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Original Research Article

Evaluation of vitamin D levels in patients with primary hypothyroidism: A cross-sectional study

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ABSTRACT

Background: Vitamin D deficiency (VDD) and hypothyroidism have been linked in research with inconsistent outcomes. Because of the controversy surrounding these two disorders and their relatively high incidence, we undertook a case-control research comparing the prevalence of VDD in hypothyroid patients (both TPO-Ab positive and negative) to euthyroid controls.

Materials and Methods: This is a cross-sectional study with a single centre. Adults (aged 18 and above) of both sexes with primary hypothyroidism (TSH >5.1 mIU/L) were included (n = 165), as were age and sex matched euthyroid (TSH 5 mIU/ml) controls (n = 165) from the outpatients' department. Biochemical parameters like Thyroid stimulating hormone (TSH), free T4 (fT4), 25 hydroxy vitamin D (Vit D) and thyroid peroxidase antibody (TPO-Ab) were tested in both the groups.

Results: The study involved 330 patients, with 165 (mean age 46± 15 years) having primary hypothyroidism (3 having subclinical hypothyroidism) and 165 (mean age 45± 17 years) being euthyroid controls. VDD was seen in 96 percent of hypothyroid patients compared to 90 percent in the control group. The hypothyroid group had a significantly lower mean Vitamin D level than the euthyroid group (12.03± 8.6 SD vs. 17.49± 11.89 SD [ng/ml]; P= 0.001). TPO-Ab was found in two-thirds of the hypothyroid group (110/165). The mean Vitamin D level in the TPO-Ab positive hypothyroid group was 10.4± 7.2 ng/ml, compared to 15.3± 10.3 ng/ml in the TPO-Ab negative group (P = 0.004). With increased TPO-Ab titers, there was a downward trend in Vitamin D levels. A direct association between Vitamin D levels and TPO-Ab, on the other hand, did not reveal any relevance.

Conclusion: Patients with hypothyroidism had considerably lower vitamin D levels than euthyroid controls. TPO-Ab positive individuals had lower vitamin D levels than TPO-Ab negative patients.

1. Introduction

Vitamin D is a fat-soluble vitamin that helps maintain calcium balance and regulates bone metabolism. Vitamin D deficiency can cause rickets and osteomalacia in children, as well as osteomalacia in adults. In the 1930s, vitamin D fortification of milk proved successful in eradicating rickets over the world. Subclinical vitamin D deficiency, on the other hand, is still common in both industrialised and developing nations, with a global prevalence of up to 1 billion people.[1] Vitamin D insufficiency in the subclinical stage is linked to osteoporosis, a higher risk of falling, and fragility fractures. Many recent research, some of which are contradictory, reveal a link between vitamin D insufficiency and cancer, cardiovascular disease, diabetes, autoimmune diseases, and depression. The majority of vitamin D deficient individuals are asymptomatic. Even minor chronic vitamin D insufficiency, on the other hand,

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can cause chronic hypocalcemia and hyperparathyroidism, which can increase the risk of osteoporosis, falls, and fractures, particularly in the elderly.[2]

Symptoms of secondary hyperparathyroidism include bone pain, arthralgias, myalgias, weariness, muscular twitching (fasciculations), and weakness in patients with a protracted and severe vitamin D insufficiency. Chronic vitamin D deficiency, which leads to osteoporosis, can cause fragility fractures. Irritability, tiredness, developmental delay, bone abnormalities, and fractures can all be symptoms in youngsters.[3] Vitamin D insufficiency (VDD) is now widely recognised as a widespread problem that is being explored as a contributing factor to a variety of disorders. Despite the abundance of sunlight in India, this sunshine vitamin is said to be insufficient in the population. Hypothyroidism is estimated to affect 11% of the Indian population, with 9% of those suffering from subclinical hypothyroidism.[4]

Thyroid hormone levels in the blood are low in hypothyroidism. Clinical (overt) hypothyroidism is identified by symptoms such as mental sluggishness, depression, dementia, weight gain, constipation, dry skin, hair loss, cold intolerance, hoarse voice irregular menstruation infertility, muscle stiffness and pain, bradycardia, hyper cholesterolaemia, combined with a raised blood level of thyroid stimulating hormone (TSH) (serum TSH levels >12 mU/L), and a low-serum thyroxine (T4) level (serum T_4 <60 nmol/L). When serum TSH levels are elevated (>4 mU/L), yet serum T4 is normal, and there are mild or no symptoms or indicators of thyroid malfunction, subclinical hypothyroidism is identified.[5] Primary hypothyroidism develops after the thyroid gland is destroyed due to autoimmunity (the most common cause) or medical intervention such as surgery, radioiodine, or radiation. Secondary hypothyroidism develops following injury to the pituitary or hypothalamus, resulting in inadequate TSH production. This review does not address secondary hypothyroidism. When tri iodothyronine (T3) levels are low, serum T4 levels are low, and TSH levels are normal or low, euthyroid ill syndrome is identified.[6]

The outcomes of investigations linking VDD to primary hypothyroidism have been inconsistent. Because of the high frequency of hypothyroidism and VDD in the Indian population, we have a unique chance to investigate the relationship between these two factors.[7] As a result, we conducted a case–control study to determine the prevalence of VDD in hypothyroid individuals. Vitamin D levels were also examined between TPO Ab positive and TPO Ab negative patients, as well as hypothyroid patients and euthyroid controls, in the study.

2. Materials and Methods

This is a single-center cross-sectional study that took place from September 2019 to July 2020 at Sheikh Bikhari Medical College, Hazaribagh, Jharkhand on 165 hypothyroid patients. Adults (aged 18 years or older) of both sexes diagnosed with primary hypothyroidism were enrolled after receiving Institutional Ethics Committee approval and written informed consent.

2.1. Inclusion criteria

1. Adults (aged 18 years or older) of both sexes diagnosed with primary hypothyroidism were included
2. Age and sex matched euthyroid controls were included.
3. Patients who gave written informed consent

2.2. Exclusion criteria

1. Patients with post-radioiodine hypothyroidism
2. Patients with hepatic or renal impairment
3. Patients taking anti-epileptic drugs
4. Patients taking vitamin D supplementation

The study excluded patients with post-radioiodine hypothyroidism, hepatic or renal impairment, anti-epileptic drugs, or vitamin D supplementation. Age and sex matched euthyroid controls were included. To detect the signs and symptoms of hypothyroidism and VDD, a complete history and clinical examination were conducted. Thyroid-stimulating hormone (TSH), free T4 (fT4), TPO-Ab titers, and 25 hydroxy Vitamin D levels were assayed for each patient in the primary hypothyroidism group. Vitamin D levels were assayed for the control group with normal TSH.

2.3. Assays and ranges

The biochemical parameters were measured in our institution's Department of Biochemistry. TSH and FT4 were measured using a chemi-luminescence enzyme immunoassay (CLIA), whereas Vitamin D was measured using an enhanced CLIA (ECLIA) (total 25 hydroxy Vitamin D). TPO-Ab was tested using a chemi-luminescent microparticle immunoassay. VDD (20 ng/ml), insufficiency cb (20–30 ng/ml), and sufficiency (>30 ng/ml) were all defined using the Endocrine Society Clinical Practice Guidelines. As per laboratory norms levels <4.2 ng/ml were classified as severe VDD. The normal ranges for TSH and fT4 were 0.5–5 IU/ml and 0.76–1.79 ng/dl, respectively, in the laboratory where tests were performed. TSH values >5.1 IU/ml were enrolled into the study.

2.4. Sample size

A sample size of 165 was computed for each group based on the previous study, with a power of 80% and an alpha error of 0.05.

2.5. Statistical analysis

For all continuous variables, the mean standard deviation (SD) and range were determined. The TSH and Vitamin D levels of the different groups were compared using the Student's t test (hypothyroid and euthyroid). The prevalence of severe VDD was compared using the Chi square test between the TPO-Ab positive and negative groups. SPSS for Windows version 18.0 was used to conduct the statistical analysis (IBM SPSS Statistics for Windows, Version 18.0. Armonk, NY: IBM Corp was used for all analyses). The value of $P < 0.05$ was considered statistically significant.

2.6. Statistically significant criteria

The value of $P < 0.05$ was considered statistically significant.

3. Results

A total of 330 patients were enrolled into the study, of which 165 (mean age 46 ± 15 years) had primary hypothyroidism (3 had subclinical hypothyroidism) and 165 (mean age 45 ± 17 years) were euthyroid controls. (Table 1) In the hypothyroid group, 96% had VDD as compared to 90% in the control group. Mean Vitamin D level was significantly lower in the hypothyroid group than the euthyroid group (12.03 ± 8.6 SD vs. 17.49 ± 11.89 SD [ng/ml]; $P < 0.001$) (Table 2). While 47 cases with hypothyroidism had severe VDD (Vitamin D levels <4.2 ng/ml) only 30 among the control group had severe VDD (odds ratio [OR] 2.04, confidence interval [CI]: 1.03–4.05, $P < 0.05$).

Two- thirds of the hypothyroid group (110/165) tested positive for TPO-Ab. The mean level of Vitamin D in the TPO-Ab positive hypothyroid group was 10.4 ± 7.2 ng/ml, compared to the TPO-Ab negative group 15.3 ± 10.3 ($P = 0.004$). One–third of those with positive antibody had severe VDD as compared to 10% (6/55) in the antibody negative group (OR 3.39, CI: 1.18–9.80; $P < 0.05$) 3.62) (Table 3).

There was a declining trend of Vitamin D levels with rising titers of TPO-Ab. (Table 4) However, a direct correlation between Vitamin D levels and TPO-Ab did not show any significance ($R = 0.15$, $P = 0.18$).

Table 1: Mean age in cases and controls

Study Groups	Mean Age ± S.D years	p value
Hypothyroids	46 ± 15	0.43
Euthyroids	45 ± 17	

Table 2: Mean vitamin D levels in cases and controls

Study Groups	Mean vitamin D level ± S.D (ng/ml)	p value
Hypothyroids	12.03 ± 8.6	0.001
Euthyroids	17.49 ± 11.89	

4. Discussion

When Vitamin D levels were compared between hypothyroid patients and euthyroid controls in this study, 93 percent were found to be inadequate. Although the prevalence of VDD did not differ substantially between hypothyroidism and euthyroidism, the group with hypothyroidism had a considerably greater proportion of severe VDD (4.2 ng/dl). In the severely VDD group, the OR for hypothyroidism was 2.04. The group with hypothyroidism had considerably lower mean Vitamin D levels. These findings suggest that people who are Vitamin D deficient are more likely to develop hypothyroidism. TPO -Ab positive patients exhibited a lower mean Vitamin D level than those with hypothyroidism and there was a significantly higher number of patients with severe VDD in this group.[8,9]

VDD is widely reported to be widespread in India, and the overall prevalence of VDD in our study backs up this claim. Vitamin D levels were lower in hypothyroidism patients, with a significant OR in individuals with severe VDD. A research of 152 hypothyroid individuals conducted in Meerut found a similar link between Vitamin D and hypothyroidism. Vitamin D levels were 16.73 12.46 ng/ml, 13.23 10.08 ng/ml, and 29.07 19.01 ng/ml in the subclinically hypothyroid, overt hypothyroid, and controls, respectively. Patients with hypothyroidism had much lower levels than controls, which matched our findings.[10,11]

TPO-Ab positivity was found in 110 of the 165 cases. Autoimmunity is the most common cause of autoimmune thyroid disease (AITD), and about 10% of people with AITD may not have detectable antibodies in their blood. A recent metaanalysis of 20 case–control studies found that AITD patients had lower mean levels of Vitamin D than controls, which is consistent with our findings.[12–14] The odds of being hypothyroid in patients with VDD was 2.99. (95 percent CI 1.88,4.74). In India, Goswami et al. found a link between TPOAb and Vitamin D, but it wasn't strong enough to infer that Vitamin D protects against thyroid autoimmunity. Another study from Benares found that AITD patients had considerably lower levels of Vitamin D. Patients with autoimmune hypothyroidism had a mean Vitamin D level of 15.67 5.61 ng/dl in this study, while non-hypothyroid controls had a Vitamin D level of 31.39 4.63 ng/dl.[15,16]

Similar findings have been found in studies from around the world. Patients with higher Vitamin D levels exhibited lower thyroid antibody titers, according to Kivity et al. These data imply that Vitamin D may have a role in the onset of autoimmunity. Immune cell dynamics, particularly dendritic cells and T lymphocytes, as well as their products, may play a role. In a recent Indian study supplementation of Vitamin D to TPO-Ab positive individuals resulted in a reduction of antibody titers, reiterating the role of Vitamin D in antibody production. It is also of interest that Vitamin

Table 3: Levels of vitamin D in hypothyroid cases

Vitamin D level	Anti TPO positive hypothyroidism (n= 110)	Anti TPO negative hypothyroidism (n= 55)	P value
< 4.2 ng/ml	33	09	0.003
4.2 to 20 ng/ml	62	33	0.004
20-30 ng/ml	10	07	0.002
>30ng/ml	5	6	0.004

Table 4: Mean levels of vitamin D among various levels of TPO-Ab positivity

Anti-TPO levels	Mean levels of Vit D (ng/ml)	P value
Less than 100 IU (n=15)	12.31± 2.43	
100-1300 IU (n= 17)	10.66 ±3.65	0.02
More than 1300 IU (n=55)	8.88 ±2.87	

D has been implicated in connective tissue diseases and studies are ongoing to define the effects of Vitamin D supplementation in these conditions.[17,18]

The control group and the inclusion of sufficient research participants are the study's key strengths. The study, on the other hand, was designed to detect VDD in hypothyroidism rather than TPO-Ab positive hypothyroidism. The paucity of prospective data and thyroglobulin antibody levels are two further drawbacks. TPO-Ab was not measured in the euthyroid control group, which could be a disadvantage.

5. Conclusion

VDD is found in both hypothyroid and euthyroid people, with considerably lower Vitamin D levels in antibody-positive hypothyroid patients, implying that decreased blood Vitamin D levels may be linked to autoimmune hypothyroidism. Large multicenter randomised controlled trials would undoubtedly aid in determining whether there is a genuine link between Vitamin D and thyroid autoimmunity, as well as the beneficial effects of Vitamin D treatment in these patients.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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