

# Vitamin D and Calcium Levels in Female Hypothyroid Patients Presenting to OPD in Sialkot: Cross Sectional Study

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## ABSTRACT:

Vitamin D is an important vitamin for bone metabolism and development of immune functions.

**Purpose:** To determine the serum levels of 25-hydroxy-vitamin D and calcium in diagnosed patients of hypothyroidism in comparison to healthy controls group.

**Study Design:** Cross sectional study.

**Methodology:** Total subjects were sixty with n=30. Control group comprised of normal females and diseased group had females that were diagnosed with hypothyroidism. Blood was collected from all the subjects from antecubital vein. Serum was separated to determine the serum levels of vitamin D and calcium.

**Statistical analysis:** Data analyzed by SPSS 22.0v. T-test applied for comparison between groups with P-value <0.05 as significant.

**Results:** Serum 25 hydroxy-vitamin D and calcium levels were significantly lower in the diseased group as compared to the control group having P-value < 0.001.

**Conclusion:** This study clearly showed that hypothyroid patients were deficient in vitamin D and calcium as they had lower serum levels when compared with healthy individuals.

**Key Words:** 25-Hydroxy-Vitamin D and Calcium, Hypothyroidism and Healthy Individuals.

## INTRODUCTION

Vitamin D is an important vitamin that plays a pivotal role in bone metabolism and immune function. It has been reported that it plays a central role in various immune processes like growth and differentiation of cells. It possess anti-inflammatory and immune-regulatory properties<sup>1</sup>. The active form of vitamin D<sub>3</sub> is 1,25-dihydroxy-cholecalciferol (calcitriol). Low vitamin D levels cause clinical issues like infectious disease, bones weakness and abnormal bone mineralization as revealed by literature review but its causal role remains a mystery<sup>2</sup>.

Thyroid hormones (T<sub>3</sub>, T<sub>4</sub> and TSH) are produced by thyroid gland. These hormones play a critical role in body homeostasis including normal body growth and energy metabolism. Due to its diverse role in human life, it is more prone to dysfunction. It is treatable if diagnosed correctly at early stage but if remains undiagnosed or untreated than an individual is at risk of various adverse effects. Regardless of advancement in medical science as well as an increase in thyroid disease awareness with highly sensitive laboratory aids for its serum hormone level measurement still cases with extreme thyroid dysfunction occur. Common thyroid dysfunctions include hypothyroidism and hyperthyroidism that usually occur due to pathological changes in thyroid gland (primary thyroid disease). Rarely, they occur from hypothalamus or pituitary (central) disorders or from peripheral causes<sup>3</sup>.

Overt hypothyroidism prevalence ranges from 0.2-5.3% in UK while its 0.3-3.7% in USA as revealed by previous study among general population<sup>3</sup>. Due to the lack of research culture at Pakistan, data regarding hypothyroidism incidence and prevalence is limited unfortunately. In our clinical setups, patients suffering from overt hypothyroidism are treated with levo-thyroxine at a dose of 1.6 µg/kg/day, later titrated to achieve optimal TSH levels (0.4–4.0 mIU/L)<sup>4</sup>.

Vitamin D deficiency is associated with various autoimmune diseases like systemic lupus erythematosus (SLE), inflammatory bowel disease and type-1 diabetes are linked with vitamin-D deficiency. Previous studies showed that supplementation with vitamin-D prevents both the onset as well as the progression of these autoimmune diseases. Moreover, patients of Hashimoto's thyroiditis have lower vitamin D levels<sup>5</sup>.

In the light of above description and lack of data on this health issue, we planned to conduct present study with aim to determine the serum levels of 25-hydroxy-vitamin D and calcium in diagnosed patients of hypothyroidism in comparison to healthy controls group.

**Objective:** To determine the serum levels of 25-hydroxy-vitamin D and calcium in diagnosed patients of hypothyroidism in comparison to healthy controls group.

**METHODOLOGY**

Present study was done at a private setting, Sialkot. Consent was taken from patients that visited the OPD of the hospital. Convenient sampling was done. Sixty patients were included with n=30. We divided the subjects in two groups. Control group included normal healthy females. Other had patients with TSH levels >7mU/mL. Levels of 20ng/ml- 50ng/ml for 25-hydroxyvitamin D was considered normal. Serum calcium levels of 8.6-10.3 mg/dl were taken as normal. The blood was collected in the non-EDTA vacutainers. Afterwards, vacutainers were centrifuged at 3000 rotations per minute to separate the serum. Serum was aspirated with the help of a micropipette and saved in the Eppendorf tubes. Labelling of the samples was done very carefully. 25-hydroxy vitamin D levels were done by enzyme linked immunosorbent assay. Alkaline phosphatase conjugated 25-hydroxyvitamin D and antibody conjugated 25-hydroxyvitamin D were added to the wells. The microplate was incubated and then washed with wash buffer. p-Nitrophenyl Phosphate reagent was added in the wells. The reaction was stopped with the stop solution. The absorbance was recorded at 405nm<sup>6</sup>. Calcium microplate assay kit was used to check the serum calcium levels.

**Statistical Analysis:** Data analyzed by SPSS 22.0v. T-test applied for comparison between groups with P-value <0.05 as significant.

**RESULTS:**

Comparison between different groups for serum vitamin-D levels were presented in table-1 as Mean ± SEM.

Table-1: Mean ± SEM for the serum 25- hydroxyvitamin D

	CONTROL Mean ± SEM	DISEASED Mean ± SEM	P value
Serum 25-hydroxyvitamin level ng/ml	39.58 ± 1.777	14.50 ± 0.621	<0.001*

\*Statistically significant.

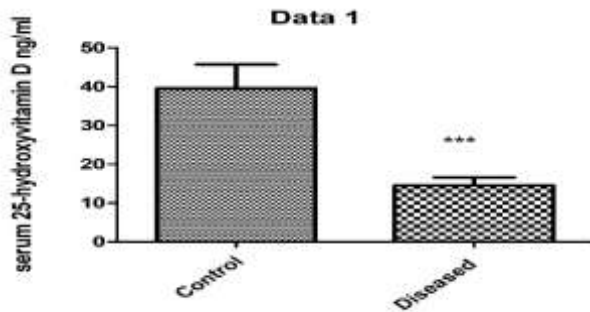


Figure-1: Graphical presentation of serum 25-hydroxyvitamin D levels \*\*\*-P value < 0.001

The serum calcium were higher in the normal controls as compared with the hypothyroid patients (p-value <0.001) as presented in table-2.

Table-2: Serum Calcium Levels as Mean ± SEM

	CONTROL Mean ± SEM	DISEASED Mean ± SEM	P value
Serum calcium levels mg/dl	8.940 ± 0.1108	7.650 ± 0.1204	< 0.001*

\*Statistically significant.

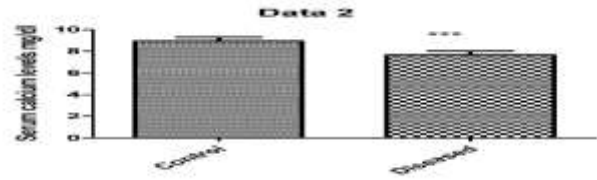


Figure-2: Graphical presentation of serum calcium levels \*\*\*-P value < 0.001

**DISCUSSION:**

Even in the presence of modern era, around 5% cases of hypothyroidism are misdiagnosed. Primary hypothyroidism comes out to be in 99% cases of thyroid dysfunction. Iodine deficiency is a major cause of hypothyroidism. However, in areas with iodine sufficiency, chronic autoimmune thyroiditis leads to thyroid failure<sup>4</sup>.

The serum biomarker for vitamin D status is its active (1,25-dihydroxy-cholecalciferol) form. Initial step of hydroxylation occur in liver followed by kidneys. Vitamin D binding proteins play an important role in transport of vitamin D and its metabolites to target cells. Its serum levels are regulated via negative feedback mechanism involving the actions of calcium, phosphorous, 1,25, di-hydroxy-vitamin-D, parathyroid hormone and fibroblast growth factor<sup>7</sup>.

Various previous studies have documented a relationship between vitamin D deficiency and autoimmune thyroid diseases with impaired vitamin D signaling especially in thyroid cancers. Literature review has reported that vitamin D deficiency play a critical role in thyroid disease development including thyroid cancers<sup>8</sup>. In our study, levels of vitamin D were lower in the patients having hypothyroidism that was diagnosed with serum TSH levels. This result was supported by the fact that lower serum calcium levels were present in hypothyroid group than healthy group. Vitamin D along with parathyroid hormone tightly regulated the serum levels of calcium and phosphorous<sup>9</sup>.

Replacing vitamin D in patients is very important to prevent the deleterious effects of the deficiency. Ergocalciferol and cholecalciferol are used for the vitamin replacement therapy. Both forms of vitamin D are fat soluble. After taking ergocalciferol<sup>10</sup>, levels rise and fall back to almost baseline in a few weeks. In contrast, after administering cholecalciferol, the levels stay high for a while. Doctors frequently prescribe 50,000 IU of vitamin D weekly for 8 weeks<sup>10</sup>. However, with appropriate supplementation of vitamin D in high risk population we can decrease considerable patient morbidity.

Due to the budgeting and resource issues we were not able to include other parameters like anti thyroid antibodies, levels of parathyroid hormone, serum phosphate levels, T<sub>3</sub> and T<sub>4</sub> levels. Another group of subjects taking levothyroxine could have been added to the study. We give the recommendation that in the future studies these parameters can be done to better understand the association of vitamin D levels and the thyroid function.

**Limitations:** Our limitations include small sample size with limited financial and human resources. No genetic workup was done.

## CONCLUSION

This study clearly showed that hypothyroid patients were deficient in vitamin D and calcium as they had lower serum levels when compared with healthy individuals.

**Author's contribution:** HM & IA: Conceptualized the study, analyzed the data, and formulated the initial draft.

AM: Contributed to the histomorphological evaluation.

TL: Contributed to the analysis of data and proofread the draft.

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