 ($P = 0.004$) (OR: 3.39, CI: 1.18–9.80; $P < 0.05$; 3.62).

Prasad *et al*.[19] conducted study in 104 subjects (52 hypothyroid and 52 healthy). Vitamin D and calcium levels were measured. Samples were used to measure TSH, fT4, fT3, Anti–TPO, and 25(OH)D levels. Low serum Vitamin D ($P < 0.0001$) was seen in hypothyroid cases 15.87 ± 5.61 ng/ml and controls Vitamin D was 31.39 ± 4.63 ng/ml. Serum calcium in hypothyroid patients was found to be 7.67 ± 8.34 mg/dl which is significantly lower ($P = 0.0344$) than that of the controls (10.16 ± 0.74 mg/dl).

Importantly, both Vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. Furthermore, a different gene has been found in the VDR which shows predisposition of people to autoimmune thyroid disease including Graves' disease and HT. 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 in turn enhances Th2 cytokine (IL-4) production.

Limitations of the Study

The study should have been carried out on a larger group of population. Major limitation of the study was that the patients were not followed up to look for the correlation of serum Vitamin D status, outcome, and prognosis of the disease.

CONCLUSION

In the present study, Vitamin D deficiency is significantly low among cases of AITD compared to control group, suggesting that lower serum Vitamin D may be related to AITD. 73% of the cases are under deficiency group, 20% are insufficient group, and 6.6% are in sufficiency group. Therefore, serum Vitamin D seems to have a role in the etiopathogenesis of AITD.

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How to cite this article: Susmitha PB, Anita C, Ramadevi M, Arundhathi B. Vitamin D Status in Hashimoto's Thyroiditis: A Case–ControlStudy in Tertiary Care Hospital. Int J Sci Stud 2021;9(5):75-78.

Source of Support: Nil, **Conflicts of Interest:** None declared.