Can Serum Osteocalcin Level be Used as a Marker to Assess Bone Remodeling Status in Hyperthyroidism?

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Authors’ contributions

This work was carried out in collaboration between all authors. Author KCV designed the study, wrote the protocol and supervised the work. Author KN carried out all laboratories work and author KR performed the statistical analysis. Author PK supported the study. Author KN wrote the first draft of the manuscript. Author KN managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to estimate the serum osteocalcin levels before and during treatment in hyperthyroidism and to compare with healthy controls.

Study Design: Case control study.

Place and Duration of Study: Department of Biochemistry, Department of Endocrinology. M.S. Ramaiah medical college, Bangalore. December 2014 to May 2015.

Methodology: Twenty five newly diagnosed hyperthyroid patients, Twenty five patients who are euthyroid during treatment and Twenty five healthy controls in the age group of 20-50yrs were enrolled in this study. Serum T3, T4, TSH were assayed by electrochemiluminescence and serum Osteocalcin was estimated by ELISA. Kruskal wallis test was used to compare T3, T4, TSH,
Osteocalcin and calcium levels among three groups. Post Hoc test was used for multiple comparisons between two groups.

**Results:** Using Kruskal wallis test, a significant difference for T3, T4, TSH and osteocalcin values was observed among the different groups (p <0.001). Post Hoc test for multiple comparisons between two groups revealed that osteocalcin levels were significantly high in newly diagnosed hyperthyroid patients compared to controls (p=0.02) indicating high bone turnover. There was also a significant difference in osteocalcin levels between newly diagnosed hyperthyroid patients and patients who are euthyroid on treatment (p= 0.04) indicating values decreased on treatment. ROC analysis showed serum osteocalcin is a good marker for assessing bone remodeling status in hyperthyroidism. (Area under curve = 86.6 and p value = 0.001)

**Conclusion:** Serum osteocalcin can be used to assess bone remodeling status in newly diagnosed hyperthyroidism and to identify residual bone resorption in the euthyroid state during treatment.

**Keywords:** Hyperthyroidism; osteocalcin; resorption; euthyroid; remodeling.

### 1. INTRODUCTION

Hyperthyroidism is an endocrinological disorder that exhibits low serum TSH levels and elevated levels of the thyroid hormones. Hyperthyroidism includes diseases that are a subset of thyrotoxicosis, caused by excess synthesis and secretion of thyroid hormone. Graves disease is an autoimmune thyroid disease in which anti TSH receptor autoantibodies cause hyperthyroidism. Hyperthyroidism is sometimes caused by paraneoplastic disease. Because of this, in these cases normalizing the thyroid function with antithyroid drugs can not improve prognosis [1].

Hyperthyroidism is accompanied by osteoporosis with increased rate of bone formation and bone resorption caused from direct stimulation of bone cells by increased level of thyroid hormones. The severity of hyperthyroidism is directly related to the alteration in the levels of biochemical markers of bone turnover and bone loss [2]. Studies have shown that euthyroid state will only partially reduce hyperthyroid osteopenia with a bone mass recovery during 6-9 months of effective treatment [3].

Osteocalcin is a non collagenous protein found in bone and dentin. It is secreted solely by osteoblasts and is pro-osteoblastic or bone building by nature. It is implicated in bone mineralization and calcium ion homeostasis [4].

Calcium is one of the abundant extracellular cations in the body and it plays an important role in bone formation. Thyroid hormones stimulate bone resorption directly there by increasing the serum calcium levels and suppressing parathyroid hormone [5].

Osteocalcin being a bone formation marker is supposed to increase in hyperthyroidism [2].

Therefore, this study was done to show the correlation between serum osteocalcin levels and serum calcium in hyperthyroidism which may in turn suggest the need for calcium supplementation to prevent osteoporosis in these patients.

### 2. MATERIALS AND METHODS

This study was conducted in department of Biochemistry, M. S. Ramaiah Medical College, Bangalore from December 2014 to May 2015. Patients attending outpatient department of Endocrinology M. S. Ramaiah Hospitals for evaluation of their thyroid status were enrolled in the study.

A case control study which included:

**Group 1:** Twenty five newly diagnosed hyperthyroid patients with Graves disease and multinodular goitre. (18 Females, 7 males)

**Group 2:** Twenty five Age and sex matched healthy controls. (18 Females, 7 males)

**Group 3:** Twenty five Patients who are euthyroid during treatment for atleast 3 months. (17 Females, 8 males)

All subjects were in the age group of 20-50 years. Written informed consent was taken from both cases and controls. Study protocol was approved by Ethical committee of the Institute. Patients of age group >50 yrs, postmenopausal women, history of osteoporosis, fractures, hepatic dysfunction, parathyroid dysfunction, renal disease, malignancies and patients who were on calcium supplements, vitamin D
supplements, steroids and thiazides were excluded from the study.

About 2 ml of venous blood was collected. Serum was separated by centrifugation and stored at -20°C until the estimation of biochemical parameters.

Serum T3, T4 and TSH were assayed by electrochemiluminescence on Roche cobas analyzer [6-8] Serum osteocalcin levels were estimated by ELISA [9].

Serum calcium levels was estimated by o-Cresolphthalein method spectrophotometrically [10,11].

3. RESULTS

The results of all variables were expressed as median values as our data did not follow normal Gaussian distribution. Kruskal wallis test was used to compare T3, T4, TSH, osteocalcin and calcium levels among three groups. Post Hoc test was used for multiple comparisons between two groups. Results were computed using

![Graphs showing comparison of median values of T3, T4, TSH, Osteocalcin and Calcium levels.](image-url)

Fig. 1. Comparison of median values of T3, T4, TSH, Osteocalcin and Calcium levels in newly diagnosed hyperthyroid patients, controls and patients who are euthyroid during treatment for hyperthyroidism
SPSS (Statistical Package for the Social Sciences) software version 20. The correlation between serum osteocalcin and hyperthyroidism was estimated by Spearman's correlation coefficient.

Median values for T3 and T4 in newly diagnosed hyperthyroid patients were found to be increased when compared to controls and patients on treatment in the euthyroid state. Patients on treatment achieved T3 and T4 values which is almost normal.

Median values for TSH in newly diagnosed hyperthyroid patients were low compared to controls and patients on treatment with a statistically significant difference of p=0.001.

Table 1. Comparison of the median values of different variables among newly diagnosed hyperthyroid patients, patients who are euthyroid during treatment for at least 3 months and controls using Kruskal Wallis test

|                  | Group 1 (Newly diagnosed hyperthyroid patients) | Group 2 (Controls) | Group 3 (Patients euthyroid on treatment) | p value |
|------------------|------------------------------------------------|--------------------|----------------------------------------|---------|
| T3 (nmol/l)      | 4.56(3.03-5.60)                                 | 1.81(1.64-1.98)    | 2.07(1.42-2.65)                        | <0.001  |
| T4 (nmol/l)      | 230.8(171.35-292.10)                            | 109.4(97.44-128.5) | 190(161-250.54)                         | <0.001  |
| TSH (µIU/ml)     | 0.005(0.005-0.010)                              | 2.56(1.61-3.23)    | 1.5(0.5-1.9)                           | <0.001  |
| Calcium (mg/dl)  | 11.33(10.76-12.21)                              | 10.3(9.2-11.19)    | 11.07(10-11.96)                        | <0.008  |
| Osteocalcin (ng/ml) | 9.9(0.35-35.55)                     | 0.001(0.001-0.15) | 0.1(0.001-2.20)                       | <0.001  |

There was a statistically significant difference for T3, T4, TSH and osteocalcin values among the different groups with p value <0.001 and for calcium among different groups with p value <0.008.

Table 2. Post Hoc test for multiple comparisons between two groups for T3, T4 and TSH

| Dependent variable | Mean difference | Std. error | Significance |
|--------------------|-----------------|------------|-------------|
| T3 (nmol/l)        |                 |            |             |
| 1                  | 2               | 3.13280    | 0.43254     | 0.001       |
|                   | 3               | 2.91440    | 0.43254     | 0.001       |
|                   | 2               | -3.13280   | 0.43254     | 0.001       |
|                   | 3               | -2.1840    | 0.43254     | 1.000       |
|                   | 1               | -2.91440   | 0.43254     | 0.001       |
|                   | 2               | 2.1840     | 0.43254     | 1.000       |
| T4 (nmol/l)        |                 |            |             |
| 1                  | 2               | 122.28520  | 16.62       | 0.001       |
|                   | 3               | 115.01440  | 16.62892    | 0.001       |
|                   | 2               | -122.28520 | 16.62892    | 0.001       |
|                   | 3               | -7.27080   | 16.6292     | 1.000       |
|                   | 1               | -115.01440 | 16.62892    | 0.001       |
|                   | 2               | 7.27080    | 16.62892    | 1.000       |
| TSH (µIU/ml)       |                 |            |             |
| 1                  | 2               | -2.4300800 | .2833591    | 0.001       |
|                   | 3               | -1.5890000 | .2833591    | 0.001       |
|                   | 2               | 2.4300800  | .2833591    | 0.001       |
|                   | 3               | .8410800   | .2833591    | 0.12        |
|                   | 1               | 1.5890000  | .2833591    | 0.001       |
|                   | 2               | -.8410800  | .2833591    | 0.012       |
T3 and T4-
There was a significant difference in T3 and T4 values between newly diagnosed hyperthyroid cases and controls as also between newly diagnosed hyperthyroid and patients who are euthyroid during treatment. There was no significant difference in T3 and T4 values between controls and patients who are euthyroid during treatment as the patients have attained T3 and T4 values which is almost normal.

TSH-
There was a significant difference in TSH values between newly diagnosed hyperthyroid patients and controls as also between newly diagnosed hyperthyroid patients and patients who are euthyroid on treatment. There was a moderate significant difference between controls and patients who are euthyroid on treatment which indicates TSH values are increasingly tending towards normal.

There was a significant difference in osteocalcin levels between newly diagnosed hyperthyroid patients and controls indicating hyperthyroid patients have a high bone turnover.

There was a significant difference in osteocalcin levels between newly diagnosed hyperthyroid patients and patients who are euthyroid on treatment indicating the values decreased on treatment. There was no significant difference between patients who are euthyroid on treatment and controls.

There was a significant difference in calcium levels between newly diagnosed hyperthyroid patients and controls. There was no significant difference in calcium levels between newly diagnosed hyperthyroid patients and patients who are euthyroid on treatment and also between patients who are euthyroid on treatment and controls.

There was a positive correlation between T3 and osteocalcin in newly diagnosed hyperthyroid patients with a correlation coefficient of 0.537.

Table 3. Post Hoc test for multiple comparisons between two groups for Osteocalcin and calcium

| Variable            | Mean difference | Std error | Significance |
|---------------------|-----------------|-----------|--------------|
| OSTEOCALCIN (ng/ml) |                 |           |              |
| 1 2                 | 24.215360       | 6.932379  | .002         |
| 3 2                 | 22.955680       | 6.932379  | .004         |
| 2 1                 | -24.215360      | 6.932379  | .002         |
| 3 2                 | -22.955680      | 6.932379  | .004         |
| 2 3                 | 1 2             | 1.25980   | 6.932379     | 1.000 |
| CALCIUM (mg/dl)     |                 |           |              |
| 1 2                 | 1.3880000       | .4172263  | .004         |
| 3 2                 | .4152000        | .4172263  | .969         |
| 2 1                 | -1.3880000      | .4172263  | .004         |
| 3 2                 | -.9728000       | .4172263  | .068         |
| 3 1                 | -.4152000       | .4172263  | .969         |
| 2 1                 | .9728000        | .4172263  | .068         |

Table 4. Spearman’s correlation between osteocalcin and T3

| Group 1 (Newly diagnosed hyperthyroid) | Osteocalcin | Calcium | Age | T3 | T4 | TSH |
|---------------------------------------|------------|---------|-----|----|----|-----|
| Osteocalcin                           | Correlation coefficient | 1.000   | .012 | .306 | .537 | .342 | .330 |
|                                      | Significance | -      | .954 | .137 | .006 | .094 | .107 |
A) Between newly diagnosed hyperthyroid patients and controls

Serum osteocalcin is a good marker for assessing bone remodeling status in hyperthyroid state. Calcium is a fair test in assessing bone remodeling between newly diagnosed hyperthyroid patients and controls.

B) Between newly diagnosed hyperthyroid patients and patients who are euthyroid during treatment

Osteocalcin is a marker which can identify patients in whom there is residual bone resorption during treatment. Calcium fails as a biomarker to assess bone remodeling during treatment.
C) Between patients who are euthyroid during treatment and controls

![ROC Curve](image)

**Osteocalcin**  
Sensitivity=60, Specificity=72, Area under curve 64.2 and p=0.08

**Calcium**  
Sensitivity=68, Specificity=60, Area under curve 66.6 and p=0.45

Area under the curve was similar for both osteocalcin and calcium. Osteocalcin levels were tending towards normal although not completely falling within the range established for the control group where the median value in euthyroid state, for osteocalcin was higher 0.1 ng/ml with an interquartile range of (0.001-2.20) as compared to 0.001 ng/ml with an interquartile range of (0.001-0.15) in controls. Considering the range established in both these groups, this study throws light on the fact that values of osteocalcin >0.15 ng/ml and < 2.20 ng/ml probably still indicate a certain degree of bone resorption although not statistically insignificant.

4. DISCUSSION

Hyperthyroidism is characterized by accelerated bone turnover, caused from direct stimulation of bone cells by increased thyroid hormones. The cycle of bone remodeling is shortened to almost 50% (from 200 to 113 days), and the proportions between bone formation and bone resorption are disturbed. Those with a history of untreated thyrotoxicosis extending for more than a year, may have severe and premature osteoporosis [11].

Triiodothyronine (T3), is responsible for major actions of thyroid hormones. T3 binds to nuclear receptors that regulate gene transcription via interaction with thyroid hormone response elements of specific genes [12].

T3 regulates the chondrogenesis bone mineralization, angiogenesis and bone matrix formation. T3 stimulates the IL-6 and IL-8, intensifies the effects of IL-1 and IL-6, augments the synthesis of osteocalcin, collagen type 1, increases proliferation, differentiation and apoptosis of osteoblasts [13,14]. Also, TSH deficiency could be partly responsible for the skeletal loss seen in thyrotoxicosis, because it has been proposed that TSH may be a direct negative regulator of bone turnover acting via the TSH receptor on both osteoblasts and osteoclasts, as TSH inhibits osteoclast formation and survival. It also inhibits differentiation of osteoblasts [15].

A similar study conducted by Barsal et al. [16] showed that there is increase in bone formation marker Osteocalcin and ALP as well as bone resorption marker, urinary calcium/ creatinine levels in Hyperthyroidism. These markers failed to normalize even after attaining euthyroidism. Hence he concluded that hyperthyroid patients have a high bone turnover of formation and resorption even after attainment of euthyroidism and osteocalcin is a sensitive marker in documenting bone remodeling during treatment of Hyperthyroidism [17].

Another study conducted by Siddiqi et al observed a fall of the bone resorption markers urinary collagen pyridinoline and deoxypyridinoline and bone formation markers osteocalcin and bone ALP after 4-8 weeks of antithyroid treatment [18,19].
The accelerated bone remodeling cycle in hyperthyroid state results in increased bone resorption and a subsequent increased release of calcium into systemic circulation [20].

High levels of serum calcium inhibit parathyroid hormone (PTH) secretion and create a negative calcium balance through prolonged urinary and fecal losses. Decreased PTH secretion causes hypercalciuria as a protective mechanism against hypercalcemia [20].

With low PTH, Vitamin D is not converted into its active form, leading to low gastrointestinal calcium and phosphorous absorption and resultant fecal calcium losses [20].

Calcium balance which was negative during the hyperthyroid status was converted to positive soon after attainment of euthyroidism which is due to healing of the metabolic bone disease and increased calcium deposition in to the bone [21,22].

Calcium levels in newly diagnosed hyperthyroid patients was in upper normal range when compared to lower normal range of controls. There was no significant difference in calcium levels in newly diagnosed hyperthyroid patients and those who are euthyroid during treatment as also between patients who are euthyroid during treatment and controls.

Osteocalcin levels in newly diagnosed hyperthyroid patients is significantly high compared to patients who are euthyroid during treatment and controls. During treatment, osteocalcin levels showed a significant fall but failed to achieve the range established for controls indicating a probable phase of residual bone resorption even though the patients were euthyroid. Therefore, estimating both Serum Osteocalcin and serum Calcium together in hyperthyroidism will reflect the bone remodeling status better.

5. CONCLUSION

Antithyroid drugs can help the patient to achieve an euthyroid state but the residual bone loss can be identified and treated only by monitoring both serum calcium levels and the levels of bone formation markers like serum osteocalcin. Such patients can be supplemented with calcium, vitamin D and bisphosphonates to improve bone mineralization and prevent osteoporosis.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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