Research Article

Assessing The Response of Bone Mineral Metabolism and Turnover Markers To Levothyroxine in Primary Hypothyroidism

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Abstract

Introduction:
Primary hypothyroidism is a common endocrine disorder with systemic effects on metabolism and skeletal health. Thyroid hormone deficiency alters calcium homeostasis, vitamin D status, and bone turnover, contributing to long-term skeletal complications.

Aim:
To evaluate thyroid function, calcium, vitamin D, and bone-specific alkaline phosphatase (BSAP) in untreated hypothyroid patients, those on long-term levothyroxine therapy, and healthy controls.

Methods:
A case-control study was conducted on 150 participants who were categorized into three groups: treated hypothyroidism (n=50), newly diagnosed untreated hypothyroidism (n=50), and healthy controls (n=50). Serum TSH, T3, T4, calcium, vitamin D, and Bone-specific alkaline phosphatase (BSAP) were measured using standard immunoassay and colorimetric methods. Statistical analysis was performed using ANOVA and chi-square tests.

Results:
Untreated hypothyroid patients showed significantly elevated TSH (12.8 ± 4.6 mIU/mL), reduced T3/T4, hypocalcemia (8.4 ± 0.7 mg/dL), vitamin D deficiency (15.5 ± 5.5 ng/mL), and elevated BSAP (25.0 ± 6.0 U/L) compared to treated patients ($p<0.05$) and controls (p 0.001). Levothyroxine therapy improved thyroid function, calcium, and vitamin D status, though BSAP remained moderately elevated compared to controls.

Conclusion:
Untreated hypothyroidism is associated with deranged thyroid function, hypocalcemia, vitamin D deficiency, and increased bone turnover. Long-term levothyroxine therapy restores biochemical euthyroidism and improves calcium-vitamin D status but does not fully normalize bone metabolism. Early diagnosis and careful monitoring of thyroid replacement and bone health markers are essential to prevent skeletal complications.

Keywords: bone turnover markers, primary hypothyroidism, thyroid function tests (TSH, T3, T4)

Introduction

Primary hypothyroidism, one of the most prevalent endocrine disorders worldwide, is characterized by inadequate thyroid hormone production due to iodine shortage, autoimmune injury, or other factors. Systemic implications of thyroid hormone deficiency include altered bone and calcium metabolism. Growth, skeletal development, and metabolism are all regulated by thyroid hormones [1].

Elevated thyroid-stimulating hormone (TSH) and decreased thyroxine (T4) and triiodothyronine (T3) levels are typical biochemical characteristics of hypothyroidism, which is diagnosed by thyroid function tests (TFTs) [2]. Long-term medication may not completely correct accompanying metabolic abnormalities, particularly those related to bone health, but levothyroxine is still the mainstay treatment for hypothyroidism and successfully restores euthyroid state in the majority of patients. [3].

Deficits in calcium and vitamin D, two essential regulators of bone mineralization, have been linked to an increased risk of osteoporosis and fractures. Metabolic bone disease and treatment results have been tracked using bone-specific alkaline phosphatase (BSAP), a marker of bone production [4, 5]. Reduced serum calcium and vitamin D levels have been associated with hypothyroidism, especially when left untreated, which can lead to compromised bone metabolism [6].

Because thyroid dysfunction, calcium homeostasis, vitamin D status, and bone turnover markers interact, it is critical to assess these parameters in hypothyroid patients—both those receiving long-term levothyroxine medication and

those who are not. The degree to which thyroid hormone replacement restores biochemical equilibrium and reduces skeletal problems can be better understood with this method.

Materials & Methods:

A present case-control study was conducted in the Department of Biochemistry, Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth Deemed To Be University, Karad. A total of 150 participants were enrolled, and they were categorized into three groups: Group I: Primary hypothyroidism cases who had been on levothyroxine therapy for at least two years with a range of 4.9 ±2.2 years (N = 50), Group II: Newly diagnosed cases of primary hypothyroidism who were yet to start thyroxine therapy (N = 50), and Group III: Normal healthy individuals serving as controls (N = 50).

Inclusion Criteria

Adults aged 18-60 years, who confirmed the diagnosis of primary hypothyroidism based on elevated TSH and low T4 levels. Group I: Patients on stable levothyroxine therapy for ≥2 years (The duration of levothyroxine therapy was in the range of 4.9 ± 2.2 years). Group II: Newly diagnosed cases with no prior thyroid hormone replacement. Group III: Normal healthy individuals serving as controls & the subjects' willingness to provide written informed consent were included in the study.

Exclusion Criteria

History of secondary or central (pituitary/hypothalamic) hypothyroidism, known metabolic bone disorders (e.g., osteoporosis, Paget's disease, osteomalacia, chronic kidney disease (eGFR < 60 mL/min/1.73 m^2), liver dysfunction, or active malignancy, use of medications known to affect bone metabolism (e.g., systemic corticosteroids, bisphosphonates, anticonvulsants, heparin), pregnant women and lactating mothers, vitamin D deficiency (<10 ng/mL) requiring active pharmacological supplementation, history of calcium or phosphate disorders were excluded from the study.

Sample Collection and Processing:

All participants underwent a thorough clinical evaluation, including detailed history, physical examination, anthropometric measurements (height, weight, BMI) and assessment of duration and symptoms of hypothyroidism. Demographic information, such as age and body mass index (BMI), was recorded. BMI was assessed by using the formula: weight in kilograms divided by height in meters squared (kg/m^2). Venous blood samples were collected after an overnight fast. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis.

Thyroid function tests (TSH, Total T4, and Total T3) & Vitamin D (25-hydroxyvitamin D) were estimated using chemiluminescent immunoassay (CLIA). Serum calcium was measured by the Arsenazo III method on an automated analyzer. Bone-Specific Alkaline Phosphatase (BSAP) was estimated using Enzyme-Linked Immunosorbent Assay (ELISA). All assays were performed in duplicate, with internal quality controls run for each batch.

The study protocol was approved by the Institutional Ethics Committee of Krishna Institute of Medical Sciences, Karad. (KIMSDU/IEC/04/2023). Written informed consent was obtained from all participants prior to enrollment. Patient confidentiality was maintained throughout the study. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Data were entered into Microsoft Excel and analyzed using SPSS Statistics version 25.0. The data has been represented as 'N' with percentage (%) calculated. Continuous variables were expressed as mean ± standard deviation (SD). Intergroup comparisons of continuous variables were performed using the independent samples t-test. Categorical variables were compared using the ANOVA and chi-square tests. A p value < 0.05 was considered statistically significant.

Results:

The majority of participants in each of the three groups were between the ages of 31 and 60 (N=31) (62% in Group I), (N=29) (58% in Group II), and (N=27) (54% in Group III). There was no statistically significant difference between the percentage of younger individuals (≤30 years) and older participants (>60 years) (p = 0.96). BMI-wise, more

people in Groups I and II were overweight (>24 kg/m²: (N=26) 52% and (N=27) 54%, respectively), whereas more people in Group III fell within the normal reference BMI range (N=30) 60%. At p = 0.07, the result was not statistically significant (Table 1).

Table 1: Baseline Clinical Profile of Study-Enrolled Participants

	Group I (n = 50)	Group II (n = 50)	Group III (n=50)	p-value
Age in years				
≤ 30	9 (18%)	11 (22%)	13 (26%)	0.96 (NS)
31 to 60	31 (62%)	29 (58%)	27 (54%)	
> 60	10 (20%)	10 (20%)	10 (20%)	
BMI in kg/m²				
< 18	3 (6%)	4 (8%)	5 (10%)	0.07 (NS)
18 to 24	21 (42%)	19 (38%)	30 (60%)	
> 24	26 (52%)	27 (54%)	15 (30%)	

Table 1: Baseline Clinical Profile of Study-Enrolled Participants

In comparison to Group I (treated hypothyroidism, 3.2 ± 1.5 mIU/mL) and Group III (healthy controls, 2.4 ± 1.2 mIU/mL), Group II (newly diagnosed hypothyroidism) had higher TSH levels (12.8 ± 4.6 mIU/mL), with a highly significant difference ($p < 0.001$). Group II (4.2 ± 1.5 µg/dL) exhibited the lowest values, indicating untreated hypothyroidism, while Group I (8.5 ± 2.1 µg/dL) and Group III (9.1 ± 2.0 µg/dL) displayed comparable total T4 levels. Similarly, Group II (70 ± 20 ng/mL), Group I (110 ± 30 ng/mL), and Group III (120 ± 30 ng/mL) had significantly different Total T3 levels ($p < 0.001$) (Table 2).

Table 2: Parameters of Thyroid Function Test (TFT) among all the groups

Parameter/Participant Group	Group I (n = 50)	Group II (n = 50)	Group III (n=50)	p-value
TSH (mIU/mL) Mean ±SD	3.2 ± 1.5	12.8 ± 4.6	2.4 ± 1.2	<0.001
Total T4 (µg/dL) Mean ±SD	8.5 ± 2.1	4.2 ± 1.5	9.1 ± 2.0	<0.001
Total T3 (ng/mL) Mean ±SD	110 ± 30	70 ± 20	120 ± 30	<0.001

The mean serum calcium levels in Group I (treated hypothyroidism), Group II (newly diagnosed hypothyroidism), and Group III (healthy controls) were 9.1 mg/dL, 8.4 mg/dL, and 9.3 mg/dL, respectively. The lowest values were seen in hypothyroid patients who were not receiving treatment, while the calcium levels in treated cases and controls were similar and somewhat higher. The statistically significant difference between groups ($p < 0.05$) indicates that untreated hypothyroidism is associated with lower serum calcium levels (Table 3, Figure 1).

Table 3: Comparison of Serum Calcium among all the groups

Serum Calcium (mg/dL)	Group I (n = 50)	Group II (n = 50)	Group III (n=50)	p-value
Mean	9.1	8.4	9.3	<0.05 (S)
±SD	0.6	0.7	0.5	

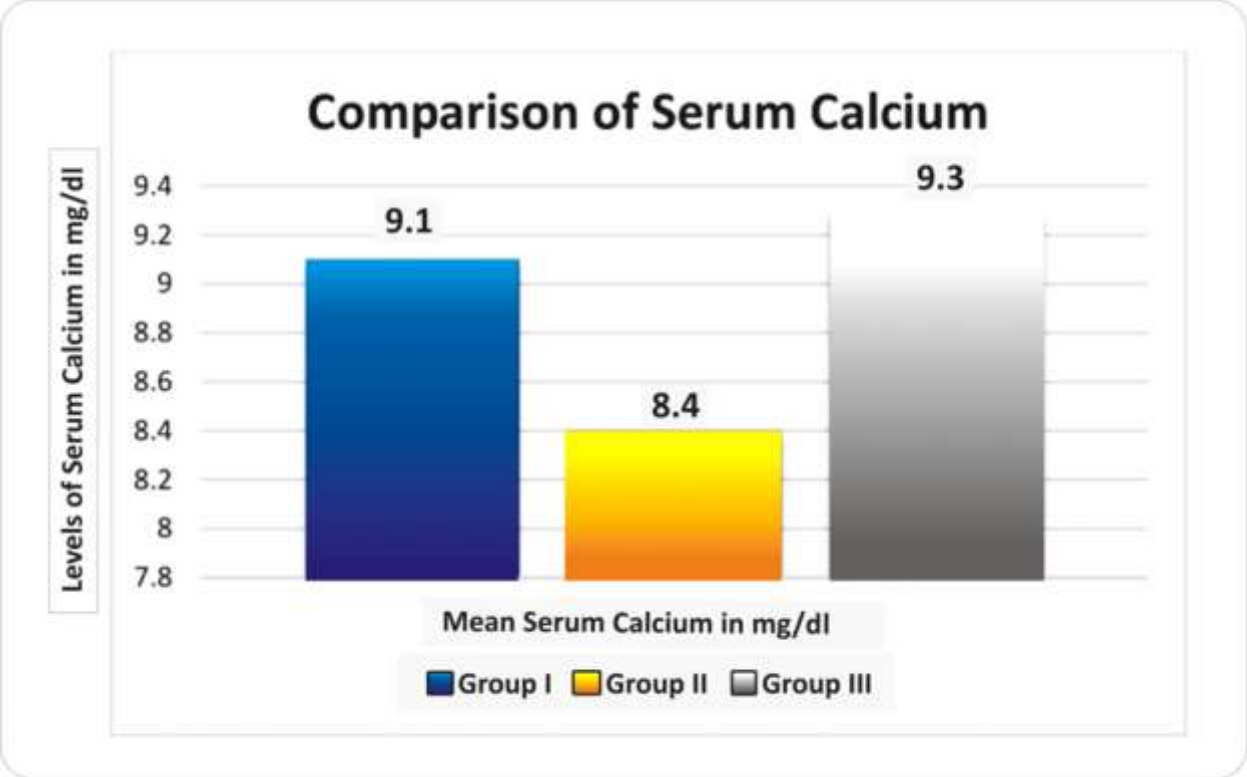

Figure 1: Comparison of Serum Calcium among all the groups

Vitamin D levels were 22.0 ng/mL in Group I, 15.5 ng/mL in Group II, and 28.0 ng/mL in Group III. Healthy controls had the highest results, whereas those with untreated hypothyroidism had the lowest. Individuals undergoing therapy for hypothyroidism showed intermediate levels. The difference was statistically significant ($p < 0.01$) (Table 4, Figure 2).

Table 4: Comparison of Vitamin D among all the groups

Vitamin D (ng/mL)	Group I (n = 50)	Group II (n = 50)	Group III (n=50)	p-value
Mean	22.0	15.5	28.0	<0.01 (S)
±SD	6.0	5.5	7.0	

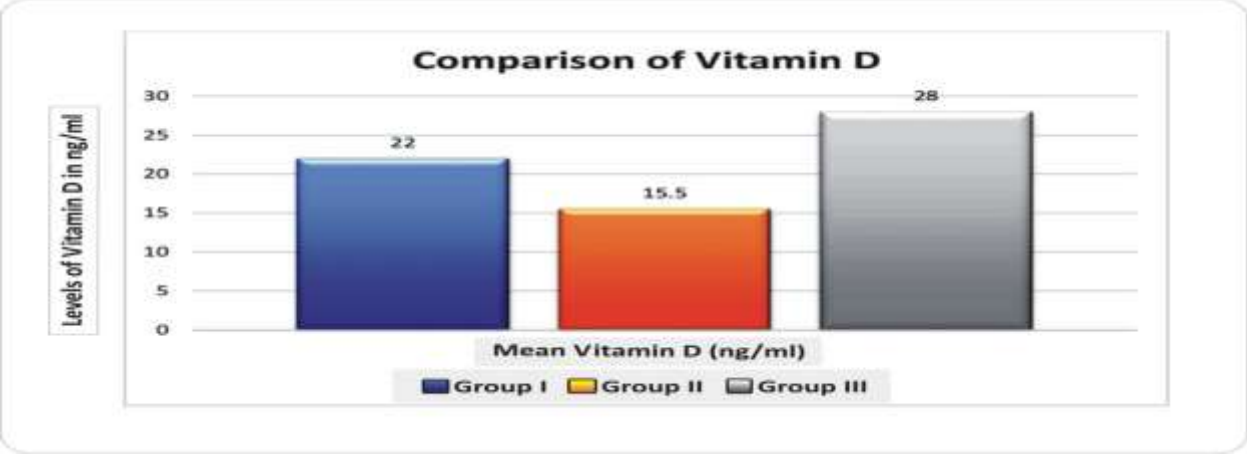

Figure 2: Comparison of Vitamin D among all the groups

Bone Specific Alkaline Phosphatase (BSAP) levels were 18.5 U/L in Group I, 25.0 U/L in Group II, and 15.0 U/L in Group III. While treated cases showed somewhat greater levels than controls, untreated hypothyroid individuals had the highest findings. The difference was statistically significant ($p < 0.01$) (Table 5, Figure 3).

Table 5: Comparison of Bone Specific Alkaline Phosphatase (BSAP) among all the groups

BSAP (U/L)	Group I (N = 50)	Group II (N = 50)	Group III (N=50)	p-value
Mean	18.5	25.0	15.0	<0.01 (S)
±SD	4.5	6.0	3.5	

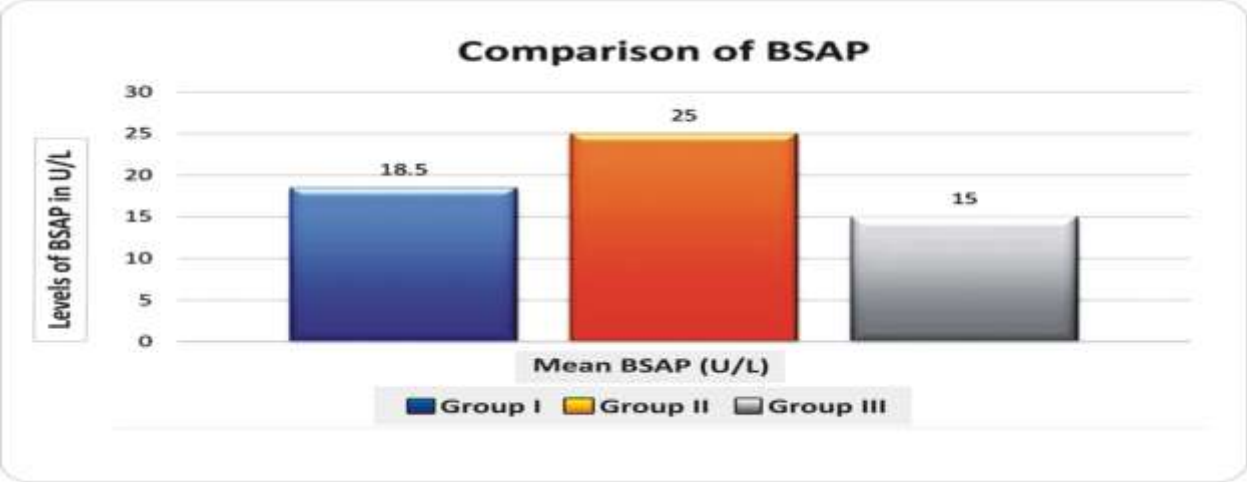

Figure 3: Comparison of Bone Specific Alkaline Phosphatase (BSAP) among all the groups

Discussion:

The current study shown that untreated hypothyroid patients had significantly greater TSH and lower T3/T4 compared to treated patients and healthy controls. This is in line with the conventional pathophysiology of hypothyroidism, which holds that decreased thyroid hormone production leads to elevated TSH levels and compensatory pituitary activity [1, 2].

Levothyroxine therapy restored T4 and partially normalized T3, which is consistent with earlier results that exogenous T4 replacement improves biochemical euthyroidism but may not fully cure peripheral T3 metabolism [3]. Van Uytfanghe et al. and Zaid M. Hadi et al. obtained similar results, emphasizing that hypothyroidism is characterized by a log-linear increase in TSH with slight decreases in T4/T3 levels [7, 8].

Serum calcium levels were significantly lower in untreated hypothyroid patients (8.4 mg/dL) compared to treated patients (9.1 mg/dL) and controls (9.3 mg/dL). This is consistent with research by Satish Kumar Yadav et al. and Diana Athokpham et al., which also discovered hypocalcemia in hypothyroid patients relative to controls [9,10]. The mobilization and absorption of calcium are directly impacted by thyroid hormones. The altered renal calcium processing and reduced intestinal calcium absorption caused by hypothyroidism lead to mild hypocalcemia. Furthermore, decreased thyroid hormone activity reduces calcium deposition in bone via impairing osteoblast function [4].

Untreated hypothyroid individuals had lower levels of vitamin D (15.5 ng/mL) than treated patients (22 ng/mL) and controls (28 ng/mL). Sheikh & Sharma found similar results, indicating that hypothyroid patients had lower vitamin D levels, which correlated with lower bone mineral density [11]. Additionally, David Ushaw emphasized that vitamin D insufficiency often coexists with hypothyroidism, aggravating bone fragility [12].

Reduced renal 1α-hydroxylase activity and impaired hepatic 25-hydroxylation, both regulated by thyroid hormones, may provide a molecular explanation for this deficiency. Lifestyle variables, such as hypothyroid patients' reduced sun exposure, may also contribute to low vitamin D status [5].

Untreated hypothyroid patients had significantly higher levels of bone-specific alkaline phosphatase (BSAP) (25 U/L) than treated patients (18.5 U/L) and controls (15 U/L). This suggests that hypothyroidism causes an increase in bone turnover. BSAP was found by Vasikaran et al. to be a trustworthy indicator of bone turnover and formation, especially in metabolic bone disease [6]. Our results are in line with studies showing that hypothyroidism modifies bone remodeling and that levothyroxine treatment partially restores turnover. Mechanistically, hypothyroidism causes a compensatory rise in BSAP by decreasing osteoblast activity while paradoxically increasing osteoclast-mediated resorption. Increased TSH may also promote osteoclastogenesis, which could lead to an imbalance in bone remodeling.

The current study shows that long-term levothyroxine therapy improves calcium and vitamin D status and restores thyroid hormone levels, even though BSAP is still somewhat higher than in controls. Levothyroxine therapy has positive skeletal effects, as evidenced by Mahmood Parham et al.'s demonstration that it dramatically increased bone mineral density within a year of treatment [13]. However, meta-analyses indicate that excessive LT4 treatment may negatively impact bone metabolism, highlighting the necessity of close dose control.

Conclusion:

Untreated hypothyroidism causes inadequate calcium, vitamin D deficiency, and increased bone-turnover markers (BSAP), all of which seriously impair bone health. Standard levothyroxine medication effectively returns these particular blood levels to normal, but it is unable to completely restore long-term, healthy bone metabolism at the cellular level. Therefore, early diagnosis, accurate hormone dosage, and ongoing, proactive calcium and vitamin D level monitoring are crucial for preventing irreversible skeletal injury. In conclusion, research emphasizes that treating hypothyroidism requires a thorough, bone-centric strategy rather than merely depending on routine thyroid hormone normalization.

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