

Reliable Earlier and Causative Biomarker in newly Diagnosed Hypothyroid Cases—Vitamin D: A Case–control Study

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ABSTRACT

Background: In Indian population there are 42 million people suffering from hypothyroidism. Hence, even though thyroid-stimulating hormone (TSH) has been used as the diagnostic marker of hypothyroidism, there arises a need to evaluate an earlier and a causative marker for hypothyroidism and thus it could be a better predictor of the disease in the earlier stages only.

Aims and objectives: The aim of the study is to identify a reliable earlier and one of the causative biomarkers of hypothyroidism.

Materials and methods: This study was conducted in 200 cases of newly diagnosed hypothyroidism and 200 controls of healthy individuals. Vitamin D, intact parathyroid hormone, TSH, triiodothyronine, thyroxine, and calcium were measured.

Results: It was observed that vitamin D was significantly decreased in hypothyroid cases. There was a negative correlation between vitamin D and TSH.

Conclusion: Vitamin D being one of the causative factors in thyroid disorders should be included as a routine parameter of analysis in hypothyroid cases and supplementation of vitamin D also to be initiated by the clinicians to avoid the progression to overt hypothyroidism in the newly diagnosed cases.

Keywords: Calcium, Hypothyroidism, Intact parathyroid hormone, Thyroid-stimulating hormone, Vitamin D.

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INTRODUCTION

In Indian population, there are 42 million hypothyroid cases.¹ Thus, there arises a need to identify an earlier causative and reliable biomarker to help in the early

diagnosis of hypothyroidism and in the therapeutic aspect also. Vitamin D, being a hormone, acts on various types of cells and plays multiple roles. Other than bone metabolism, it has a causative role in various diseases^{2,3} like diabetes mellitus,⁴ cancer,⁵ multiple sclerosis,⁶ autoimmune diseases,⁷ and atherosclerosis.⁸ Vitamin D not only acts through the immune-mediated process but also acts on the thyroid follicular cells by inhibiting directly the thyrotropin-mediated stimulation of iodine uptake in dose-dependent manner.⁹ A population-based study has observed low thyroid-stimulating hormone (TSH) among younger individuals.¹⁰ Hence, the reliability of TSH in the diagnosis of hypothyroidism in younger individuals is affected. Thus, in the present study, the relation between TSH and vitamin D with triiodothyronine (T3) and thyroxine (T4) at the time of diagnosis of hypothyroid cases has been evaluated.

MATERIALS AND METHODS

Two hundred cases of newly diagnosed hypothyroidism and 200 healthy individuals as controls were included in this case–control study. Written consent was taken from all the participants in the study. Complete history was taken. They belonged to the age group of 15 to 30 years. None of the participants were started on treatment for hypothyroidism. Patients with renal diseases, hepatic disease, rheumatologic disease, diabetes mellitus, alcoholics, dermatologic diseases, and child birth in last 1 year were excluded from the study. Control groups of people were those who visited the hospital for health checkup and the volunteers of blood donations. Venous samples were collected after 12 hours of fasting in plain tubes. For all the participants of the study, plasma glucose (glucose oxidase and peroxidase method), serum creatinine (modified Jaffe's kinetic method), and blood urea (urease method) were evaluated to rule out the basic pathology and it was done in Beckman Coulter AU 480. Calcium was done by o-cresolphthalein method. The TSH and intact parathyroid hormone (iPTH) were done by chemiluminescence in Beckman access 2. Vitamin D [25(OH)D] was done by high performance liquid chromatography method. Vitamin D of <30 ng/mL was considered as insufficiency and vitamin D of <20 ng/mL

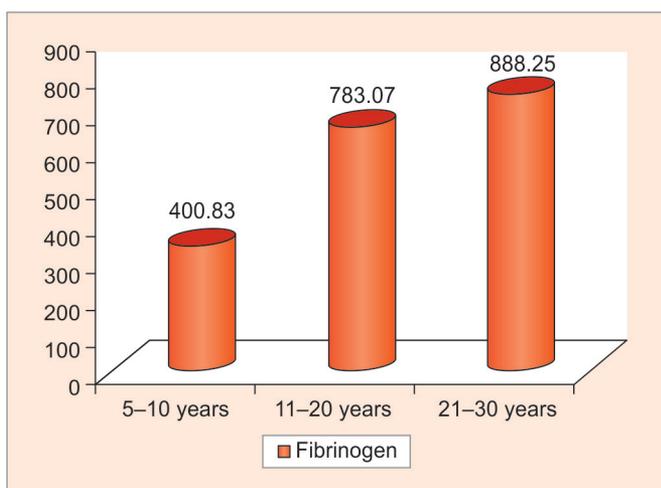
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Table 1: Characteristics of the physical parameters in the study group

Parameters	Controls n = 200	Cases n = 200	p-value
Sex	M – 43% (86) F – 57% (114)	M – 40% (80) F – 60% (120)	>0.05
Age (years)	20 ± 8.1	21 ± 7.3	>0.05

**Graph 1:** Characteristics of calcium, vitamin D, and iPTH in the study group

was considered as deficiency.¹¹ The reference range were 1.2–4.4 pg/mL for T3, 0.8–2.0 ng/dL for T4, and 0.5–5.0 mU/L for TSH.¹² The statistical analysis was done using the Statistical Package for the Social Sciences, version 20. Mean, standard deviation (SD), p-value, and Pearson's correlation were calculated; p-value <0.05 was considered as significant and indicated by * mark.

RESULTS

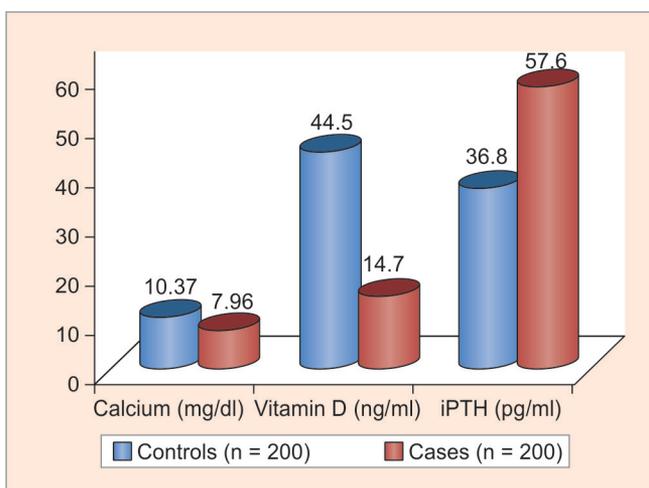
The mean, SD, p-value, and Pearson's correlation (r) were calculated for the age, sex, serum calcium, serum vitamin D, serum iPTH, and thyroid profile. Age group of the cases and controls were matching. In age and sex categories, there were no statistical significance and p-value was >0.05. For serum calcium, serum vitamin D, serum iPTH, and thyroid profile, p-values were <0.000 and hence statistical significance was seen. The characteristics of the physical parameters and the p values are shown in Table 1.

The characteristics of the biochemical parameters and the p values are shown in Table 2 and Graphs 1 and 2. The TSH and iPTH values were higher in the hypothyroid cases (6.92 ± 0.97 and 57.6 ± 3.30 respectively). The mean values of serum calcium and vitamin D were lower in hypothyroid cases: 7.92 ± 1.77 and 14.79 ± 2.11 respectively.

The comparison of TSH, serum calcium, and vitamin D values according to the sex distribution was statistically insignificant and it is shown in Table 3 and Graph 3. In

Table 2: Characteristics of the biochemical parameters in the study group

Parameters	Controls n = 200	Cases n = 200	p-value
Serum calcium (mg/dL)	10.37 ± 1.55	7.92 ± 1.77	0
Serum vitamin D (ng/mL)	44.53 ± 14.91	14.79 ± 2.11	0
Serum iPTH (pg/mL)	36.8 ± 1.91	57.6 ± 3.30	0
Serum TSH (mU/L)	3.66 ± 0.91	6.92 ± 0.97	0
Serum T3 (pg/mL)	2.94 ± 1.01	1.08 ± 1.02	0
Serum T4 (ng/dL)	1.59 ± 0.30	0.64 ± 0.46	0

**Graph 2:** Characteristics of thyroid profile in the study group**Table 3:** Levels of parameters according to sex in the hypothyroid patients

Parameters	Cases		p-value
	Male (n = 80)	Female (n = 120)	
Serum calcium (mg/dL)	7.92 ± 2.13	7.89 ± 1.55	0.988
Serum iPTH (pg/mL)	58.4 ± 3.12	56.3 ± 3.01	0.498
Serum vitamin D (ng/mL)	15.58 ± 2.27	14.27 ± 1.89	0.213
Serum TSH (mU/L)	6.80 ± 1.02	7.05 ± 0.96	0.597

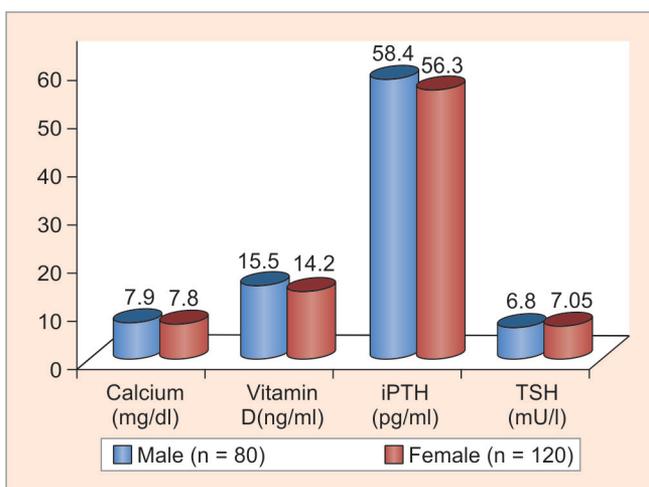
**Graph 3:** Levels of parameters according to sex in the hypothyroid patients

Table 4: Correlation between parameters in cases

Parameters	Vitamin D
Calcium	r = (0.82)
Intact PTH	r = (0.76)
TSH	r = (-0.52)
T3	r = (0.63)
T4	r = (0.04)

hypothyroid cases, TSH was higher and serum calcium and vitamin D were on lower side and were statistically significant with p-value <0.000.

In control group, there is a positive correlation between the vitamin D and each of calcium (r = 0.76) and T3 (r = 0.59). In these cases, there was a significant negative correlation between vitamin D and TSH (r = -0.52). There was also a significant negative correlation between calcium and TSH (r = -0.40). The correlations among the parameters are shown in Table 4.

DISCUSSION

Vitamin D, being a steroid hormone, not only plays a role in bone metabolism but also has a role in the pathogenesis of diabetes mellitus, hypertension, cancer, and autoimmune diseases. Vitamin D acts on 36 cell types that have vitamin D receptors (VDRs) including the thyroid gland. The vitamin D deficiency causes the abnormal functioning of the thyroid gland and presents with the symptoms of the hypothyroidism.^{8,13} Moreover, in the hypothyroid patients, the intestinal motilities were sluggish and it causes the malabsorption of vitamin D and it goes as a vicious cycle.¹⁴ A recent study has shown that the vitamin D deficiency leads to Graves' disease and the other autoimmune diseases of the thyroid gland.^{15,16} In India, it was thought that vitamin D deficiency was very uncommon as it is located between 8.4°N and 37.6°N latitude receiving a lot of sunshine.¹⁷ But many epidemiological studies have shown that in Indian population, 50 to 90% people have vitamin D deficiency due to the poor oral intake of calcium.³ Vitamin D gene polymorphism plays a main role in the altered action of the VDRs in the thyroid gland cells. Hence, the presentation of either hypothyroidism or even Graves' disease may be seen in patients depending on the basic gene polymorphism.¹⁸ Thus vitamin D gene polymorphism causes thyroid diseases and it has been revealed that vitamin D plays one of the causative roles in thyroid disorders. Vitamin D [1,25-(OH)₂D] is not used as the indicator of the vitamin D status in the serum as it has the short half-life of 15 hours only.¹⁹ Moreover, vitamin D [1,25-(OH)₂D] values will decrease in the serum of cases with severe vitamin D deficiencies.^{20,21} Hence, usually serum concentration

of 25(OH)D is measured as it has longer half-life of 15 days, which was followed in the present study also. Even though increased TSH is used as the marker of hypothyroidism for long time, it is only a feedback mechanism to reduce T3 and T4 and it will elevate during the time the disease has already progressed. But vitamin D playing the role in the pathogenesis of the disease will get altered at the early stages of the hypothyroidism.

In the present study, serum calcium and vitamin D levels were on lower side in cases when compared with the controls. Intact PTH was increased as a compensatory mechanism to reduce calcium and vitamin D. There was also a significant negative correlation between TSH and vitamin D levels in the cases at the time of diagnosis of hypothyroidism. In vitamin D deficiency individuals, iPTH increases as they are not able to maintain the serum calcium levels. In this study also, iPTH levels were elevated in accordance with the vitamin D levels and calcium levels. The target cells of iPTH are less responsive to the iPTH in hypothyroid cases, which also aggravates the vitamin D deficiency in the hypothyroid cases.²² This study shows the decreased levels of vitamin D in females than males, but it was not significant. The other studies have also mentioned the nonsignificant decrease in vitamin D levels among males and females.^{23,24} A study conducted in Japan has shown vitamin D deficiency in 40% females and 20% males with thyroid disorders, which was statistically significant (p < 0.005).¹⁴

Thus, in cases of hypothyroidism, the vitamin D levels should be analyzed as a routine to treat one of the causative factors of the disease pathology. Moreover, vitamin D is one of the modifiable risk factors for hypothyroidism and hence it is necessary to include vitamin D analysis as a routine in hypothyroid cases and to initiate the treatment of vitamin D. On following the analysis of vitamin D as a routine in practice, it becomes possible to diagnose hypothyroid cases in the early stages and to prevent the progression of the disease.

CONCLUSION

Hypothyroid cases have vitamin D deficiency at the time of diagnosis and there is an important role of vitamin D in the pathogenesis of thyroid disorders. There is a significant negative correlation between vitamin D and TSH in newly diagnosed hypothyroid cases, indicating the causative role of vitamin D in hypothyroidism. Hence, there arises a need to analyze vitamin D in all cases of hypothyroidism and to supplement vitamin D as a routine treatment to protect the cases from developing overt hypothyroidism in the future.

LIMITATIONS AND RECOMMENDATIONS

The present study has not attempted to exclude the effect of smoking, body mass index, and other factors which could alter the TSH levels. Moreover, only the newly diagnosed cases have been included in the study and attempts should be taken to follow the therapeutic effect of vitamin D on hypothyroid cases.

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