

## The Study of Serum Calcium and 25-OH Vitamin D Levels in Newly Diagnosed Hypothyroid Patients

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**Abstract:** Introduction: Among various endocrine problems, thyroid disorders are the most common worldwide. Thyroid hormone is a central regulator of body functions. Vitamin D deficiency is also a global health problem and its role as an immune modulator is already established. Recent evidence has demonstrated an association between low vitamin D status and autoimmune diseases such as Hashimoto's thyroiditis and Grave's disease, and impaired vitamin D signaling in thyroid cancers. The main role of vitamin D is regulating bone metabolism and calcium homeostasis. Therefore, the aim of this study is to assess levels of 25-OH Vitamin D and serum Calcium in newly diagnosed hypothyroid patients. Materials & Methods: Total 70 newly diagnosed hypothyroid patients and 65 healthy controls were included in the study. Sera were collected from them and serum calcium, 25-OH vitamin D, T3, T4, fT3, fT4 & TSH level were estimated in the study groups. Result: Patients with hypothyroidism showed significant decrease in serum calcium & 25-OH vitamin D level ( $p < 0.001$ ). When correlated with TSH level, the correlation was negative for both serum calcium & vitamin D. Correlation of 25-OH vitamin D with serum calcium and thyroid hormones were significantly positive. Conclusion: The present study indicates relationship of thyroid hormones and serum calcium and vitamin D in hypothyroid patients. We suggest that hypothyroid patients should be regularly monitored for serum calcium and 25-OH vitamin D level.

**Key words:** Hypothyroid, TSH, fT3, fT4, 25-OH vitamin D

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### I. Introduction

Primary hypothyroidism is a frequent syndrome, whose prevalence is 0.5–2.0% among women and around 0.2% among men.<sup>[1]</sup> According to several authors, recently the number of patients with autoimmune diseases with hypothyroidism have increased by 2.1%.<sup>[1]</sup> Hypothyroidism is state of thyroid hormone deficiency or their impaired functions with compensatory thyroid enlargement.<sup>[2]</sup> Impaired production of thyroid hormones primarily due to thyroid abnormality or iodine deficiency; sometimes may be secondary to pituitary or hypothalamic disorders. Hypothyroidism is associated with weight gain, cold intolerance, constipation. Thyroid hormones have important biological effects such as regulation of body hemodynamic, thermoregulation, and various metabolisms. It influences almost all metabolisms in the body including carbohydrate, proteins, lipids, and maintenance of water and electrolyte homeostasis, which are well-established.<sup>[3,4]</sup>

Vitamin D deficiency is a global health problem.<sup>[5]</sup> Over a billion people worldwide are vitamin D deficient or insufficient.<sup>[5]</sup> Vitamin D deficiency has been shown to be associated with autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), multiple sclerosis (MS) and type 1 diabetes (T1DM), and that vitamin D supplementation prevents the onset and/or development of these autoimmune diseases.<sup>[6]</sup> Furthermore, it was reported that patients with Hashimoto's thyroiditis, an autoimmune thyroid disease had lower vitamin D levels.<sup>[7]</sup>

Vitamin D mediates its effects through binding to vitamin D receptor (VDR) and thus activation of respective genes<sup>[8]</sup> So a lower level of vitamin D is likely to aggravate the systemic abnormalities associated with hypothyroidism<sup>[9]</sup> It is present in the serum in either of its two forms: 25 hydroxycholecalciferol [25(OH) D] or 1, 25 dihydroxycholecalciferol [1, 25(OH) D]. The measurement of 25(OH) D was preferred to 1, 25(OH) D in many studies as it has fairly long circulating half-life of 15 days<sup>[10]</sup> and reflects the total vitamin D content of the body.<sup>[11]</sup> Several studies have reported low serum levels of vitamin D in hypothyroid patients which in turn may lead to some musculoskeletal complaints in these patients.<sup>[12,13]</sup>

The study was conducted to evaluate calcium and 25-OH Vitamin D status in newly diagnosed hypothyroid patients and also its association with thyroid hormones in patients with Hypothyroidism attending tertiary care centre (RMCH & RC, Hapur).

## II. Materials & Methods

This study was a hospital based cross sectional study conducted in the department of Biochemistry at RMCH &RC, comprising of 70 newly diagnosed and untreated cases of hypothyroidism and 65 age and sex matched controls were taken . Institutional Ethical clearance was taken. The objectives of the study were explained to all eligible subjects. Informed consent of all subjects included in the study was obtained for involvement in study groups and for venipuncture.

### 2.1.1 Inclusion criteria

The diagnosis was based on decreased serum T3 and T4 levels associated with increased TSH levels. All patients suffering from hypothyroidism were diagnosed and confirmed by the physician based on freeT3 (Normal: 2.4-4.2 pg/ml), freeT4 (Normal: 0.7-1.4 ng/dl) and TSH (Normal: 0.34-4.25  $\mu$ IU/ml) levels of the patients.<sup>[14]</sup>

### 2.1.2 Exclusion criteria

Patients with post radioiodine hypothyroidism, hepatic or renal dysfunction, on anti- epileptic medications or vitamin D supplementation were excluded from the study.

The Endocrine Society Clinical Practice Guidelines were used to define VDD (<20 ng/ml), insufficiency (20–30 ng/ml) and sufficiency (>30 ng/ml).<sup>[15]</sup>

A 3ml of venous blood is drawn from each volunteer using a disposable plain vacutainer system in fasting condition. Serum is separated within half an hour by centrifugation and stored at 2-8°C temperature till analysis is done. Thyroid hormones assay and Vitamin D level were done by Fluorometric enzyme immunoassay (FEIA) method on TOSHO AIA-360 Immunoanalyzer machine. Determination of serum Calcium levels using spectrophotometric method by ERBA CHEM-7 semiautoanalyzer. This method is based on formation of Ca<sup>+</sup> ions violet complex with o-cresol-phthalein complex in alkaline medium.

The collected data were statistically analyzed by using SPSS statistics (Statistical Package for Social Sciences) software version 20.0. Correlations were done using Pearson's Correlation for numerical parametric data. P value < 0.05 was considered statistically significant.

## III. Results

Clinical data was studied to find out the age and sex distribution of hypothyroidism in the selected population. It was observed that a majority of the patients (n=42) were females and (n=28) of the patients were males (p<0.01).

**Table1.** Distribution of patients according to different age-groups

| Age-group (years) | Frequency | Percentage (%) |
|-------------------|-----------|----------------|
| 20-39             | 19        | 27.1           |
| 40-59             | 38        | 54.2           |
| 60-79             | 11        | 15.7           |
| >80               | 2         | 2.8            |

Among 70 patients, 27.1% (n=19) fall in age-group 20-39 years category, 54.2% (n=38) in 40-59 years category, 15.7% (n=11) in 60-79 years category and 2.8% (n=2) in  $\geq$  80 years category. We also observed that highest number (54.2%) of patients belonged to the age group of 40-59 yrs. (Table.1)

**Table2.** Biochemical parameters of the hypothyroid patients & healthy controls

| Parameters              | Controls (Mean $\pm$ SD) | Cases (Mean $\pm$ SD) | p value |
|-------------------------|--------------------------|-----------------------|---------|
| fT3 (pg/mL)             | 3.12 $\pm$ 0.89          | 2.34 $\pm$ 0.06       | < 0.001 |
| fT4 (ng/dL)             | 1.07 $\pm$ 0.08          | 0.61 $\pm$ 0.56       | < 0.001 |
| T3 (ng/dL)              | 126.25 $\pm$ 8.53        | 88.76 $\pm$ 6.54      | < 0.001 |
| T4 ( $\mu$ g/dL)        | 8.56 $\pm$ 1.57          | 5.17 $\pm$ 0.76       | < 0.001 |
| TSH ( $\mu$ IU/mL)      | 2.76 $\pm$ 0.42          | 6.76 $\pm$ 4.16       | < 0.001 |
| 25-OH Vitamin D (ng/mL) | 52.03 $\pm$ 10.26        | 13.98 $\pm$ 2.09      | < 0.001 |
| Serum Calcium (mg/dL)   | 10.53 $\pm$ 0.96         | 7.87 $\pm$ 1.28       | < 0.001 |

TSH = thyroid stimulating hormone; fT3 = free triiodothyronine; fT4 = free thyroxine

Table 2. Shows the mean values of thyroid function parameters and 25-OH Vitamin D and serum calcium level of study subjects. fT3, fT4, T3, T4 and TSH levels were 2.34  $\pm$  0.06 pg/mL, 0.61  $\pm$  0.56 ng/dL, 88.76  $\pm$  6.54 ng/dL, 5.17  $\pm$  0.76  $\mu$ g/dL, and 6.76  $\pm$  4.16  $\mu$ IU/mL respectively. Serum 25-OH vitamin D & serum

calcium levels were found to be  $13.98 \pm 2.09$  ng/mL,  $7.87 \pm 1.28$  mg/dL respectively (fig.1). We found a significant decrease in ( $p < 0.001$ ) fT3, fT4, T3, and T4 were found in hypothyroid patients as compare to healthy controls whereas serum TSH level was significantly higher in patients ( $p < 0.001$ ). Serum 25(OH) vit D and serum calcium levels were significantly lower in hypothyroid patients than in controls ( $p < 0.001$ ). Figure.1 is depicting mean 25-OH vitamin D status in study groups.

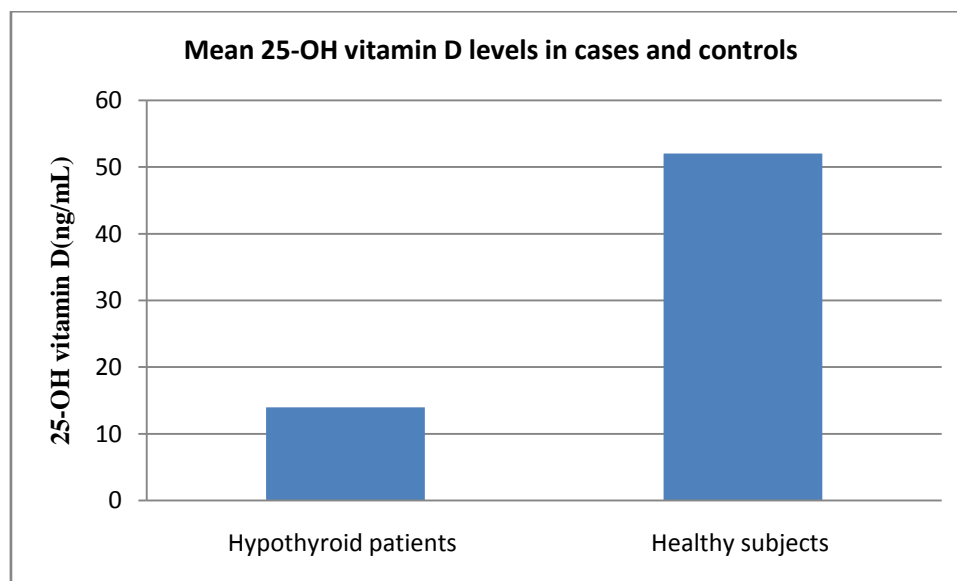


Fig.1. Distribution of mean 25-OH vitamin D levels in study subjects

Table 3. Mean  $\pm$  SD of serum 25(OH) vit D, Calcium and TSH levels in hypothyroid patients according to sex

| Parameters<br>Mean $\pm$ SD | Male =23         | Female=47        | p- value |
|-----------------------------|------------------|------------------|----------|
| 25(OH) vit D ng/mL          | 14.58 $\pm$ 2.27 | 13.27 $\pm$ 1.89 | p >0.05  |
| Calcium levels (mg/dL)      | 7.92 $\pm$ 2.13  | 7.89 $\pm$ 1.55  | p >0.05  |
| TSH ( $\mu$ U/mL)           | 6.20 $\pm$ 4.02  | 6.95 $\pm$ 4.96  | p >0.05  |

On comparing according to the sex distribution, in hypothyroid patients serum calcium and 25-OH vitamin D levels were insignificantly decreased in females than male patients ( $p > 0.05$ ). When serum TSH levels in hypothyroid patients were compared regarding to the sex, we noticed a non-significant difference between males and females patients ( $p > 0.05$ ). (Table.3).

Table 4. Correlation coefficient values of thyroid hormones and TSH with 25-OH Vitamin D & Serum Calcium.

| Parameters                           | Coefficient of correlation (r) | p value |
|--------------------------------------|--------------------------------|---------|
| 25(OH) vit D vs Serum Calcium Levels | 0.477                          | < 0.001 |
| 25(OH) vit D vs TSH                  | -0.567                         | < 0.001 |
| 25(OH) vit D vs T3                   | 0.554                          | < 0.001 |
| Serum Calcium vs TSH                 | - 0.459                        | < 0.001 |

Table.4 There were significant positive correlations between serum 25-OH vitamin D and each of serum calcium levels ( $r=0.477$ ,  $p < 0.001$ ) in hypothyroid patients. Serum TSH level is negatively correlated to 25-OH vitamin D ( $r=-0.567$ ,  $p < 0.001$ ) but T3 shows significantly positive correlation ( $r=0.554$ ,  $p < 0.001$ ). The correlation between serum calcium vs TSH in patients was significantly negative ( $r= -0.459$ ,  $p < 0.001$ ).

#### IV. Discussion

This study was conducted to assess the relationship between hypothyroidism and serum electrolyte level among the patients attending RMCH&RC. This study revealed more than three fourth (67.1%) of the hypothyroid patients were female. Majority (54.2%) of them fall in age group 40 to 59 years. This indicates that hypothyroidism is more common in middle-aged females. This finding is supported by earlier statistics which suggested much higher prevalence of hypothyroidism in women compared to men. We observed vitamin D deficiency in 51.72% of subjects irrespective of thyroid hormone status. These findings were in accordance with the findings of many authors.<sup>[16,17]</sup>

In present study we observed a significant difference in serum calcium levels between the studied groups with lower level seen in hypothyroid patients where it insignificantly decreased in females than male patients. Al-Hakeim HK. et al<sup>[18]</sup> conducted a study showed a significant changes in ionized Ca, but not total Ca means that the physiologically active form of Ca is affected, while the overall concentration of Ca is still significantly unchanged. Disturbance of calcium homeostasis were frequently observed with thyroid dysfunction.<sup>[19]</sup> Thyroxine normally regulates blood calcium levels by releasing calcium extra cellular.<sup>[20]</sup> In hypothyroidism, less thyroxine in the bloodstream and thus less thyroxin entry into the cells leading to decreased extra cellular calcium release.<sup>[21]</sup>

The present study showed that vitamin D level was significantly lower in hypothyroid patients compared to the controls. We recorded a significant positive association between Vitamin D and calcium levels in both groups. Vitamin D and calcium serum levels had negative correlation when compared to TSH levels. These results suggested that there may be a significant association between vitamin D deficiency and hypothyroidism. It also states a putative role of vitamin D as a potential modifiable risk factor for hypothyroidism. In order to function, vitamin D must bind to VDR which is found in several cell types including thyroid gland<sup>[8,22]</sup> Studies have shown that patients of autoimmune thyroid disease have several VDR polymorphisms that affect its expression and activation.<sup>[23]</sup> So vitamin D plays a role in maintaining a euthyroid state by interacting with its receptor in the thyroid gland. Although a causal relationship could not be established.

## V. Conclusion

We found in our study that hypothyroid patients are suffered from Vitamin D deficiency and hypocalcaemia. The positive correlation of Vitamin D and calcium with thyroid hormones and negative correlation with TSH, suggest that vitamin D to be supplemented to all hypothyroid patients including the subclinical cases. It is also recommended that all newly diagnosed hypothyroid patients should be screened for 25-OH vitamin D and serum calcium.

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