

SERUM OSTEOCALCIN LEVELS IN HYPERTHYROIDISM: A CASE CONTROL STUDY

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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.[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]

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

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

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

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49 **2. MATERIAL AND METHODS**

50 This study was conducted in department of Biochemistry, M.S.Ramaiah medical college,
51 bangalore from December 2014 to May 2015. Patients attending outpatient department of
52 Endocrinology M.S.Ramaiah hospitals for evaluation of their thyroid status were enrolled in
53 the study.

54 A case control study which included

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

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

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

60 All subjects were in the age group of 20-50 years. Written informed consent was taken from
61 both cases and controls. Study protocol was approved by Ethical committee of the Institute.

62 Patients of age group >50yrs, postmenopausal women , history of osteoporosis, fractures,
63 hepatic dysfunction, parathyroid dysfunction, renal disease, malignancies and patients who
64 were on calcium supplements, vitamin D supplements, steroids and thiazides were excluded
65 from the study.

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

68 Serum T3,T4 and TSH were assayed by electrochemiluminescence on Roche cobas
69 analyzer.[6,7,8] Serum osteocalcin levels were estimated by ELISA.[9]

70 Serum calcium levels was estimated by *o*-Cresolphthalein method spectrophotometrically.
71 [10,11]

72 3. RESULTS

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74 The results of all variables were expressed as median values as our data did not follow
75 normal Gaussian distribution. Kruskal wallis test was used to compare T3,T4,TSH,
76 osteocalcin and calcium levels among three groups. Post Hoc test was used for multiple
77 comparisons between two groups. Results were computed using SPSS (Statistical Package
78 for the Social Sciences)software version 20. The correlation between serum osteocalcin and
79 hyperthyroidism was estimated by Spearman's correlation coefficient.

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81 **Table 1: Comparison of the median values of different variables**
82 **among newly diagnosed hyperthyroid patients, patients who are**
83 **euthyroid during treatment for atleast 3 months and controls using**
84 **kruskal wallis test.**

	Group1(Newly diagnosed hyperthyroid patients)	Group2(Controls)	Group3(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

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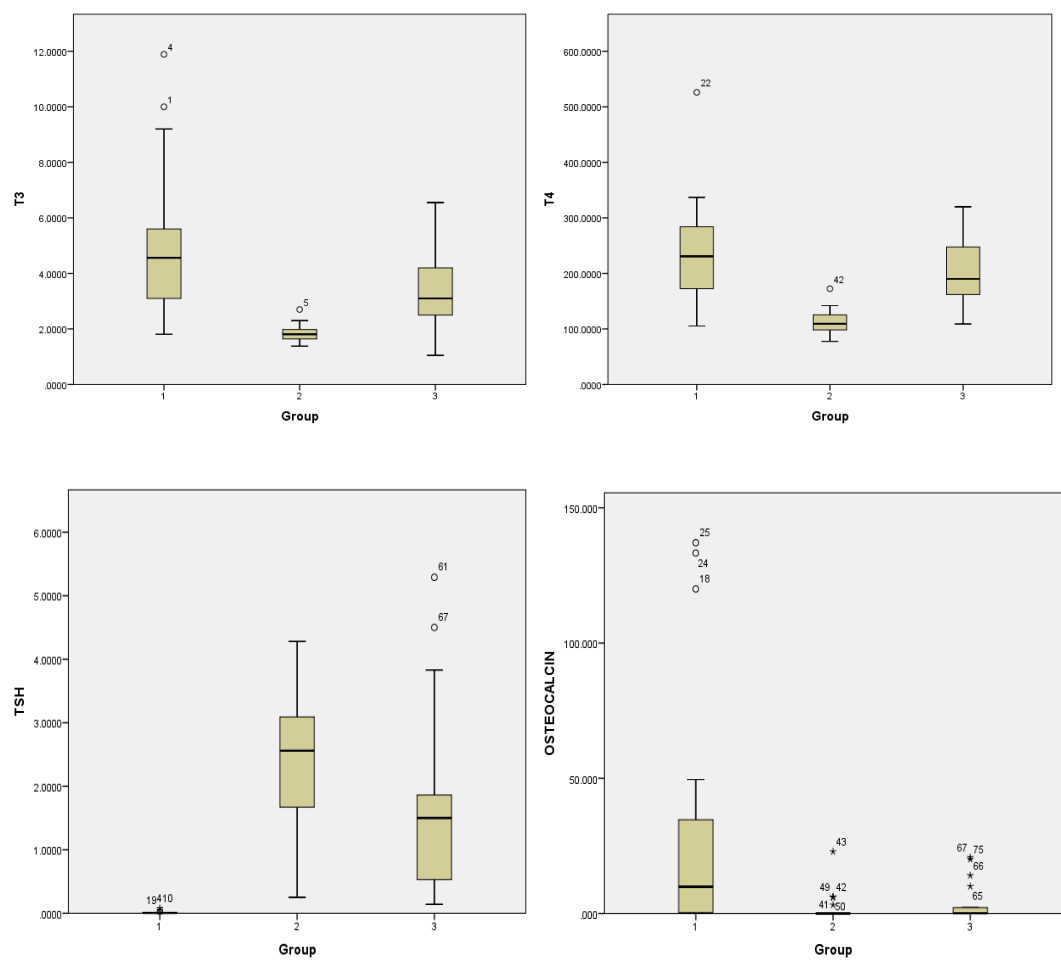
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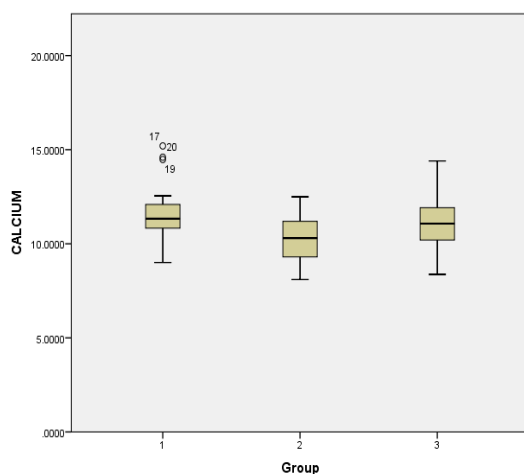
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There was a statistically significant difference for T3, T4, TSH and osteocalcin values among the different groups with p value <0.001 and for calcium among different groups with p value < 0.008.

Figure 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.





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

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

107 Osteocalcin levels were elevated in hyperthyroid state compared to controls. In those
108 patients who are euthyroid during treatment, serum osteocalcin levels decreased
109 considerably but were found to be marginally high as compared to the control group.

110 Median values obtained for calcium in group 1 was 11.33(10.6- 12.21), group 3 was
111 11.07(10-11.96) and in controls 10.3(9.2-11.19). This indicates that compared to the controls
112 the calcium concentration was highest in the serum in newly diagnosed hyperthyroid patients
113 and tending towards normal in patients who are euthyroid during treatment but higher than
114 that of controls.

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120 **Table 2:- Post Hoc test for multiple comparisons between two groups**
 121 **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	1	-3.13280 [*]	0.43254	0.001
		3	-.21840	0.43254	1.000
	3	1	-2.91440 [*]	0.43254	0.001
		2	.21840	0.43254	1.000
T4 (nmol/l)	1	2	122.28520 [*]	16.62	0.001
		3	115.01440 [*]	16.62892	0.001
	2	1	-122.28520 [*]	16.62892	0.001
		3	-7.27080	16.62892	1.000
	3	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	1	2.4300800 [*]	.2833591	0.001
		3	.8410800 [*]	.2833591	0.012
	3	1	1.5890000 [*]	.2833591	0.001
		2	-.8410800 [*]	.2833591	0.012

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125 **T3 and T4-**

126 There was a significant difference in T3 and T4 values between newly diagnosed
127 hyperthyroid cases and controls as also between newly diagnosed hyperthyroid and patients
128 who are euthyroid during treatment. There was no significant difference in T3 and T4 values
129 between controls and patients who are euthyroid during treatment as the patients have
130 attained T3 and T4 values which is almost normal.

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132 **TSH-**

133 There was a significant difference in TSH values between newly diagnosed hyperthyroid
134 patients and controls as also between newly diagnosed hyperthyroid patients and patients
135 who are euthyroid during treatment.

136 There was a moderate significant difference between controls and patients who are
137 euthyroid during treatment which indicates TSH values are increasingly tending towards
138 normal.

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141 **Table 3: Post Hoc test for multiple comparisons between two groups for Osteocalcin**
142 **and calcium.**

Variable			Mean difference	Std error	Significance
OSTEOCALCIN(ng/ml)	1	2	24.215360*	6.932379	.002
		3	22.955680*	6.932379	.004
	2	1	-24.215360*	6.932379	.002
		3	-1.259680	6.932379	1.000
	3	1	-22.955680*	6.932379	.004
		2	1.25980	6.932379	1.000

CALCIUM(mg/dl)	1	2	1.3880000 *	.4172263	.004
		3	.4152000	.4172263	.969
	2	1	-1.3880000 *	.4172263	.004
		3	-.9728000	.4172263	.068
	3	1	-.4152000	.4172263	.969
		2	.9728000	.4172263	.068

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

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

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

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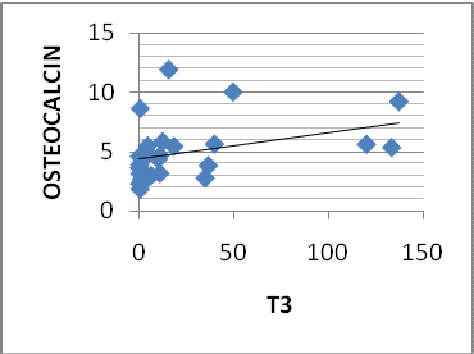
156 **Table 4: Spearman's correlation between osteocalcin and T3.**

Group 1(Newly diagnosed hyperthyroid)		Osteocalcin	Calcium	age	T3	T4	TSH
Osteocalcin	Correlation coeffecient	1.000	.012	.306	.537**	.342	-.330
	Significance	-	.954	.137	.006	.094	.107

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

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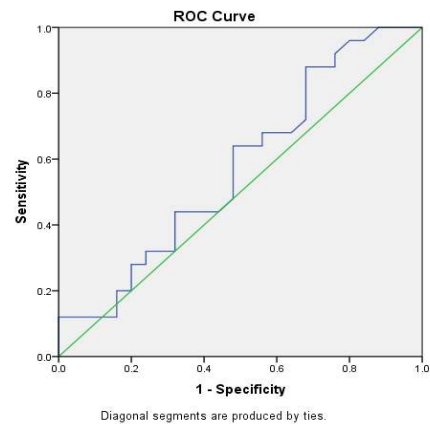
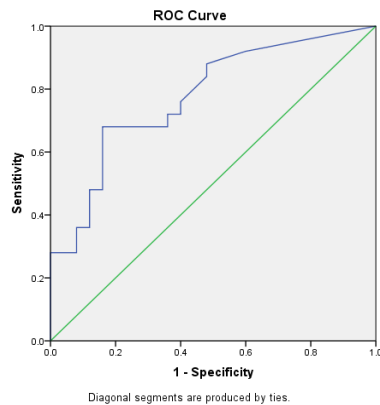
165 **Figure 2: ROC analysis**

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A) Between newly diagnosed hyperthyroid patients and controls.	
Osteocalcin	Calcium
Sensitivity=84, Specificity=80, Area under curve 86.6 and p=0.001.	Sensitivity=68, Specificity=68, Area under curve 75.7 and p=0.002.
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

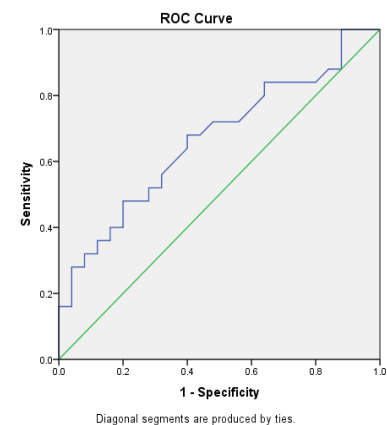
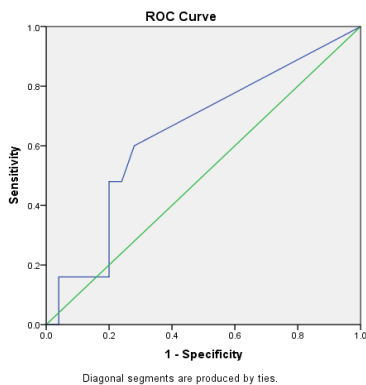
Calcium

Sensitivity=68, Specificity=68, Area under curve 77.7 and $p=0.001$.

Sensitivity=68, Specificity=64, Area under curve 57.4 and $p=0.36$.

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



Osteocalcin

Calcium

Sensitivity=60, Specificity=72, Area under curve 64.2 and $p=0.08$.

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 0.1ng/ml with an interquartile range of (0.001-2.20) as compared to 0.001ng/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 >0.15ng/ml and < 2.20 ng/ml probably still indicate a certain degree of bone resorption although not statistically insignificant.

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171 **DISCUSSION**

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

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

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

190 A similar study conducted by Barsal et al showed that there is increase in bone formation
191 marker Osteocalcin and ALP as well as bone resorption marker, urinary calcium/ creatinine
192 levels in Hyperthyroidism. These markers failed to normalize even after attaining
193 euthyroidism. Hence he concluded that hyperthyroid patients have a high bone turnover of
194 formation and resorption even after attainment of euthyroidism and osteocalcin is a sensitive
195 marker in documenting bone remodeling during treatment of Hyperthyroidism. [15]

196 Another study conducted by Siddiqi et al observed a fall of the bone resorption markers
197 urinary collagen pyridinoline and deoxypyridinoline and bone formation markers osteocalcin
198 and bone ALP after 4-8 weeks of antithyroid treatment.[16]

199 The accelerated bone remodeling cycle in hyperthyroid state results in increased bone
200 resorption and a subsequent increased release of calcium into systemic circulation.[17]

201 High levels of serum calcium inhibit parathyroid hormone (PTH) secretion and create a
202 negative calcium balance through prolonged urinary and fecal losses. Decreased PTH
203 secretion causes hypercalciuria as a protective mechanism against hypercalcemia .[17]

204 With low PTH, Vitamin D is not converted into its active form, leading to low gastrointestinal
205 calcium and phosphorous absorption and resultant fecal calcium losses.[17]

206 Calcium balance which was negative during the hyperthyroid status was converted to
207 positive soon after attainment of euthyroidism which is due to healing of the metabolic bone
208 disease and increased calcium deposition in to the bone. [18]

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

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

219 **4. CONCLUSION**

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

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230 **CONSENT (WHERE EVER APPLICABLE)**

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232 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**

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285 **APPENDIX**

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