Original Article

Serum vitamin D levels in primary hypothyroidism

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Abstract

Introduction: Hypothyroidism is a common endocrine disorder with a prevalence rate of 10.95% in India. Primary hypothyroidism is the dysfunction at the level of thyroid gland, leading to decrease secretion or action of thyroid hormones. Vitamin D deficiency/ insufficiency are very prevalent in general population. In recent years the role of Vitamin D in body was extensively researched. It has been seen that besides having role in bone metabolism, Vitamin D is involved with pathology of various chronic diseases like diabetes, psoriasis, autoimmune diseases. This study was aimed to see the relationship between primary hypothyroidism and serum Vitamin D deficiency/insufficiency. **Method:** It was a case control study including 57 newly diagnosed hypothyroid cases of age group 20-50 years and 29 age and sex matched controls. Patients were selected based on serum TSH levels according to ATA guidelines. Any other underlying disorder was ruled out after taking proper medical history. Serum levels of 25(OH)D was measured by ELISA. TSH and thyroid hormones were measured by ECLIA. **Result:** The statistical analysis was done using SPSS version 20.0 software for Windows. The hypothyroid cases showed a median value with an inter-quartile range of 14.9(13.4-21.35) for Vitamin D (ng/mL). The median value with an inter-quartile range for Vitamin D in controls was26.2 (20.45-32.8). Comparing the serum Vitamin D levels between hypothyroid group and controls, a significant p value of <0.001 was observed. Vitamin D showed a moderately negative correlation (r= -0.347) with TSH with a p value of 0.008 in hypothyroid group. **Conclusion:** This study showed that though Vitamin D insufficiency was present in control group but this was progressed to deficiency in hypothyroid group. The decrease in serum Vitamin D levels was proportional to increase in serum TSH levels.

Keywords: Hypothyroidism, Vitamin D, Serum TSH, 25(OH) D.

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Received Date: 18/10/2015 Revised Date: 20/11/2015 Accepted Date: 14/12/2015

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	DOI: 20 December 2015

INTRODUCTION

Hypothyroidism is a common endocrine disorder that occurs due to the deficiency of thyroid hormone or its effect on peripheral tissue. The prevalence of hypothyroidism in adults is around 10.95% in India.[1] Hypothyroidism is classified as[2]

1. Primary Hypothyroidism-Due to insufficient production of thyroid hormone by thyroid gland.
2. Secondary Hypothyroidism-Due to insufficient production of TSH from pituitary gland.
3. Tertiary Hypothyroidism-Due to inadequate secretion of TRH from hypothalamus.

Primary hypothyroidism is most common among all types. The aetiology of this could be nutritional (Iodine deficiency), autoimmune (Hashimoto's thyroiditis), iatrogenic (radio-iodine ablation) and idiopathic.[3] Vitamin D is a fat soluble vitamin present in two forms cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2). Both forms can be found in foods or supplements, however vitamin D_3 can be synthesized in skin from 7-dehydrocholesterol on exposure to Ultra Voilet B (UVB) rays. The provitamin produced is activated after hydroxlation at 25 and 1 alpha position by 25-hydroxlasein liver and 1-alpha hydroxylase in kidney respectively.[4]

Serum 25(OH) D level is a better indicator of vitamin D status[4] than 1, 25$(OH)_2$ D because-

1. 25(OH) D is the main circulating form of vitamin D.
2. Serum 25(OH) D has longer half life as compared to 1,25 $(OH)_2$D.

How to site this article: R Priya, K CVasudha, P Kalra. Serum vitamin D levels in primary hypothyroidism. *MedPulse – International Medical Journal.* December 2015; 2(12): 953-957. http://www.medpulse.in (accessed 22 December 2015).

3. The technique to measure serum 25(OH) D is simpler than the more complicated methods for measuring serum 1, 25 $(OH)_2$ D.

Vitamin D belongs to the family of steroid hormones having its own nuclear hormone receptor.[5] Classically the role of vitamin D is on calcium/phosphorus metabolism and on skeletal system. Recent studies have seen its non-skeletal effects including immunomodulatory effect. The demonstration of vitamin D receptor in monocytes, dendritic cells and activated T cells indicates significant interaction between vitamin D and the immune system.[6] Vitamin D insufficiency/deficiency has become a global health problem. It has been seen in various studies that an inverse relationship exists between serum vitamin D levels and autoimmune thyroiditis. Results of the study done by Goswami R *et al*[7] indicated the presence of a significant inverse association between 25 (OH) D levels and TPOAb positive autoimmune thyroid disease. In the study done by Kivity *et al*[8] they showed that 79% of HT patients have 25(OH) D < 10ng/mL. Vitamin D has been shown to influence thyrocytes directly by attenuating TSH stimulated iodide uptake and cell growth.[9] Vitamin D receptor (VDR) and Thyroid hormones receptors (TR) both are members of nuclear hormone receptor family which includes receptor for glucocorticoids, mineralocorticoids, sex hormones, and vitamin A metabolites or retinoids. Both VDR and TR after binding with forms heterodimers with Retinoid-X-Receptor (RXR). The complex then binds with specific Hormone Responsive Element (HRE), VDRE (vitamin D responsive element) and TRE (thyroid hormone response element) respectively for vitamin D and thyroid hormone. The association of ligand with these receptors results in dissociation of co-repressor. The liganded receptor is capable of binding one or more co-activators with high affinity. This results in recuirtment of RNA polymerase II and activation of gene transcription.[10] As vitamin D and thyroid hormone has similar mechanism of action at molecular level, there is a possibility of association of vitamin D deficiency and hypothyroidism. This association is yet to be definitely proved.ChaiourkitL[11] ***et al*** has seen in their population based health study that higher 25 (OH) D levels were independently associated with lower TSH in younger individuals. As Vitamin D affects thyroid hormone action in varied way, this study was done to see the relationship between primary hypothyroidism and serum Vitamin D levels.

MATERIALS AND METHODS

It was a case control cross sectional study done at M S Ramaiah Medical College and Hospitals, Bangalore for a period of one year. Ethical clearance was obtained from College's Ethical Committee for this study. Written consent was taken from all participants in this study. Eighty six subjects were included in this study. They were classified into two main groups:

Group I "control group": Itincluded 29 apparently healthy individuals [Male (21.03%) and Female (78.95%)], their median ages with interquartile range were 31(26.5-35.5) years. They were not complaining of any chronic medical diseases, with normal clinical examinations, there was no history of thyroid diseases or any chronic illness which may interfere with results. They were not on Vitamin D supplements.

Group II "Hypothyroid patients": It included 57patients [Male (20.69%) and Female (79.31%)], their median ages with interquartile range were 31(25.5-35.5) years. They were diagnosed as hypothyroid patients if serum TSH level was higher than 4.5 mU/L with normal or lower levels of serum total T_4.[12]

All participants included in this study were subjected to the followings: Complete history taking. Complete clinical examination. Laboratory investigations, including:

Routine investigations

Serum T_3, T_4 and TSH, with reference range (1.3‑3.1 nmol/Lfor T_3), (66‑181 nmol/L for T_4) and (l0.270-4.20 μIU/mL for TSH). These analytes were estimated on Cobas 6000 e601 automated analyzer by Electrochemiluminenscense (ECLIA) method.

Research investigations

Estimation of serum 25 (OH) D levels using Enzyme linked immunosorbent assay (ELISA) kit method. The kit was from Calbiotech Inc. USA. This method was basedon the principle of competitive binding. A fixed amount of biotin-labeled vitamin D competes with the endogenous Vitamin D in the sample, standard, or quality control serum for a fixed number of binding sites on the anti-Vitamin D antibody. Bound Vitamin D-Biotin was detected with Streptavidin-HRP (SA-HRP).Addition of TMB Reagent and incubation at room temperature for 30 minutes, resulted the development of blue color. The color development was stopped with the addition of stop solution, and the absorbance was measured spectrophotometrically at 450 nmwavelength. Vitamin D deficiency was defined as a serum level of 25(OH) D of ≤ 20 ng/ml and insufficiency as a serum levelbetween >20 ng/ml and <30ng/ml and normal ≥ 30 ng/ml.[13]

RESULTS

Results were statistically analyzed by SPSS 20.0 for Windows. All the variables were presented as median with interquartile range (25^{th} percentile-75^{th} percentile) due to non-gaussian distribution of variables. The Kolmogrov- Smirnov method was used to test for normality of data distribution. Mann Whitney U test was

used to compare the results between the studied groups. The associations between the variables in a group were analyzed using Spearman test as correlation coefficient (r) and their significance (p value). Results were considered significant when p value was <0.05. The median values with interquartile range of all studied parameters, in all studied groups are shown in table1.There was no statistical difference (P > 0.05) between groups regarding age.

Table 1: Table showing the demographic data and laboratory parameters of the study group

	Control group (n=29) Median(Interquartile range)	Hypothyroid group (n=57) Median (Interquartile range)	p-value
Age (yrs)	31(26.5-35.5)	31(25.5- 37.5)	0.76
Total T_3(nmol/L)	1.79(1.54-1.92)	1.45(1.11-1.73	<0.001*
Total T_4(nmol/L)	105.2(96.31- 119.05)	87.66(57.07-101.36)	<0.001*
TSH(µIU/mL)	2.27(1.69- 2.9)	11.9(6.63-75.68)	<0.001*
Vitamin D(ng/mL)	26.2(20.45-32.8)	14.9(13.4-21.35)	<0.001*

Serum Vitamin D levels in control group was showing median value of 26.2 ng/mL with interquartile range of 20.45-32.8 ng/mL.Serum Vitamin D levels in hypothyroid group was showing median value of 14.9 ng/mL with interquartile range of 13.4-21.35 ng/mL The control group range was showing insufficiency or sufficiency but hypothyroid group has median value and range showing deficiency in cases. Using Mann Whitney U test for comparison between the two group, Vitamin D showed a p value of <0.001. Similar results were found for serum T_3, T_4 and TSH. The intragroup spearman correlation coefficient derived between Vitamin D and other parameters is shown in table (2).

Table 2: The correlation of different variables with Vitamin D in different groups

	Control group	Hypothyroid group
Total T_3(nmol/L)	r = 0.09 p = 0.62	r = 0.26 p = 0.05
Total T_4(nmol/L)	r = 0.10 p = 0.60	r = 0.27 p = 0.04
TSH(µIU/mL)	r = -0.03 p = 0.87	r = -0.34 p = 0.008

In control group no correlation was not significant between Vitamin D and other parameters. The hypothyroid group has shown a significant correlation. The thyroid hormones showed a weak positive correlation with Vitamin D, this correlation was statistically significant (p value of 0.05 and 0.04 for T_3 and T_4 respectively). Serum TSH showed a negative correlation with r value of 0.34 and a strong statistically significant p value of 0.008. The scatter plots derived for showing correlation of TSH with Vitamin D in hypothyroid group is shown in figure (1).

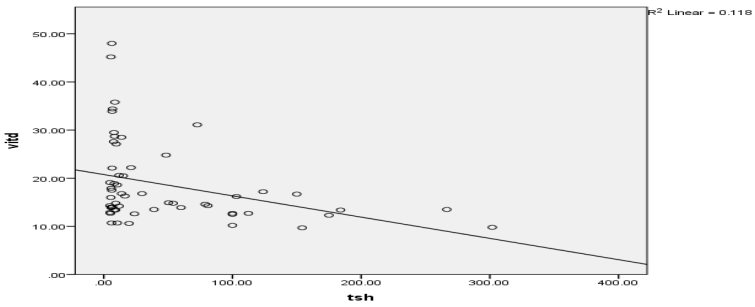

Figure 1: Correlations between serum TSH and Vit D Levels in hypothyroid patients

Gender distribution in different groups is shown in figure (2) and (3) as percentage.

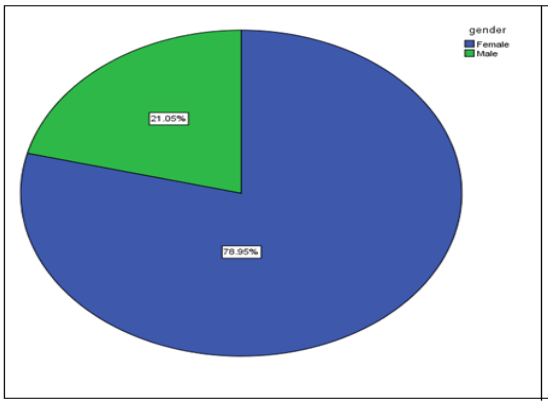

Figure 2

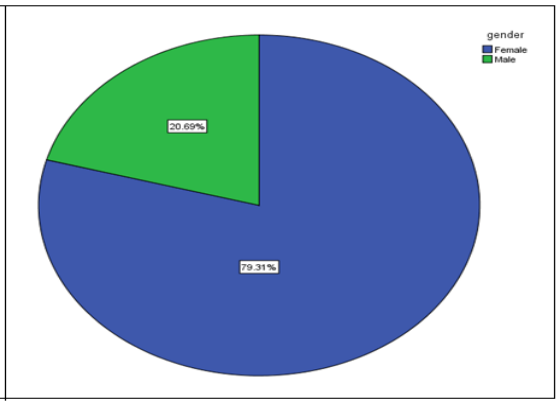

Figure 3

Legend

Figure 2: Pie diagram showing gender distribution in control group

Figure 3: Pie diagram showing gender distribution in hypothyroid group

DISCUSSION

Vitamin D is known for its primary role inbone and mineral homeostasis, and it has been shown recently that its deficiency is associated with various diseases such as cardiovascular disease, cancer, infection, and adiposity as well as osteoporosis. Few studies have been conducted to find any significant association between the levels of Vitamin D and hypothyroidism and to determine whether Vitamin D deficiency involves in the pathogenesis of hypothyroidism or rather a consequence of the disease. These studied yielded conflicting results. The present study showed serum Vitamin D deficiency in hypothyroid group. The decrease in Vitamin D was proportional to increase in serum TSH levels. This increase in TSH levels with fall in Vitamin D observed in the present study was in accordance with a study done by Zhang *et al*[14] on Chinese population where they found that high Vitamin D status was associated with low levels of TSH irrespective of thyroid hormones status. On correlating Vitamin D with TSH in hypothyroid group a significant correlation was seen. This finding from this study suggests a possible association of hypovitaminosis D with hypothyroidism. This observation is consensus with a study done by Mackway *et al*[15] in Saudi Arabia where it was observed that hypovitaminosis D in hypothyroid cases was significantly associated with the degree and severity of disease. Vitamin D insufficiency prevalent in general population was reflected in this study also with presence of insufficient subjects in control group. Though the cases were selected without any gender bias, a female predominance was seen in each group. This is in concurrence with a study done by Unnikrishnan *et al*[1] for the prevalence of hypothyroidism in India. In an experimental study done by Byron Richards[16] to see the effect of Vitamin D deficiency on thyroid gland, he reported that a lack of Vitamin D contributed to the possibility of low thyroid hormones. Two mechanisms may explain the low levels of Vitamin D in patients with hypothyroidism. First, the low levels of vitamin D may be due to poor absorption of Vitamin D from the intestine. Second, the body may not activate Vitamin D properly. Importantly, both Vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. A different gene in the Vitamin D receptor was shown to predispose people to autoimmune thyroid diseases including Graves' disease and Hashimoto's thyroiditis.[17]

CONCLUSION

Hypothyroidism is one of the commonest endocrine disorders. Primary hypothyroidism is the disorder of thyroid gland causing decreased synthesis and secretion of thyroid gland. It is characterized by an increase in serum TSH levels. Iodine deficiency and autoimmunity are the common causes of this condition. Both Vitamin D hormone receptors and Thyroid hormone receptors belong to nuclear hormone receptor family and have the same mechanism of action. Both the receptors are expressed on most of the tissues. The role of Vitamin D in the mechanism of auto immune disease is being studied extensively and one of the roles postulated is in the pathogenesis of hypothyroidism. The current study was undertaken to see the association of serum Vitamin D levels with serum TSH in primary hypothyroidism. The hypothyroid cases were chosen based on the serum TSH levels as already mentioned. Serum thyroid hormones and serum TSH was assayed by ECLIA. Serum Vitamin D and serum TPO Ab was assayed by ELISA method. This was a case control study involving eighty six subjects. The statistical analysis was done using Mann Whitney U test. The correlations were presented as Spearman correlation coefficient. The results showed serum Vitamin D insufficiency/ deficiency in most of the study subjects. The decrease in Vitamin D levels was more evident in hypothyroid cases as compared to euthyroid controls. A good correlation between Vitamin D and TSH in hypothyroid cases was observed. The findings of this study suggest the screening of Vitamin D insufficiency/ deficiency in hypothyroid cases and supplementation of the same for effective response to therapy.

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Source of Support: None Declared
Conflict of Interest: None Declared