Biomarker Insights

Utility of C-terminal Telopeptide in Evaluating Levothyroxine Replacement Therapy-Induced Bone Loss

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ABSTRACT

BACKGROUND: Levothyroxine (LT4) therapy has shown to have effects on bone metabolism though its deleterious effect on bone remodeling is debatable. This study was aimed at assessing the diagnostic utility of the bone remodeling marker C-terminal telopeptide (CTx) in detecting early bone loss.

MATERIALS AND METHODS: In this case–control study, 84 premenopausal women of 30–45 years of age were selected. Out of them, 28 were recently diagnosed of hypothyroidism (not on LT4), 28 were on LT4 replacement therapy (100–200 μg/day) for more than five years, and 28 had euthyroid. Plasma CTx levels were estimated. Bone mineral density (BMD) was measured by quantitative ultrasound (QUS) method. Pearson's coefficient of correlation and ANOVA were used for statistical analysis.

RESULTS: CTx was most elevated in LT4-treated group (0.497 ± 0.209 ng/mL). It showed a significant negative correlation with T-score and Z-score of BMD values. In the treatment group of more than 150 μg/day, CTx showed significantly negative correlation with TSH ($r = -0.462$, $P = 0.047$).

CONCLUSION: LT4 therapy induces bone loss in hypothyroid patients. CTx levels can measure such bone loss along with BMD. Regular monitoring of CTx with adjustment in LT4 doses may help delay osteoporosis induced by prolonged LT4 replacement therapy.

KEYWORDS: hypothyroidism, bone loss, C-terminal telopeptide, LT4 – levothyroxine therapy, Bone mineral density, bone remodeling, osteoporosis.

Introduction

Hypothyroidism is a state of thyroid hormone deficiency.[1] Its overall incidence is 2–15% in the general population.[2] Thyroid hormone preparations are widely used to correct hypothyroidism of various etiologies. In many cases of hypothyroidism, slightly supraphysiological doses of thyroxine (levothyroxine (LT4)) are administered resulting in suppression of TSH (thyroid-stimulating hormone). It has been observed that long-term treatment with LT4 is associated with ill effects such as reduced bone mass and increased incidence of fractures.[2] A study done by De Rosa et al.[3] suggests that suppressive doses of LT4 significantly increase the bone turnover and lead to a reduction in bone mineral density (BMD), more in cortical bone, in both pre- and post-menopausal hypothyroid women. The possible reason behind this is that, in the presence of excess thyroid hormone, bone formation and bone resorption both increase, but bone resorption exceeds bone formation resulting in bone loss.[4]

There are various indices available to measure such bone loss. BMD measurement by densitometry is the gold standard method.[5,6] It is measured by various techniques such as dual energy X-ray absorptiometry (DEXA) and quantitative ultrasound (QUS). Though DEXA is the most reliable method of BMD estimation, QUS has also proven to be an equivalent predictor of fracture risk in patients of osteoporosis. QUS has an advantage that it provides information not only on

bone mass but also on bone elasticity and structure.[7] BMD is expressed as *T*-score and *Z*-score. *Z*-score being a better indicator of bone loss in premenopausal women is preferred over *T*-score to detect bone loss in this group.[6]

Although BMD measurement is a definitive indicator of bone status, its use has been limited to only diagnosis and not to monitor the bone loss, mainly because of its high cost.

Several biochemical markers are available to measure such bone loss. Markers of bone turnover such as osteocalcin, bone-specific alkaline phosphatase, tartrate-resistant acid phosphatase, urine hydroxyproline and deoxypyridinoline, serum and urine N-terminal and C-terminal telopeptides (CTx) of type I collagen cross-links have been studied extensively for their use in measuring bone loss induced by postmenopausal osteoporosis or other causes.[8]

Among the aforementioned markers, CTx has gained considerable interest in monitoring the response to bisphosphonate therapy in cases of osteoporosis. Osteoclasts produce a number of proteolytic enzymes capable of degrading the organic bone matrix, thus releasing calcium and a large variety of collagen breakdown products into the serum. CTx is one of the collagen breakdown product. Though it has been used to measure bone loss in hyperthyroidism and LT4 suppressive therapy, data of its use in LT4 replacement therapy-induced bone loss are still limited.[8]

This study was aimed at evaluating the attributes of CTx in subjects with LT4 replacement therapy-induced bone loss and to assess its diagnostic utility as a cheaper and more convenient alternative to the conventional investigations.

Material and Methods

Study population. A case–control study was conducted at KMC Hospital (KMCH), Ambedkar Circle and KMCH, Attavar, Mangalore from November 2011 to October 2012. On the basis of T3, T4, and TSH levels, a total of 84 premenopausal women in age group of 30–45 years were selected and divided into three groups: Group 1 (28 hypothyroid women on LT4 therapy (100–200 μg/day) for minimum five years), Group 2 (28 premenopausal women of newly diagnosed hypothyroidism as first controls to match the hypothyroidism), and Group 3 (28 age-matched euthyroid women as second

controls) (Fig. 1). Informed consent was obtained from the subjects. The study was approved by research and ethics committee of the institution.

Post-thyroidectomy patients on suppressive doses of LT4; patients with bone and joint disorders, and hypertension; patients on anticonvulsant, chemotherapeutic agents, glucocorticoids, and oral contraceptive pills; patients on vitamin D and calcium for last four months or more; patients with long-term immobilization, malignancy, lung diseases; pregnant and lactating women; and patients with hysterectomy were excluded from the study.

Blood samples were collected in the fasting state in EDTA vacutainer. Samples were immediately centrifuged, and plasma was separated and stored at −20 °C until analysis. Serum CTx was measured using cobas e 411 hormone analyzer by electrochemiluminescence immunoassay (ECLIA) based on the sandwich principle[9,10] using Elecsys β-CrossLaps kits by Roche diagnostics, Mannheim as per the manufacturer's instructions. BMD of all the subjects was measured at the orthopedics outpatient department using the QUS method with the portable ultrasound device at the heel.[11]

Statistical analysis. Data were analyzed using IBM SPSS Statistics version 20. Data are presented as mean ± SD. Comparison of group means was done using ANOVA, Tukey's method. Pearson's correlation was done to evaluate correlation between variables. *P* value <0.05 was considered significant.

Results

Baseline characteristics are shown in Table 1. There is no significant difference in age among the three groups. TSH and T3 showed significant difference, with TSH being highest in the newly diagnosed hypothyroid individuals. T4 did not show any significant difference. CTx levels were elevated in premenopausal hypothyroid women on long-term LT4 therapy and was significantly higher than newly diagnosed hypothyroid and euthyroid individuals (Table 2). BMD was significantly lower in treatment group when compared to other two groups (Table 2). When the patients were divided into three groups based on their duration of treatment, women on treatment for 11–15 years had highest elevation in CTx levels, and it was significantly higher than the other two groups
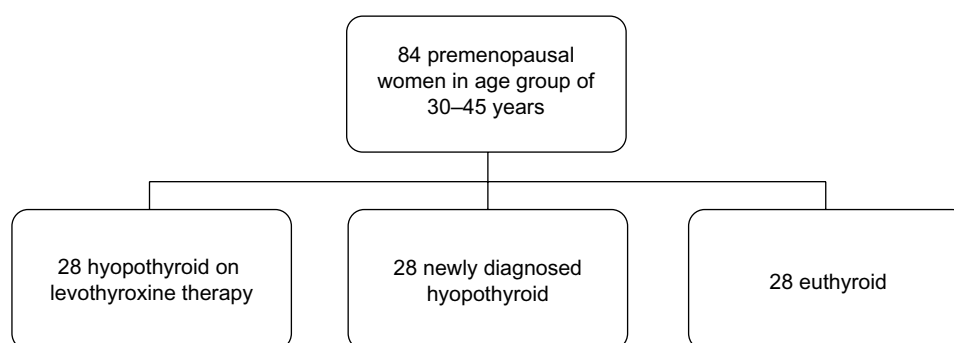

Figure 1. Study group.

84 premenopausal women in age group of 30–45 years

28 hyopothyroid on levothyroxine therapy

28 newly diagnosed hyopothyroid

28 euthyroid

Table 1. Baseline characteristics of study group.

	ON TREATMENT (GROUP I) N = 28	NEWLY DIAGNOSED (GROUP II) N = 28	EUTHYROID (GROUP III) N = 28	*P* VALUE
Age (Years)	40.25 ± 5.31	38.03 ± 5.71	37.96 ± 6.34	0.252
TSH (µIU/ml)	3.91 ± 2.88	11.74 ± 12.47	2.42 ± 0.83	<0.0001
T3 (ng/ml)	1.19 ± 0.32	1.18 ± 0.33	1.49 ± 0.51	0.01
T4 (µg/dl)	8.29 ± 2.28	7.32 ± 2.06	8.70 ± 2.09	0.07

Notes: *P* value was calculated using ANOVA. *P* < 0.05 was considered significant.
Abbreviations: TSH, Thyroid Stimulating hormone; T3, Triiodothyronine; T4, Thyroxine.

(Table 3). When correlation was studied between CTx and *T*-score (Fig. 2), a significantly negative correlation ($r = -0.66$, $P < 0.0001$) was found. Similarly, *Z*-score also showed a significant negative correlation with CTx ($r = -0.56$, $P = 0.001$) (Fig. 3). Distribution of CTx values in groups according to dosage showed a significant negative correlation ($r = -0.462$, $P = 0.047$) between CTx and TSH in hypothyroid women taking more than 150 µg of LT4 per day (Fig. 4). Correlation between CTx and dosage of LT4 was found to be significantly positive ($r = 0.68$, $P < 0.0001$).

Discussion

This study aimed at understanding the role of CTx in measuring LT4-induced bone loss, if any. It was observed that thyroid hormone does have a potent effect on bone turnover as BMD values of the treatment group was in the osteopenic range (*T*-score between –1 and –2.5). There have been various hypotheses put forth to explain this bone loss. According to some workers, the mechanism behind thyroid hormone being associated with accelerated bone turnover is its ability to increase the osteoclastic activity and the ratio of resorptive to formative bone surface.[12–14] This is supported by studies showing thyroid hormone's stimulating effect on

Table 2. Distribution of C-terminal telopeptide and BMD in study group.

VARIABLES	ON TREATMENT (GROUP I) N = 28	NEWLY DIAGNOSED (GROUP II) N = 28	EUTHYROID (GROUP III) N = 28	*P* VALUE
C-terminal telopeptide (ng/ml)	0.497 ± 0.209	0.220 ± 0.079	0.244 ± 0.094	<0.0001
T- score	−2.27 ± 0.48	-0.17 ± 1.19	0.16 ± 1.17	<0.0001
Z- score	−1.78 ± 0.45	0.95 ± 1.25	0.95 ± 0.85	<0.0001

Notes: *P* value was calculated using ANOVA. *P* < 0.05 was considered significant.

Table 3. Distribution of variables amongst the patients on levothyroxine therapy based on duration of treatment.

	5 YEARS (N = 14)	6–10 YEARS (N = 9)	11–15 YEARS (N = 5)	*P* VALUE
C-terminal Telopeptide (ng/ml)	0.408 ± 0.124	0.487 ± 0.216	0.827 ± 0.063	<0.0001
T-score	−1.95 ± 0.37	−2.42 ± 0.33	−2.82 ± 0.28	<0.0001
Z-score	−1.52 ± 0.36	−1.88 ± 0.33	−2.22 ± 0.23	<0.003

**Note:** *P* value <0.05 was considered significant.

bone resorption in organ culture mediated by a nuclear T3 receptor.[15–17] In contrast, some of the studies have shown that thyroid hormone acts on osteoblasts, which in turn indirectly mediate osteoclastic bone resorption.[18] On the other hand, some workers believe that TSH may also have a direct effect on bone turnover, mediated via the TSH receptor on osteoblast and osteoclast precursors.[19] However, these findings are not supported by some of the studies where bone loss appeared independent of TSH levels in the mice lacking specific TR isoforms.[20]

In addition to the direct effect, thyroid hormones may also have an indirect effect on bone turnover by involvement of various cytokines and growth factors, including interleukin-6, interleukin-8, prostaglandin E2, insulin-like growth factor-1, matrix metalloproteinase 13, and matrix metalloproteinase 9.[21–23]

LT4 and BMD. Our study showed reduced BMD in patients on LT4 replacement therapy for more than five years (average of 6.7 years) with minimum dosage of 100 µg/day. Although the BMD values did not cross the cut off for the diagnosis of osteoporosis, they were still in the osteopenic range (as explained by their *T*-scores). The bone loss was more in patients on treatment for more than 10 years.

Although there is no doubt about decrement in BMD in post-menopausal women, more especially in those on long-term LT4 therapy, it is still debated in premenopausal women.[3,24,25] As the available data show, some of the workers have found significant decrement in BMD,[26,27] while some have failed to find any changes after carefully monitored LT4 therapy.[28] Two early cross-sectional studies in premenopausal women demonstrated that LT4 suppressive therapy resulted in lowered BMD of cortical-rich bone.[29,30]

While most studies support the deteriorating effect of LT4 suppressive therapy on BMD, bone loss because of replacement doses of LT4 has always been debatable. Three prospective trials have demonstrated a reduction in BMD over the treatment period.[31–33] The longest trial where patients were followed for one year after the initiation of therapy supports the finding in the current study. Many other studies have shown significant bone loss after a minimum of 7.5 years of LT4 replacement therapy as evidenced by a significant attenuation in speed of sound and stiffness index of the ultrasound measurements at the heel region.[34,35] The technique used to
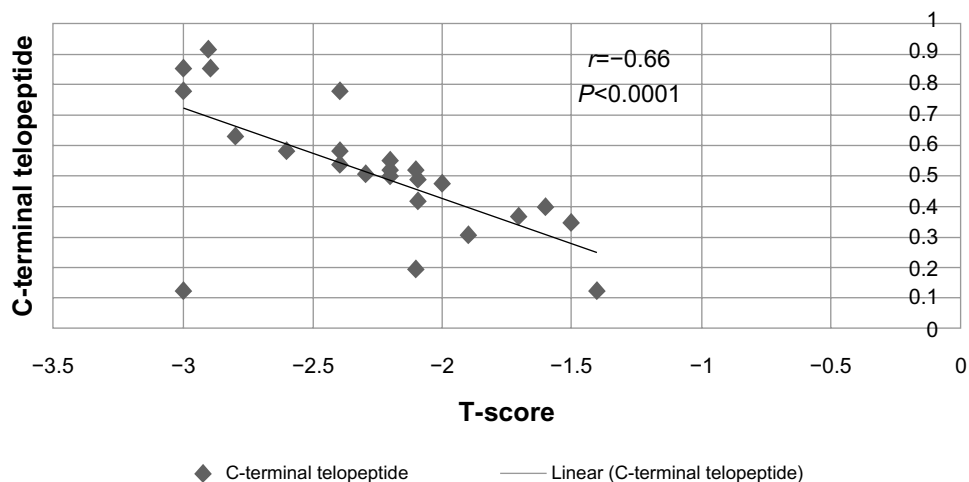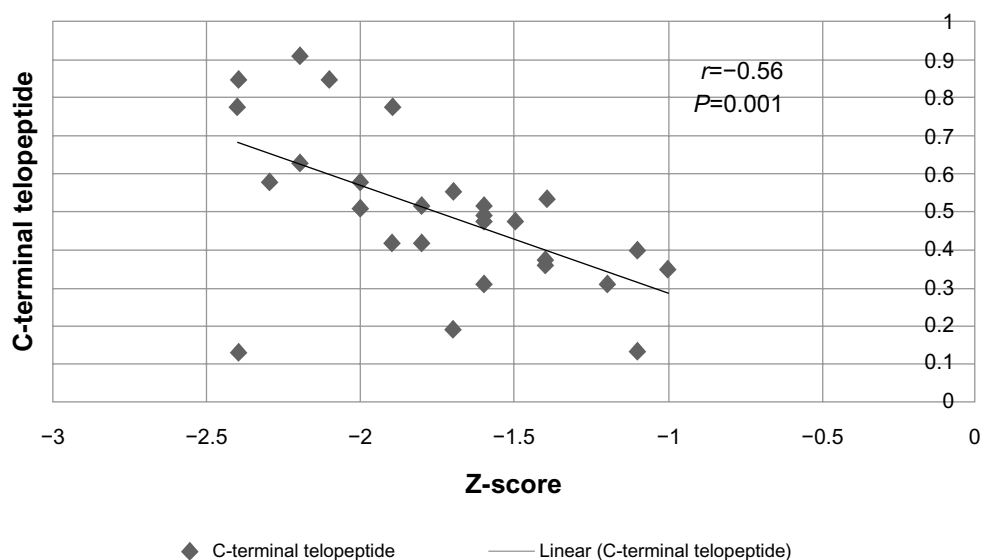
Figure 2. Correlation between C-terminal telopeptide and T-score in patients on levothyroxine treatment.

measure BMD in these studies was similar to our study as were the findings.

LT4 therapy and CTx. Where there is increased bone turnover, osteoclasts degrade type I collagen and release CTX molecules into the circulation.[36,37] CTx is being widely proposed to monitor bone loss in post-menopausal osteoporosis and has proven to be a promising diagnostic marker.[38,39] Significant data are available of its utility in measuring bone resorption in LT4 suppressive therapy. In a randomized controlled trial, Meier et al.[31] found elevated bone turn over markers DPD (deoxypyridino-line), PYD (pyridinoline), and CTx in the patients taking LT4. No change was observed in patients on placebo. Mikosch et al.[40] Sijanovic and Karner,[41] and Schneider et al.[42] also concluded that CTx is a better bone resorption marker in LT4 suppressive therapy group. Data of its use to detect replacement therapy-induced bone loss are limited.

In this study, CTx was significantly elevated in hypothyroid patients on LT4 therapy, especially in those on treatment for more than 10 years. In addition, there was a significant correlation between dose and duration with CTx levels. It has been noticed that in countries like India, LT4 replacement therapy is poorly monitored mostly because of lack of awareness among a part of patients regarding its possible side effects, and they frequently develop hyperthyroidism on prolonged therapy. Studies have shown elevated bone markers in hyperthyroidism, and hence persistent unmonitored elevated thyroid hormone levels in LT4-treated patients may cause bone loss, which can be detected by bone markers like CTx. CTx also showed significant negative correlation with BMD, more with the T-score and less with the Z-score. Although there are not many studies explaining these correlations in the LT4 replacement therapy group, a study done in premenopausal

Figure 3. Correlation between C-terminal telopeptide and Z-score in patients on levothyroxine treatment.
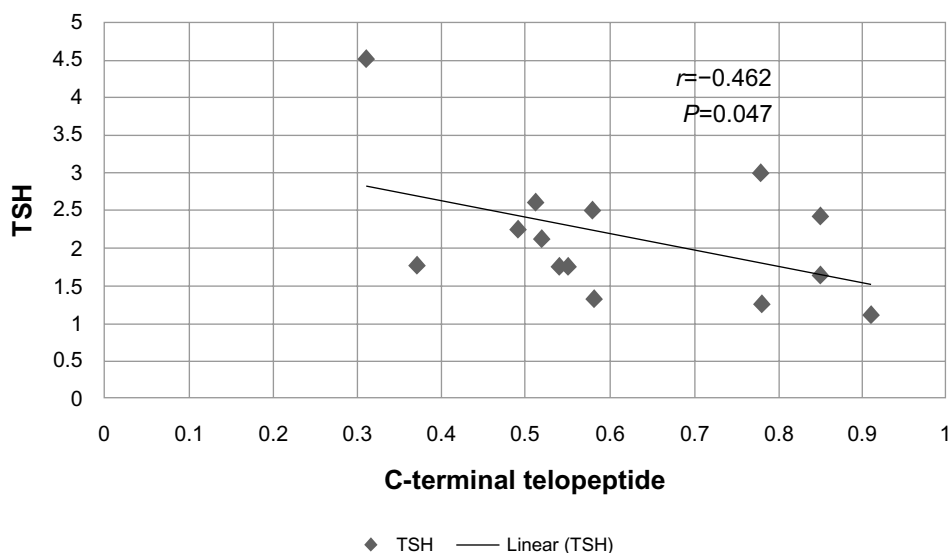
Figure 4. Correlation between CTx and TSH in dose group of more than 150 µg/day.

women taking LT4 suppressive therapy showed a significant negative correlation between CTx and BMD.[41]

In this study, CTx did not show any correlation with TSH in total LT4-treated cases, but it showed significant negative correlation in the group with dosage of 150 µg/day or above ($r = -0.462$, $P = 0.047$). Our findings are supported by a study done by Heemstra et al.[43] in which they used CTx as bone turnover marker to evaluate the role of TSH independent of T3 and T4 in bone metabolism and found inversely proportional relationship of CTx with TSH. Although low TSH levels suggest hyperthyroidism and hence with lowering of TSH levels, bone markers should elevate, in this study patients on LT4 therapy maintained TSH levels in euthyroid range (Table 1); hence, no correlation was found in the whole group. On the other hand, when stratified according to dosage, a significant negative correlation was found in those on higher dose of LT4 though the number of subjects was less.

Although it is being proposed as a reliable marker for most of the bone resorption-related disorders, its role has been widely debated because of variations in its levels with age, sex, smoking status, ovulation, concurrent drug use, exercise, circadian rhythms, renal function, and fasting states.[44] In this study, samples were taken in the morning in fasting states, and hence the possible variability in the results was minimized. Moreover, the patients on calcium supplements, corticosteroids, and antiepileptic drugs were excluded. Patients suffering from kidney diseases were also excluded. All the subjects had regular menstruation cycles, and therefore patients with ovulatory dysfunction were excluded negating the effects of ovulatory dysfunction on CTx.

Strengths and Limitations

Adequate sample size, strict patient selection criteria, and careful handling of samples strengthen the reliability of this study. Although information on the etiology of hypothyroidism from majority of the subjects was obtained, the etiology was not correlated with our findings because of unawareness of some of the subjects toward the exact etiology of their disorder. We propose a randomized controlled trial with strict dose regimen of LT4 to further substantiate the present findings.

Conclusion

To summarize, LT4 induces bone resorption in hypothyroid individuals especially those on long-term therapy. CTx provides a reliable assessment of the bone status in the above patients and may be used alongside BMD measurement. We conclude that there should be a careful monitoring of LT4 therapy to delay the bone loss by timely monitoring of markers like CTx.

Author Contributions

Conceived and designed the experiments: ALC. Analyzed the data: ALC. Wrote the first draft of the manuscript: ALC. Contributed to the writing of the manuscript: PM, RB, VDS. Agree with manuscript results and conclusions: RB, ST, MSR. Jointly developed the structure and arguments for the paper: PM, VDS, ALC, AH. Made critical revisions and approved final version: PM, VDS. All authors reviewed and approved of the final manuscript.

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