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Serum Cytokines and Bone Metabolism in Patients With Thyroid Dysfunction

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ABSTRACT

Hyperthyroidism is associated with increased bone turnover. Besides the hormones of calcium metabolism, locally produced factors are important in maintaining normal bone metabolism. Interleukin-6 (IL-6), in particular, has a major influence on bone turnover. In this study, serum IL-6 and tumor necrosis factor-alpha (TNF-α) levels, as well as bone turnover markers and relationships between them, were investigated in hyperthyroidism and hypothyroidism. A total of 20 female patients with hyperthyroidism, 15 with subclinical hyperthyroidism, 16 with hypothyroidism, and 15 with subclinical hypothyroidism constituted the patient groups. In all, 15 age-matched healthy female volunteers were recruited as controls. When compared with controls, serum TNF-α levels showed no significant difference in any of the patient groups ($P>.05$). In the groups with hyperthyroidism and subclinical hyperthyroidism, IL-6 levels were significantly higher compared with control group values ($P<.05$). Hyperthyroid patients showed higher levels of alkaline phosphatase (ALP) and osteocalcin, and a higher urinary deoxypyridinoline/creatinine ratio, compared with controls ($P<.05$). In subclinical hyperthyroidism, only ALP was found to be higher compared with control values. No significant correlations were made in any group between serum IL-6 or TNF-α level and bone turnover markers. Results suggest that serum IL-6 level and markers of bone turnover rate seem to be

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increased in hyperthyroidism. This finding may support the role of IL-6 in induction of bone turnover in hyperthyroid states.

Keywords: hyperthyroidism; hypothyroidism; IL-6; TNF-α; bone turnover marker

INTRODUCTION

The term *cytokine* is applied to any of a rapidly growing number of small, nonstructural proteins or glycoproteins that serve as chemical messengers between cells and are involved in such processes as cell growth and differentiation, tissue repair and remodeling, and regulation of immune response. Cytokines participate in normal growth and development,[1] and interest is growing in these molecules as their roles in the pathophysiology of a diverse spectrum of diseases become apparent.

Thyroid hormones, which are known to be important modulators of developmental processes, are required for normal growth and skeletal maturation.[2] On the other hand, hyperthyroidism is characterized by an increase in bone turnover accompanied by a negative calcium balance and reduced bone mineral density[3]; however, the mechanisms by which thyroid hormones affect bone cell metabolism remain unclear. Locally produced factors such as cytokines have been suggested to play significant roles in maintaining bone metabolism.[3,4] Interleukin-6 (IL-6), in particular, has a major influence on bone turnover. In this study, serum IL-6 and tumor necrosis factor-alpha (TNF-α) levels and bone turnover markers were investigated in hyperthyroidism and hypothyroidism.

MATERIALS AND METHODS

A total of 81 female subjects were enrolled into the study. Thyroid dysfunction was diagnosed in 66 of these patients. Patients were divided into 4 groups: subclinical or overt hyperthyroid (n=15 and n=20, respectively), and subclinical or overt hypothyroid (n=16 and n=15, respectively). Hyperthyroid patients had toxic adenoma or toxic multinodular goiter, whereas those with hypothyroidism had undergone ineffective surgical treatment or insufficient l-thyroxine replacement therapy. Patients with inflammatory or autoimmune thyroid disease were excluded from the study. A group of 15 female volunteers who had no thyroid dysfunction and no ongoing disease served as control subjects.

Venous blood samples (total, 5 mL) were drawn and then centrifuged at 2000 rpm for 10 min in a refrigerated centrifuge to separate serum. Each serum was divided into 2 aliquots. One of the aliquots was stored at –70°C in plastic tubes until assayed for IL-6 and TNF-α. Total T3 (TT3), total T4 (TT4), free T3 (FT3), free T4 (FT4), thyroid-stimulating hormone (TSH), calcium (Ca), phosphorus (P), and alkaline phosphatase (ALP) were analyzed from the fresh samples. A total of 2 mL of venous blood was collected into iced heparinized and ethylenediaminetetraacetic acid (EDTA) tubes for analysis of osteocalcin (OC) and intact parathyroid hormone (iPTH), respectively. Iced heparinized and EDTA tubes were kept on ice throughout the procedure. In addition, urine samples were collected from first or second morning voiding for analysis of deoxypyridinoline (DPD) from each subject.

Serum TT3, TT4, FT3, FT4, TSH, TNF-α, and IL-6, and plasma OC, plasma iPTH, and urinary DPD levels were measured with the use of commercial Immulite kits; these are solid-phase, 2-site chemiluminescent immunometric assays in an Immulite Autoanalyzer (Immulite; Diagnostic Products Corporation, Los Angeles, Calif). Serum Ca, P, and ALP, and urinary Ca and urinary creatinine levels were determined by routine methods with commercial kits on a modular autoanalyzer (Roche, Tokyo, Japan).

All data were reported as means with standard error (SE). Group data were analyzed through 1-way analysis of variance (ANOVA). Between-group differences were ascertained through post hoc least significant difference (LSD) testing. Correlation analysis was performed with Pearson's test.

RESULTS

Study results are given in the Table. When compared with those of controls, serum TNF-α levels showed no significant difference in any patient group ($P>.05$). In both groups with hyperthyroidism, IL-6 levels were significantly higher compared with those of controls ($P<.05$). In the group with overt hyperthyroidism, ALP, OC, and urinary deoxypyridinoline/creatinine (DPD/Cr) ratio were higher than those of controls ($P<.05$). In subclinical hyperthyroidism, only ALP was found to be higher when compared with the control group. No significant correlations were made between serum IL-6 or TNF-α level and bone turnover markers in any group.

DISCUSSION

Thyroid hormones are necessary for normal skeletal growth. However, excessive quantities may result in bone loss, the mechanism of which has not been fully elucidated. In recent years, the importance of locally produced factors like cytokines in bone metabolism has been revealed. A major cytokine, IL-6, has been shown to stimulate osteoclast formation and differentiation, leading to bone resorption.[5-7] IL-6 is produced in various tissues (ie, from bone to thyroid and blood mononuclear cells).[5] Bartalena et al[8] demonstrated that IL-6 can be regarded as a useful marker of thyroid destructive processes in the absence of nonthyroidal illness, which may further increase serum concentrations of cytokines. Investigators showed that amiodarone-induced thyrotoxicosis is associated with elevated serum IL-6.[9] In addition, Lakatos et al[5] claimed that IL-6 production of mononuclear cells in hyperthyroid women was higher than in controls. Fenkci et al[10] reported that elevations in serum IL-6 occurring in hyperthyroidism may result from several mechanisms, such as an autoimmune inflammatory condition, excessive thyroid hormones, or bone resorption induced by thyrotoxicosis. It is apparent that IL-6 levels are increased in hyperthyroidism, regardless of which mechanisms are involved; in turn, elevated IL-6 may contribute to differentiation and proliferation of osteoclasts, leading to increased bone resorption.

Measured Parameters in All Groups (mean±SE) and Comparisons With Controls

Parameter	Control	Overt Hyperthyroidism	Subclinical Hyperthyroidism	Overt Hypothyroidism	Subclinical Hypothyroidism
T_3, pg/mL	116.65±7.04	150.48±12.10*	106.55±10.36	62.39±8.75†	84.04±3.45*
T_4, ng/dL	7.31±0.28	12.78±1.34†	8.10±0.77	3.30±0.54‡	7.97±0.57
TSH, μIU/mL	1.27±0.19	0.015±0.004†	0.03±0.009‡	31.41±6.20†	8.43±1.49†
FT_3, pg/mL	3.56±0.15	6.42±0.43†	3.45±0.32	2.55±0.27*	3.34±0.23
FT_4, ng/dL	1.64±0.44	2.21±0.14*	1.19±0.11	0.53±0.06†	1.32±0.08
PTH, pg/mL	43.27±7.22	35.91±4.21	39.63±7.34	57.95±13.48	56.83±15.19
Urinary Ca/Cr	0.11±0.03	0.16±0.03	0.10±0.01	0.11±0.02	0.11±0.02
Ca, mg/dL	9.31±0.18	9.83±0.17*	9.63±0.14	9.21±0.19	9.52±0.15
P, mg/dL	3.69±0.14	3.47±0.19	3.52±0.20	3.55±0.20	3.75±0.12
ALP, U/L	170.07±8.88	245.53±21.03*	283.82±39.87‡	150.67±14.25	232.15±33.55
OC, ng/mL	7.00±1.33	13.21±1.81*	11.18±1.71	6.18±0.81	11.03±3.37
IL-6, pg/mL	2.16±0.15	3.93±0.29*	4.28±0.19‡	2.59±0.24	2.17±0.28
TNF-α, pg/mL	7.42±1.88	11.17±2.95	9.26±3.24	7.32±0.97	5.23±1.81
Urinary DPD/Cr, nM/mM	10.41±0.89	18.14±2.72‡	14.81±2.34	10.46±1.19	10.84±0.98

*P<.05; †P<.001; ‡P<.01.
All groups were compared with the control group.

Bone turnover is characterized by 2 metabolic processes: formation of new bone by osteoblasts, and degradation of aged bone by osteoclasts. Bone mass depends on the balance between bone formation and bone resorption. Biochemical markers of formation or resorption help the clinician to discern the balance between them. In this study, OC and ALP were used as markers of bone formation, and urinary Ca and urinary DPD were considered markers of bone resorption. It was found that, compared with controls, ALP and OC levels and urinary DPD/Cr ratios were higher in patients with overt hyperthyroidism, whereas only ALP was higher in patients with subclinical disease. Increased bone turnover was evident in hyperthyroidism. However, serum cytokine levels and bone turnover markers failed to show any correlation between groups. In accordance with current findings, Siddiqi et al[3] found no evidence of a direct relationship between osteotrophic cytokines and markers of bone resorption, although elevated IL-6 and IL-8 levels were revealed in thyrotoxicosis. Celik et al[11] suggested that the deleterious effects of hyperthyroidism on bone metabolism might be mediated by cytokines.

In this study, no significant differences were noted in levels of serum TNF-α among groups with hyperthyroidism, subclinical hyperthyroidism, hypothyroidism, and subclinical hypothyroidism. In addition, no correlation was seen between levels of TNF-α and bone turnover markers. Administration of recombinant human TNF-α to man, rat, or mouse has been reported to decrease serum T3 and/or T4 concentrations to levels similar to those seen in patients with euthyroid sick syndrome in systemic nonthyroidal illness.[12-14] However, data associated with serum TNF-α levels in various thyroid diseases are inconclusive. Chopra et al[15] have reported that TNF-α levels in hypothyroidism and hyperthyroidism are not different from those of controls. Siddiqi et al[3] also failed to find any change in levels of TNF-α in thyrotoxicosis. In contrast, Celik et al[11] showed increased TNF-α levels among patients with Graves' disease, as well as increased TNF-α levels in hyperthyroidism.

In conclusion, serum IL-6 levels and bone turnover rates seem to increase in hyperthyroidism. This finding supports the suggestion that IL-6 may induce increased bone turnover in hyperthyroidism. Additional studies are needed to explain the clinical importance of cytokines in thyroid dysfunction.

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