Original Article *Özgün Araştırma*

DOI: 10.4274/Tjem.2238

Plasma Osteoprotegerin Levels Before and After Treatment of Thyroid Dysfunctions

Tiroid Disfonksiyonlarında Tedavi Öncesi ve Sonrası Plazma Osteoprotegerin Seviyeleri

Didem Özdemir, Selçuk Dağdelen, Aydan Usman

Hacettepe University School of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey

Abstract

Purpose: Osteoprotegerin (OPG) is a soluble decoy receptor for the receptor activator of nuclear factor kappaB ligand, thereby inhibiting bone resorption. In this study, we aimed to evaluate plasma OPG levels in patients with thyroid dysfunctions and determine whether its levels change after restoration of euthyroidism.

Material and Method: OPG levels were studied at the time of diagnosis and after the restoration of euthyroidism at least for 8 weeks in patients diagnosed with overt thyrotoxicosis and hypothyroidism.

Results: Seventeen hypothyroid, 17 thyrotoxic patients and 17 age-, sex- and body mass index-matched healthy controls were analyzed. Mean basal plasma OPG levels were 5.42±2.66, 5.04±1.62 and 5.24±0.93 pmol/l in thyrotoxic, hypothyroid and healthy controls, respectively (p=0.844). After restoration of euthyroidism, OPG was 5.52±2.37 pmol/l in thyrotoxic and 4.33±1.37 pmol/l in hypothyroid patients, indicating no significant difference compared to baseline values (p=0.846 and p=0.109, respectively). We also did not observe any correlation between basal OPG levels and basal thyrotropin and thyroid hormone levels.

Discussion: Thyroid dysfunctions seem to affect bone functions by mechanisms other than OPG, however, more clinical studies with larger sample sizes are needed to clarify the underlying mechanisms of thyroid dysfunction-related changes in bone metabolism. *Turk Jem 2013; 17: 102-7*

Key words: Osteoprotegerin, hypothyroidism, thyrotoxicosis

Özet

Amaç: Osteoprotegerin (OPG), reseptör aktivatör nükleer faktör kappa B ligand için yalancı reseptör görevi görür ve bu şekilde kemik yıkımını inhibe eder. Bu çalışmanın amacı tiroid disfonksiyonu olan hastalarda plazma OPG seviyelerinin değerlendirilmesi ve ötiroidizm sağlandıktan sonra OPG seviyesinde değişiklik olup olmadığının belirlenmesidir.

Gereç ve Yöntem: Aşikar hipotiroidi ve tirotoksikoz tanısı alan hastalarda tanı anında ve en az 8 hafta ötiroidizm sağlandıktan sonra OPG seviyeleri ölçüldü.

Bulgular: Çalışmaya 17 hipotiroid, 17 tirotoksik hasta ve 17 yaş, cinsiyet ve vücut kitle indeksi açısından eşleştirilmiş sağlıklı kontrol grubu alındı. Ortalama bazal plazma OPG seviyeleri tirotoksik hastalarda 5,42±2,66 pmol/l, hipotiroid hastalarda 5,04±1,62 pmol/l ve kontrol grubunda 5,24±0,93 pmol/l bulundu (p=0,844). Ötiroidizm sağlandıktan sonra ortalama OPG seviyeleri tirotoksik hastalarda 5,52±2,37 pmol/l, hipotiroid hastalarda 4,33±1,37 pmol/l olarak bulundu. Her iki grupta da tedavi öncesi ve sonrası OPG seviyelerinde anlamlı değişiklik olmadı (tirotoksik hastalar için p=0,846 ve hipotiroid hastalar için p=0,109). Bazal OPG ile bazal tirotropin ve tiroid hormonları arasında anlamlı korelasyon saptanmadı.

Tartışma: Bu çalışmanın sonuçlarına gore tiroid fonksiyon bozukluklarında görülen kemik metabolizma değişikliklerinde OPG rol almamaktadır. Tiroid disfonksiyonlarının kemik üzerine etkilerinin hangi mekanizmalarla oluştuğunun belirlenmesi için daha fazla sayıda hastanın alındığı klinik çalışmalara ihtiyaç vardır. *Turk Jem 2013; 17: 102-7*

Anahtar kelimeler: Osteoprotegerin, hipotiroidi, tirotoksikoz

Address for Correspondence/Yazışma Adresi: Didem Özdemir MD, Hacettepe University School of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey
Gsm: +90 505 232 05 37 E-posta: sendidem202@yahoo.com **Received/Geliş Tarihi:** 22/01/2013 **Accepted/Kabul Tarihi:** 19/08/2013

Introduction

Bone remodeling is under regulation of various hormones, cytokines, transcription factors and intracellular signaling proteins. Receptor activator of nuclear factor kappaB ligand (RANKL) is a hematopoietic growth factor that plays a role in osteoclastogenesis via activating receptor activator of nuclear factor kappaB (RANK) in osteoclasts. Osteoprotegerin (OPG) protects the skeleton from excessive bone resorption by acting as a decoy receptor for RANKL and preventing it from binding to its receptor RANK (1,2). It is expressed in many tissues apart from osteoblasts, including heart, kidney, liver, spleen and bone marrow. In general, up-regulation of RANKL is associated with down-regulation of OPG, such that the ratio of RANKL to OPG changes in favor of osteoclastogenesis. OPG also appears to protect large blood vessels from medial calcification, based on the observation of renal and aortic calcification occurring in OPG knockout mice (3). There are also studies showing an association between high plasma levels of OPG and cardiovascular diseases, diabetes and chronic renal failure (4,5).

It is believed that high circulating thyroid hormones induce a high turnover state in bone with increased activity of osteoblasts and osteoclasts. The osteoclast stimulation, however, predominates and structural integrity of the skeleton decreases in hyperthyroidism. General belief is that reduction in bone mineral density in overt thyrotoxicosis is due to the effect of high circulating levels of thyroid hormones, not the effect of suppressed thyrotropin (TSH) levels. However, in a recent study, Abe et al. demonstrated a critical role for TSH in maintenance of bone mass independently from circulating thyroid hormones (6). In their study, TSH receptor (-/-) knockout mice were shown to have high bone turnover osteoporosis, revealing that TSH is a negative regulator of bone turnover. They speculated that the skeletal loss that occurs in hyperthyroidism is due to low TSH levels, not as opposed solely to high thyroid hormones. Patients with hypothyroidism may actually have a higher than normal bone mass prior to thyroxine replacement. Data from cell and organ cultures have identified the role of T3 in the production of cytokines, growth factors and markers of bone turnover (7). Therefore, altered T3 is thought to be the cause of decreased bone turnover in hypothyroidism.

Plasma OPG levels in patients either with hyperthyroidism or with hypothyroidism, separately, were investigated in a few studies reporting inconsistent results. High basal OPG levels were observed with both of these thyroid dysfunctions and lower OPG levels were shown with treatment of both conditions (8-12). However, there are no publications investigating OPG levels in overt hypo and hyperthyroidism in the same study in the literature. In this study, our aim was to evaluate OPG levels in overt thyrotoxicosis and hypothyroidism before and after restoration of euthyroidism in a descriptive matched case-control study.

Materials and Methods

Patients diagnosed with overt thyrotoxicosis and hypothyroidism in our clinic were screened for the study. All patients and control subjects gave informed consent and local ethics committee approval was obtained in accordance with the ethical standards of the Helsinki Declaration. Inclusion criteria were as follows: ≥18 years old, newly diagnosed overt hypothyroidism or thyrotoxicosis without medication, body mass index (BMI)<30 kg/m^2. Patients with a previous history of bone fracture, taking medication for osteopenia or osteoporosis (bisphosphonates, vitamin D, calcium supplementation, etc.), using oral contraceptives or estrogen hormone replacement therapy, patients with known diabetes and/or using antidiabetic medications including thiazolidinediones and patients with organ failure or malignancy were excluded. Patients with central hypothyroidism were excluded as well. Patients were matched with healthy controls according to age, sex and BMI. All female patients and controls were premenopausal.

Complete physical examination was performed by the same physician in all subjects. As an open study, laboratory parameters of TSH, free T3 (FT3), free T4 (FT4), anti-thyroid peroxidase antibody (anti-TPO), and antithyroglobulin antibody (anti-Tg) levels were checked at the time of diagnosis and after the restoration of euthyroidism with appropriate therapy provided at least for 8 weeks. Healthy control subjects were tested once. Blood samples were taken after an overnight fasting from an antecubital vein between 08.30 and 09.30 am at resting position. Serum concentrations of TSH, FT3 and FT4 were measured with electrochemiluminescence immunoassay. Anti-TPO and Anti-Tg were measured using radioimmunoassay and chemiluminescent sequential immunometric assay, respectively. Plasma samples for OPG were obtained also at the time of diagnosis and after the restoration of euthyroidism at least for 8 weeks in patients and for once in control group and stored at -80 °C. After collection of all samples, they were thawed for once and studied in the same day. The time from freezing of the first sample to thawing of all the samples was approximately 10 months. OPG levels were determined by enzyme immunoassay using an ELISA kit produced by BioVendor® Research and Diagnostic Products. Presented reference range was 4.1±2.3 pmol/l. Limit of detection, defined as concentration of analyte giving absorbance higher than mean absorbance of blank plus 3 standard deviation was calculated from the real human OPG values in wells and was 0.13 pmol/l. Intraassay and interassay coefficient of variations were 2.4%-7.0% and 3.4%-7.4%, respectively. The antibodies used in this method were specific for human OPG with no detectable cross-reactivity to human RANKL.

Overt thyrotoxicosis and hypothyroidism were diagnosed in compliance with the American Association of Clinical Endocrinologists Medical Guidelines for Clinical Practice for the evaluation and treatment of hyperthyroidism and hypothyroidism (13). Normal values for TSH were 0.27-4.2 mIU/ml, for FT3 3.1-6.8 pmol/l, for FT4 12-22 pmol/l, for Anti-TPO 0-30 IU/ml and for Anti-Tg 0-20 IU/ml. Where needed, thyroid ultrasonography, scintigraphy and radioactive iodine uptakes were performed to define the underlying cause and to plan the management. Patients with thyrotoxicosis were administered propylthiouracil (dosage tapered down from 300 mg/day) and/or propranolol (40-120 mg/day for 2 weeks) according to underlying pathology to maintain thyroid functions within normal limits. Hypothyroid patients were administered L-thyroxine and initial dosage was

chosen considering age and cardiovascular risk. Thyroid function tests were re-evaluated in thyrotoxic and hypothyroid patients in every 3-4 weeks.

All data were analyzed with SPSS (Statistical Packing of Social Science for Windows) 9.05. The Kolmogorov-Smirnov test was used to find out whether parameters were normally distributed or not in each group. All parameters except basal TSH and Anti-TPO were normally distributed in patients with hypothyroidism and thyrotoxicosis. In control group, anti-TPO and anti-Tg were not normally distributed, while other parameters were normally distributed. Comparison of means between 3 groups was made using the One-way ANOVA for parametric variables, and the Kruskall-Wallis test for nonparametric variables. The Chi-square test was used to investigate the difference between the groups regarding the categorical variables. For paired data, statistical analyses before and after therapy were carried out with the paired student t-test for parametric variables and the Wilcoxon test for nonparametric variables. Pearson's correlation and Spearman's correlation analysis were performed in order to document possible associations between parametric and nonparametric variables, respectively.

Results

Twenty patients with overt hypothyroidism and 23 patients with overt thyrotoxicosis were included in the study. Data of 17 hypothyroid, 17 thyrotoxic patients and 17 control cases were analyzed, because 3 hypothyroid and 6 thyrotoxic patients were not euthyroid at the end of the study. Mean age, sex distribution and anthropometric measures were similar in all groups. Baseline characteristics of patients and control group are shown in Table 1.

Underlying causes of hypothyroidism were Hashimoto's thyroiditis in 14 patients, postoperative hypothyroidism in 2 and postradioactive iodine hypothyroidism in 1 patient. Thyrotoxicosis was due to Graves' disease in 14 patients, subacute thyroiditis in 2 patients and autonomous thyroid nodule in 1 patient. Anti-TPO and anti-Tg autoantibodes were measured in all patients and controls. Anti-TPO was positive in 15 patients with hypothyroidism, 8 patients with thyrotoxicosis and in 1 subject in the control group. Anti-Tg was positive in 13, 7 and 1 patients with hypothyroidism, thyrotoxicosis and control group, respectively (Table 1). There was no significant correlation between basal OPG and anti-TPO and anti-TG levels (rs=-0,102, p=0.477 for anti-TPO and rs=-0,214, p=0.131 for anti-TG). Table 2 compares the clinical and biochemical characteristics of the patients before and after treatment of thyroid dysfunctions. In both groups, BMI did not change with restoration of euthyroidism.

Plasma OPG levels were (mean±stdev) 5.42±2.66, 5.04±1.62 and 5.24±0.93 pmol/l in thyrotoxic, hypothyroid and healthy controls, respectively. Although, baseline OPG levels were lowest in patients with hypothyroidism and highest in patients with thyrotoxicosis, no statistically significant difference among study groups and controls were detected (Table 3). Mean OPG levels after restoration of euthyroidism in thyrotoxic and hypothyroid patients were (mean±stdev) 5.52±2.37 and 4.34±1.37 pmol/l, respectively. OPG seemed to increase in thyrotoxic and decrease in hypothyroid patients with treatment, however, no significant alteration was detected compared to baseline state in both groups (p=0.846

Table 1. Baseline characteristics of patients and controls

	Hypothyroidism (n=17)	Thyrotoxicosis (n=17)	Controls (n=17)	p
Age	41.1±11.8	37.6±11.0	38.2±10.2	0.613
BMI (kg/m^2)	25.4±3.1	23.4±3.7	25.6±2.5	0.093
TSH (mIU/ml)				
Mean	85.15±22.77	0.01±1.11	1.58±0.16	<0.001
Median	101 (26.84-101)	0.005 (0.005-0.041)	1.64 (0.44-269)	<0.001
FT3 (pmol/l)				
Mean	2.30±0.93	18.35±12.05	4.94±0.12	<0.001
Median	2.90 (0.537-3.090)	11.85 (6.90-44.0)	(4.94-4.1-5.65)	<0.001
FT4 (pmol/l)				
Mean	5.48±2.51	50.08±29.50	15.75±0.53	<0.001
Median	5.64 (1.43- 11.08)	35.02 (23.1-100.0)	16.1 (12.1-19.0)	<0.001
Anti-TPO (IU/ml)				
Mean	2287.23±1081.21	762.91±1085.48	19.29±46.91	<0.001
Median	3000 (7-3000)	76 (0-3000)	7.6 (3.6-201)	0.002
Anti-Tg (IU/ml)				
Mean	683.12±980.68	173.42±336.43	24.18±87.6	0.006
Median	249 (0-3950)	43 (0-1300)	0 (0-363)	0.011
Anti-TPO positivity (n)	15	8	1	<0.001
Anti-Tg positivity (n)	13	7	1	<0.001

BMI: Body mass index, TSH: Thyrotropin, FT3: Free T3, FT4: Free T4, Anti-TPO: Anti-thyroid peroxidase antibody, anti-Tg: Antithyroglobulin antibody, NA: Not applicable

and p=0.109, respectively) (Table 3). The mean change in OPG in hypothyroid patients was -0.71±1.71 pmol/l, while the mean change in thyrotoxic patients was +0.10±2.17 pmol/l (p=0.234).

Analyzing all groups together, there was no correlation between basal OPG levels and basal TSH and thyroid hormone levels (rs=-0.002 and p=0.990 for TSH, rs=0.057 and p=0.692 for fT3, rs=0.030 and p=0.832 for fT4). Changes in TSH, FT3 and FT4 levels (before-after the restoration of euthyroidism) showed no correlation with changes in OPG levels (Table 4).

Discussion

In the present study, we found no significant difference in basal OPG levels in both hypothyroid and thyrotoxic patients compared with euthyroid healthy subjects. Additionally, achievement of euthyroidism in both groups did not result with any change in OPG levels. In the literature, there are few studies investigating OPG in thyroid dysfunctions, but with inconsistent results. To our knowledge, there are 3 clinical trials studying OPG levels in hypothyroid patients. In all, OPG was found to be higher than in controls and

Table 2. Characteristics of thyrotoxic and hypothyroid patients before and after restoration of euthyroidism

	Hypothyroidism			Thyrotoxicosis		
	Basal	**Post-treatment**	**p**	**Basal**	**Post-treatment**	**P**
BMI (kg/m^2)	25.4±3.1	24.8±3.0	0.103	23.4±3.7	24.1±4.3	0.125
TSH (mIU/ml)						
Mean	85.15±22.77	1.86±1.25	<0.001	0.01±1.11	2.06±1.10	<0.001
Median	101 (26.84-101.0)	1.82 (0.29-4.20)	<0.001	0.005 (0.005-0.041)	1.73 (0.38-4.14)	<0.001
FT3 (pmol/l)						
Mean	2.30±0.93	4.89±0.80	<0.001	18.35±12.05	4.60±0.63	<0.001
Median	2.90 (0.54-3.09)	4.79 (3.44-6.42)	<0.001	11.85 (6.90-44.0)	4.58 (3.19-6.13)	<0.001
FT4 (pmol/l)						
Mean	5.48±2.51	17.90±2.54	<0.001	50.08±29.50	14.42±1.80	<0.001
Median	5.64 (1.43-11.08)	17.86 (12.73-21.85)	<0.001	35.02 (23.1-100.0)	13.71 (12.31-17.32)	<0.001

BMI: Body mass index, TSH: Thyrotropin, FT3: Free T3, FT4: Free T4

Table 3. Basal and post-treatment plasma osteoprotegerin levels in patients and control group

	Hypothyroidism	**Thyrotoxicosis**	**Controls**	**p**
Basal OPG levels (pmol/l)	5.04±1.62	5.42±2.66	5.24±0.93	0.844
Post-treatment OPG levels (pmol/l)	4.34±1.37	5.52±2.37	-	0.083
p	0.109	0.846	NA	-

OPG: Osteoprotegerin, NA: Not applicable

Table 4. Correlation between changes in osteoprotegerin and changes in thyrotropin, free T3 and free T4 levels after treatment of thyroid dysfunctions

	Hypothyroidism						Thyrotoxicosis					
ΔOPG	ΔTSH		ΔFT3		ΔFT4		ΔTSH		ΔFT3		ΔFT4	
	r	p	r	p	r	p	r	p	r	p	r	p
	-0.580	0.825	-0.356	0.161	-0.209	0.421	0.341	0.180	0.339	0.183	0.280	0.276

OPG: Osteoprotegerin, TSH: Thyrotropin, FT3: Free T3, FT4: Free T4,

r: Pearson correlation coefficient

$\Delta = \frac{\text{Last volue-basal volue}}{\text{Basal volue}}$

two of them showed decreasing OPG levels after achievement of euthyroidism (8,9,14). An additional study compared OPG levels in TSH suppressed state and after withdrawal of L-thyroxine in patients with differentiated thyroid cancer and reported increased OPG when intentional hypothyroidism occurred (10). These studies suggested that OPG acting as an inhibitor of osteoclastogenesis may constitute a link between hypothyroidism and the decrease in bone resorption.

If so, thyrotoxicosis - a disease known to be related with increased bone turnover in favor of osteoclastogenesis - is expected to be associated with decreased OPG levels. On the contrary, OPG in overt thyrotoxicosis has been assessed only in two trials yet, and found to be also increased in cumulatively 114 patients with Graves' disease and 21 patients with autonomous thyroid nodules (11,12). Additionally, OPG was reported to decrease with restoration of euthyroidism in both studies. Besides, the relationship between subclinical hyperthyroidism and OPG was examined in differentiated thyroid cancer patients receiving L-thyroxine therapy for suppression of TSH and conflicting results were obtained. Although some authors declared increased OPG in exogenous TSH suppression (15,16), some suggested no difference compared to healthy subjects (17,18). However, the common finding of all was the lack of change in OPG levels after institution of high TSH with administration of rhTSH.

The precise mechanism by which thyroid hormone dysfunctions change serum OPG has not been expounded yet in existing studies. It was shown that OPG and RANKL are also produced in the thyroid gland by thyroid follicular cells, and OPG mRNA and protein secretion are regulated by cytokines and TSH (1). In hyperthyroidism, one of the possible explanations for increased OPG was induction of in vitro expression of OPG mRNA in bone cells by T3 (19).

Findings of our study were not consistent with most of the previous studies showing high OPG in both hypothyroidism and hyperthyroidism. Similar trends of change in OPG levels in two conditions which are known to have contrary effects on bone metabolism give rise to more questions on the results of those previous publications. There is evidence that TSH regulates bone function by mechanisms different from those used by RANKL/OPG cytokines. Abe et al. showed that osteoclast formation is increased in TSH receptor -/- mice although RANKL expression was decreased, considering lack of effect of RANKL on osteoclastogenesis (6). They also suggested that TNF-alpha is a proosteoclastic signal mediating the effects of TSH receptor deletion because they found that expression of TNF-alpha is enhanced dramatically in vivo and that a neutralizing antiTNF antibody inhibits the enhanced osteoclastogenesis ex vivo in TSH receptor -/- bone marrow cell cultures. Giusti et al. did not find an acute direct effect of TSH on OPG and RANKL in patients with a history of differentiated thyroid cancer, thus, confirming the view that the inhibitory role of TSH on osteoclastogenesis is achieved through mechanisms other than osteoblast-osteoclast cross-talk via the OPG/RANK/RANKL system (17). Moreover, recently, it has been shown that, circulating OPG is not correlated with serum TSH receptor antibodies in patients with Graves' hyperthyroidism (11). These data provide clues that, TSH does not regulate OPG production in bone, although some regulation may occur in other tissues. Lack of any significant change in OPG levels during thyroid dysfunctions in our study seems to be relevant in accordance with those experimental data, described above. None of the previous clinical publications investigated overt hypo and hyperthyroid patient populations in the same study with the same methods. Exclusion of methodological selection bias while evaluating any possible change in OPG levels in hypothyroidism and thyrotoxicosis in our study population might explain inconsistency of our data with the literature.

Menopausal status, autoimmunity and inflammation are three important factors that might modify serum OPG levels. Postmenopausal women without estrogen replacement therapy displayed 2 to 3 fold higher RANKL expression index on bone marrow stromal cells and lymphocytes compared to postmenopausal women on estrogen therapy (20). Premenopausal status of our study population might have masked possible difference in OPG levels across the study groups. In addition, our patient groups were heterogeneous in terms of etiologies of hypothyroidism and thyrotoxicosis. Hofbauer et al. detected OPG mRNA levels three times more abundant in surgical thyroid specimens of Graves' disease compared with other thyroid diseases suggesting a possible role of autoimmunity on serum OPG levels (1). Conducting a study investigating OPG levels in hypothyroid or hyperthyroid patients with the same etiology to exclude influence of autoimmunity and inflammation may be more elucidative. It is generally impossible to know for how long patients with thyroid dysfunctions have these disorders. In this study, euthyroidism was maintained in 7.17±4.0 months in thyrotoxic and in 4.7±2.49 months in hypothyroid patients. The durations of thyroid dysfunctions and the time elapsed after achievement of euthyroidism might be too short for significant changes to occur.

Recently, Riches et al. showed the presence of a neutralizing autoantibody against OPG in a patient with celiac disease, autoimmune hypothyroidism and high-turnover severe osteoporosis (21). Consequently, they demonstrated the presence of this antibody in 3 of 15 patients with celiac disease, but not in any of 14 patients with primary hypothyroidism and 10 healthy controls. They suggested a possible association of OPG antibodies with the development of osteoporosis in patients with celiac disease. Although no patient with autoimmune hypothyroidism was positive for OPG antibody in that study, possible confounding effect of an antibody should still be considered in our patient groups which included autoimmune thyroid diseases. We think that evaluation of bone turnover markers would help us to make more precise implications about the effects of thyroid dysfunctions on bone metabolism, yet the purpose of our study was not directed to this subject. As another limitation of our study, we could not measure RANKL concentration before and after restoration of euthyroidism in order to calculate RANKL bioactivity index (RANKL/OPG ratio). Finally, small number of cases in our groups may be considered as another limitation.

In conclusion, we demonstrated that OPG levels in hypothyroid and thyrotoxic patients are similar with that in euthyroid subjects and achievement of euthyroidism in these patients does not

change OPG levels. Impact of thyroid dysfunctions on bone metabolism seems to be regulated by mechanisms other than OPG. However, further studies with larger sample size are needed to come to a definite conclusion about the effects of TSH or thyroid hormones on OPG, RANKL and bone turnover.

References

1. Hofbauer LC, Kluger S, Kühne CA, et al. Heufelder AE. Detection and characterization of RANK ligand and osteoprotegerin in the thyroid gland. J Cell Biochem 2006;86:642-50.
2. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003;423:337-42.
3. Bucay N, Sarosi I, Dunstan CR, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998;12:1260-8.
4. Collin-Osdoby P. Regulation of vascular calcification by osteoclast regulatory factors RANKL and osteoprotegerin. Circ Res 2004;95:1046-57.
5. Knudsen ST, Foss CH, Poulsen PL, et al. Increased plasma concentrations of osteoprotegerin in type 2 diabetic patients with microvascular complications. Eur J Endocrinol 2003;149:39-42.
6. Abe E, Marians RC, Yu W, et al. TSH is a negative regulator of skeletal remodeling. Cell. 2003;115:151-62.
7. Britto JM, Fenton AJ, Holloway WR, Nisholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 1994;134:169-76.
8. Nagasaki T, Inaba M, Jono S, et al. Increased levels of serum osteoprotegerin in hypothyroid patients and its normalization with restoration of normal thyroid function. Eur J Endocrinol 2005;152:347-53.
9. Guang-da X, Hui-ling S, Zhi-song C, Lin-shuang Z. Changes in plasma concentrations of osteoprotegerin before and after levothyroxine replacement therapy in hypothyroid patients. J Clin Endocrinol Metab 2005;90:5765-8.
10. Botella-Carretero JI, Alvarez-Blasco F, San Millán JL, Escobar-Morreale HF. Thyroid hormone deficiency and postmenopausal status independently increase serum osteoprotegerin concentrations in women. Eur J Endocrinol 2007;156:539-45.
11. Amato G, Mazziotti G, Sorvillo F, Piscopo M, Lalli E, Biondi B, Iorio S, Molinari A, Giustina A, Carella C. High serum osteoprotegerin levels in patients with hyperthyroidism: effect of medical treatment. Bone. 2004;35:785-91.
12. Mochizuki Y, Banba N, Hattori Y, Monden T. Correlation between serum osteoprotegerin and biomarkers of bone metabolism during anti-thyroid treatment in patients with Graves' disease. Horm Res 2006;66:236-39.
13. Baskin HJ, Cobin RH, Duick DS, et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of hyperthyroidism and hypothyroidism. Endocr Pract. 2002;8:457-69.
14. Guang-da X, Hui-ling S, Jie H. Changes in endothelial function and its association with plasma osteoprotegerin in hypothyroidism with exercise-induced silent myocardial ischaemia. Clin Endocrinol 2008;69:799-803.
15. Martini G, Gennari L, De Paola V, et al. The effects of recombinant TSH on bone turnover markers and serum osteoprotegerin and RANKL levels. Thyroid 2008;18:455-60.
16. Mazziotti G, Sorvillo F, Piscopo M, Cioffi M, Pilla P, Biondi B, Iorio S, Giustina A, Amato G, Carella C. Recombinant human TSH modulates in vivo C-telopeptides of type-1 collagen and bone alkaline phosphatase, but not osteoprotegerin production in postmenopausal women monitored for differentiated thyroid carcinoma. J Bone Miner Res. 2005;20:480-6.
17. Giusti M, Cecoli F, Ghiara C, et al. Recombinant human thyroid stimulating hormone does not acutely change serum osteoprotegerin and soluble receptor activator of nuclear factor-kappa beta ligand in patients under evaluation for differentiated thyroid carcinoma. Hormones 2007;6:304-13.
18. Giusti M, Cecoli F, Fazzuoli L, et al. Serum osteoprotegerin and soluble receptor activator of nuclear factor kappaB ligand levels in patients with a history of differentiated thyroid carcinoma: a case-controlled cohort study. Metabolism 2007;56:699-707.
19. Varga F, Spitzer S, Klaushofer K. Triiodothyronine (T3) and 1,25-dihydroxyvitamin D3 (1,25D3) inversely regulate OPG gene expression in dependence of the osteoblastic phenotype. Calcif Tissue Int 2004;74:382-7.
20. Eghbali-Fatourechi G, Khosla S, Sanyal A, et al. Role of RANK ligand in mediating increased bone loss in early postmenopusal women. J Clin Invest 2003;111:1221-30.
21. Riches PL, McRorie E, Fraser WD, Determann C, van't Hof R, Ralston SH. Osteoporosis associated with neutralizing autoantibodies against osteoprotegerin. N Engl J Med 2009;361:1459-65.