Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 62; Numer/Number 4/2011
ISSN 0423–104X

Interleukin-6 and its considerable role in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in postmenopausal women

Interleukina 6 i jej istotna rola w patogenezie zaburzeń obrotu kostnego w przebiegu tyreotoksykozy u kobiet po menopauzie

Janusz Mysliwiec, Malgorzata Adamczyk, Agnieszka Nikolajuk, Maria Gorska

Department of Endocrinology, Diabetology and Internal Diseases, Medical University of Bialystok, Poland

Abstract

Background: Thyrotoxicosis is more frequent in postmenopausal women than in the general population, effectively accelerating bone turnover. Interleukin-6 has been shown to be involved in the pathogenesis of bone disorders. Thus, the aim of the present study was to assess the role of IL-6 and its soluble receptor in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in oestrogen-deficient women.
Material and methods: The study was carried out in 40 subjects with toxic nodular goitre in three groups: Group 1 — 13 premenopausal females, mean age 36 ± 15 years (PremTx→PremEu); Group 2 — 12 postmenopausal females, mean age 66 ± 14 years (PostTx→PostEu); and Group 3 — 15 males, mean age 45 ± 21 years (MTx→MEu). Overt thyrotoxicosis and euthyreosis after treatment with thyrostatics were confirmed by thyrotropin, free thyroxine and free triiodothyronin concentrations. Serum levels of bone turnover markers: TRACP5b and osteocalcin as well as serum IL-6 and IL-6sR were determined using ELISA kits.
Results: TRACP5b/osteocalcin quotient was significantly elevated in the PostTx females compared to the PremTx women ($p < 0.02$). There was a positive correlation between serum TRACP5b and osteocalcin in the studied patients ($R = 0.45$, $p < 0.001$). Levels of serum IL-6 values were significantly elevated in PostTx: 3.0 (2.14–6.40) and MTx: 2.24 (1.60–5.10), compared to PremTx females: 1.39 (0.96–2.14) ($p < 0.01$ and $p < 0.05$ respectively). There were significant positive correlations between IL-6 and IL-6sR concentrations ($R = 0.22$, $p < 0.05$) and between IL-6sR and TRACP5b serum levels ($R = 0.23$, $p < 0.05$).
Conclusions: The results of our study suggest that interleukin-6 plays a considerable role in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in oestrogen-deficient women. **(Pol J Endocrinol 2011; 62 (4): 299–302)**

Key words: *IL-6, IL-6sR, hyperthyrosis, osteoporosis, menopause*

Streszczenie

Wstęp: Nadczynność tarczycy występuje częściej u kobiet po menopauzie w porównaniu z populacją ogólną, skutecznie przyspieszając obrót kostny. Wykazano, że interleukina 6 (IL-6) odgrywa istotną rolę w regulacji obrotu kostnego. Uwzględniając ten fakt, celem obecnej pracy była próba oceny roli IL-6 i jej rozpuszczalnego receptora w patogenezie zaburzeń obrotu kostnego w przebiegu tyreotoksykozy u kobiet po menopauzie.
Materiał i metody: Badanie przeprowadzono u 40 osób z nadczynnym wolem guzkowym w 3 grupach: 1 — 13 kobiet przed menopauzą w wieku 36 ± 15 lat (PremTx→PremEu), 2 — 12 kobiet po menopauzie w wieku 66 ± 14 lat (PostTx→PostEu) i 3 — 15 mężczyzn w wieku 45 ± 21 lat (MTx→MEu). Stan czynnościowy tarczycy potwierdzono oznaczeniem TSH, fT3 i fT4 w surowicy. Markery obrotu kostnego: TRACP5b i osteokalcyna oraz IL-6 i IL-6sR w surowicy, oznaczono zestawami ELISA.
Wyniki: Iloraz TRACP5b/osteokalcyna był istotnie zwiększony u kobiet PostTx w porównaniu z grupą PremTx ($p < 0,02$). Stwierdzono dodatnią korelację między TRACP5b i osteokalcyną ($R = 0,45$, $p < 0,001$). Stężenie IL-6 było istotnie zwiększone w grupie PostTx: 3,0 (2,14–6,40) i MTx: 2,24 (1,60–5,10) w porównaniu z odnotowanym u kobiet z grupy PremTx: 1,39 (0,96–2,14) (odpowiednio: $p < 0,01$ i $p < 0,05$). Wykazano istotną dodatnią korelację pomiędzy IL-6 i IL-6sR ($R = 0,22$, $p < 0,05$) oraz pomiędzy IL-6sR i TRACP5b ($R = 0,23$, $p < 0,05$).
Wnioski: Podsumowując, wyniki obecnej pracy wskazują, że IL-6 odgrywa ważną rolę w patogenezie zaburzeń obrotu kostnego w przebiegu tyreotoksykozy u kobiet po menopauzie. **(Endokrynol Pol 2011; 62 (4): 299–302)**

Słowa kluczowe: *IL-6, IL-6sR, nadczynność tarczycy, osteoporoza, menopauza*

Introduction

The early years of the menopause are usually characterised by a loss of bone mass that leads to reduced skeletal strength and increased susceptibility to fractures. Thyrotoxicosis is more frequent in women of postmenopausal age than in the general population, effectively accelerating bone turnover. In thyrotoxicosis, the duration of remodelling cycles is reduced, with maintained duration of resorption and shortened

Janusz Mysliwiec, MD, PhD, Department of Endocrinology, Diabetology and Internal Diseases, Medical University of Bialystok, ul. M. Sklodowskiej-Curie 24 A, 15–276 Bialystok, Poland, tel: +48 (48) 85 746 82 39, fax: +48 (85) 744 76 11, e-mail: janusz.mysliwiec@umwb.edu.pl

formation, resulting in a loss of about 10% of mineralised bone per cycle [1].

Oestrogen depletion in the menopause is accompanied by an increased production of osteotrophic factors, such as interleukin-1 (IL-1), IL-6 and tumour necrosis factor [2, 3]. Those cytokines stimulate the differentiation of myeloid precursor cells into osteoclasts, cells that are responsible for bone resorption [4].

Interleukin-6 has been shown to be involved in the pathogenesis of several bone diseases characterised by a negative balance between bone resorption and formation, such as Paget's disease, renal osteodystrophy and postmenopausal osteoporosis [5–7]. Moreover, in ovariectomised mice, IL-6 appeared to be crucial in bone turnover acceleration and a selective inhibitor of IL-6 activity has been documented to suppress bone mass reduction [8, 9]. Recently, we have seen experimental data suggesting an important role of IL-6 in thyrotoxicosis-related disturbances in bone turnover [10].

Interleukin-6 exerts its full effect through binding to both of its glycoprotein receptors present on target cells: IL-6R and gp130. A complex of IL-6 and a soluble form of IL-6R (IL-6sR) cleaved from the cell membrane may connect to membrane gp130 that leads to biological action [11].

This current study was an attempt to assess the role of IL-6 and its soluble receptor in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in oestrogen-deficient women.

Material and methods

The study was carried out in forty subjects with toxic nodular goiter divided into three groups:
Group 1 — 13 premenopausal females, mean age 36 ± 15 years (PremTx→PremEu);
Group 2 — 12 postmenopausal females, mean age 66 ± 14 years (PostTx→PostEu);
Group 3 — 15 males, mean age 45 ± 21 years (MTx→MEu).

Overt thyrotoxicosis and euthyreosis after treatment with thyrostatics was confirmed by thyrotropin, free thyroxine and free triiodothyronin concentrations. No acute infections were observed in the studied subjects three weeks prior to the study.

Serum was collected before treatment and in stable euthyrosis state and kept frozen at minus 70°C until used. The serum levels of bone turnover markers: osteoclast-derived tartrate-resistant acid phosphatase form 5b (TRACP5b) and osteocalcin were determined by enzyme-linked immunosorbent assay commercial kits produced by Immunodiagnostics Systems Ltd, Boldon, UK: TRACP5b (BoneTRAP Assay; sensitivity 0.5 U/l; intra-assay precision 6.6%; inter-assay precision 7.2%), osteocalcin (N-MID Osteocalcin ELISA; sensitivity 0.5 ng/ml; intra-assay precision 4.2%; inter-assay precision 2.2%) and BAP (OstaseBAP; sensitivity 0.5 ng/ml; intra-assay precision 4.2%; inter-assay precision 2.2%). The serum levels of IL-6 and its soluble receptor (IL-6sR) were estimated using R&D Systems, Minneapolis, MN, USA commercial kits: IL-6 (Quantikine; sensitivity 0.7 pg/ml; intra-assay precision 2.0%; inter-assay precision 3.8%) and IL-6sR (Quantikine; sensitivity 1.5 pg/ml; intra-assay precision 2.3%; inter-assay precision 4.7%).

The statistical significance was estimated by Mann-Whitney test. To evaluate relationships between variables, Spearman's test was performed using Statistica 9.0 for Windows XP (StatSoft, Tulsa, OK, USA).

Results

Figure 1 shows medians, interquartiles and total ranges of the quotient of serum concentrations of TRACP5b (U/L) and osteocalcin (ng/ml) in examined groups. TRACP5b/osteocalcin quotient was significantly elevated in PostTx females compared to PremTx females ($p < 0.02$). TRACP5b and osteocalcin concentrations analysed separately were not significantly different between groups. All TRACP5b and osteocalcin results in medians and interquartiles are given in Table I. There was a positive correlation between serum TRACP5b and osteocalcin in the studied patients ($R = 0.45$, $p < 0.001$) (Figure 2).

Interleukin 6 serum concentration (in pg/ml) is shown as median and interquartile values in Figure 3. Levels of serum IL-6 values were significantly elevated

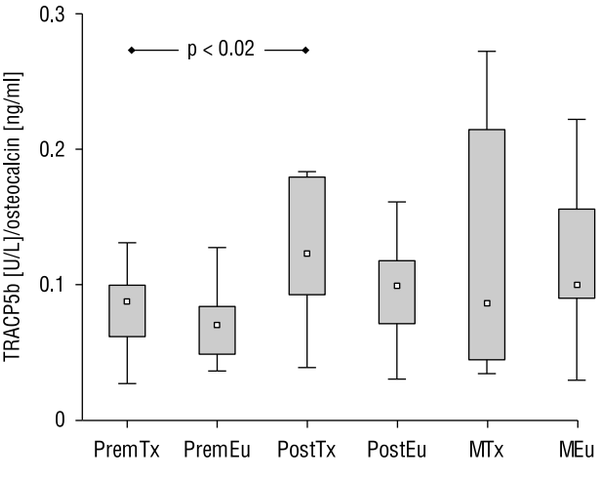

Figure 1. *Medians, total and interqurtiles ranges of the quotient of serum concentrations of TRACP5b (U/L) and osteocalcin (ng/ml) in premenopausal and postmenopausal females as well as in males — in thyrotoxicosis and in euthyrosis*

Rycina 1. *Mediany, zakresy całkowite i międzykwartylowe ilorazu stężeń surowiczych TRACP5b (U/L) i osteokalcyny (ng/ml) u kobiet przed menopauzą i po niej oraz u mężczyzn — w tyreotoksykozie i w eutyreozie*

Table I. *Medians and interquartile ranges of TRACP5b and osteocalcin serum concentrations in premenopausal and postmenopausal females, as well as in males, in thyrotoxicosis and in euthyrosis*

Table I. *Mediany i zakresy międzykwartylowe TRACP5b i osteokalcyny surowiczych stężeń u kobiet przed menopauzą i po niej oraz u mężczyzn — w tyreotoksykozie i w eutyreozie*

	PremTx	PremEu	PostTx	PostEu	MTx	MEu
TRACP5b [U/L]	1.56 1.24–1.07	1.84 1.46–2.08	1.82 1.4–2.74	2.20 1.68–2.64	2.28 1.32–3.51	1.81 1.25–2.45
Osteocalcin [ng/ml]	23.6 16.0–28.6	20.7 13.3–32.9	15.0 11.9–40.5	25.7 20.6–32.9	19.4 10.1–33.0	13,6 9.0–23.2

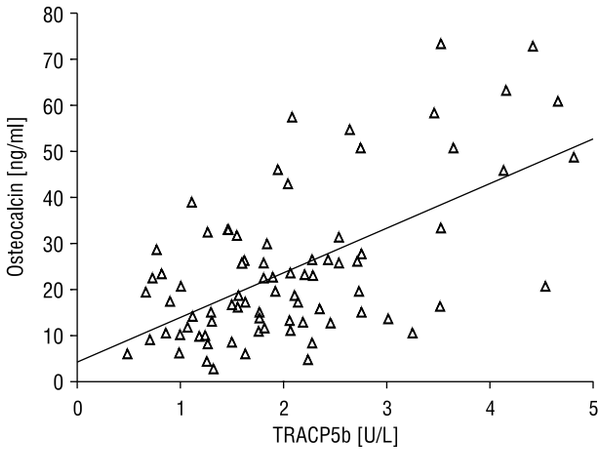

Figure 2. *Positive correlation between serum TRACP5b and osteocalcin (R = 0.45, p < 0.001)*

Rycina 2. *Dodatnia korelacja pomiędzy stężeniem TRACP5b i osteokalcyny (R = 0,45, p < 0,001)*

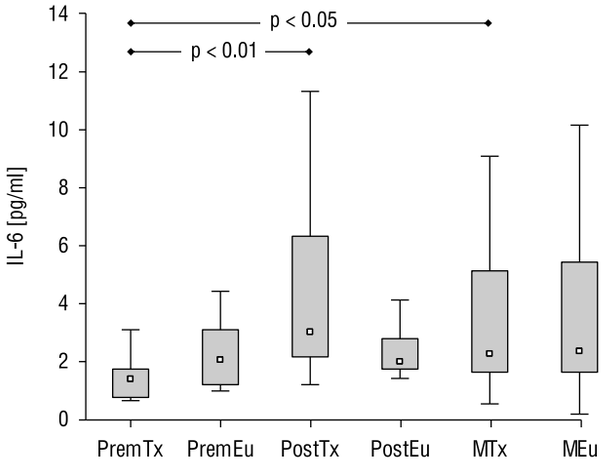

Figure 3. *Medians, total and interqurtiles ranges of IL-6 (pg/ml) serum concentrations in premenopausal and postmenopausal females as well as in males- in thyrotoxicosis and in euthyrosis*

Rycina 3. *Mediany, zakresy całkowite i międzykwartylowe stężeń surowiczych IL-6 (pg/ml) u kobiet przed menopauzą i po niej oraz u mężczyzn — w tyreotoksykozie i w eutyreozie*

in PostTx: 3.0 (2.14–6.40) and MTx: 2.24 (1.60–5.10) compared to PremTx females: 1.39 (0.96–2.14) ($p < 0.01$ and $p < 0.05$ respectively). In PremEu, IL-6 serum concentrations were 2.14 (1.20–3.40), in PostEu: 2.4 (1.8–3.8) and in MEu: 3.58 (1.60–6.50). Interleukin-6 soluble receptor levels were not significantly different in the studied groups. In PremTx, IL-6sR level was 45.0 (38.3–52.8), in PremEu 47.9 (40.4–54.9), in PostTx 48.6 (42.8-59.1), in PostEu 48.4 (40.8–55.8), in MTx 46.1 (41.2–49.2) and in MEu 50.9 (41.3–57.5).

There were significant positive correlations between IL-6 and IL-6sR concentrations ($R = 0.22$, $p < 0.05$) and between IL-6sR and TRACP5b serum levels ($R = 0.23$, $p < 0.05$).

Discussion

In the present study, significantly elevated TRACP5b/osteocalcin quotient in postmenopausal thyrotoxic females in comparison with premenopausal women may reflect bone resorption overbalance as far as TRACP5b predominances over osteocalcin-established bone formation factor. Accelerated bone resorption is accompanied by proportional bone formation increase, as shown by a positive correlation between serum TRACP5b and osteocalcin values in the present study. Taking this into account, these results suggest that bone reconstruction does not keep up with accelerated bone resorption in estrogen deficient females burdened with thyrotoxicosis. Although thyroid hormones are necessary for normal skeletal growth, their excess leads to accelerated bone turnover, diminished bone density and increased risk of fractures [12, 13]. Thyrotoxicosis is accompanied by 12-15% reduction of bone mineral density, predominantly of cortical bone, and in consequence greatly increases the risk for hip fracture, especially in postmenopausal women [14]. Bone formation and resorption markers have been shown to be elevated in patients with hyperthyroidism and significant correlations have been observed between bone metabolism indicators and plasma thyroid hormones levels [15, 16]. In experimental thyrotoxicosis model bone formation markers are additionally increased owing to

triiodotyronine, that has been implicated in the enhanced production of osteocalcin, alkaline phosphatase and I collagen [17]. Osteocalcin is an osteoblast product that constitutes the most abundant noncollagenous protein present in bone and its serum levels closely correlate with histomorphometric parameters of bone formation [18].

The precise mechanism by which thyroid hormones regulate osteoblasts and osteoclasts function at a cellular level remains unclear. Osteoblasts were suggested to mediate the action of triiodotyronine on osteoclastic bone resorption releasing osteotrophic cytokines [19, 20]. The results of the studies carried out on osteoblast cell cultures have shown that triiodotyronine induces interleukin-6 expression [21, 22]. Our previous experimental data suggest that IL-6 plays a crucial role in thyrotoxicosis-related disturbances of bone turnover in mice, determining the imbalance between bone resorption and bone formation caused by excess of thyroid hormones, predominantly by inhibition of bone formation [10]. However, data from in vivo studies on the possible role of IL-6 in the pathogenesis of disturbances of bone formation thyrotoxicosis-related remain equivocal [23, 24].

In the present study levels of serum IL-6 values were significantly elevated in estrogen-deficient thyrotoxic females (and thyrotoxic men) in comparison to premenopausal women. Moreover significant positive correlations of IL-6sR levels and both: IL-6 and TRACP5b serum levels may suggest that IL-6 exerts its effect via its soluble receptor and that consequence of this interaction is bone resorption augmentation. IL-6 may exert its inhibitory effect on bone formation directly through gp130-STAT 1/3 signaling or indirectly by influencing balance between osteoprotegerin (OPG) and receptor activator of nuclear factor B (RANK) and its ligand (RANKL) [25, 26].The influence of IL-6 on RANK-RANKL/OPG was suggested to be more powerful effect leading to increased bone mass loss [27, 28]. The results of our recent experimental study suggest that IL-6 plays a key role in stimulation of RANKL-RANK/OPG system and this effect is strongly enhanced in conditions of accelerated bone turnover such as thyrotoxicosis especially when accompanied by another factor that enforces bone resorption: estrogen depletion [29].

To sum up, the results of the present study suggest that interleukin-6 plays a considerable role of in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in estrogen-deficient women.

References

1. Murphy E, Williams GR. The thyroid and the skeleton. Clin Endocrinol 2004; 61: 285–298
2. Manolagas SC, Jilka RL Bone marrow, cytokines and bone remodelling. N Engl J Med 1995; 332: 305–311
3. Pacifici R. Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. J Bone Miner Res 1996; 8: 1043–1051
4. Roodman GD. Paget's disease and osteoclast biology. Exp J Hematol 1996; 27: 1229–1241
5. Neale SD, Schulze E, Smith R, Athanasou NA. The influence of serum cytokines and growth factors on osteoclast formation in Paget's disease. Q J Med 2002; 95: 233–240
6. Sanchez MC, Bajo MA, Selgas R, Mate A, Sanchez-Cabezudo MJ, Lopez-Barea F, Esbrit P, Martinez ME. Cultures of human osteoblastic cells from dialysis patients: influence of bone turnover rate on in vitro selection of interleukin-6 and osteoblastic cell makers. Am J Kidney Dis 2001, 37: 30–37
7. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE and Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C- reactive protein and markers of bone resorption in postmenopausal women. J Clin Endocrinol Metab 2003; 88: 255–259
8. Poli V, Balena R, Fattori E et al. Costantini F. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. EMBO J 1994; 13: 1189–1196
9. Hayashi M, Rho MC, Enomoto A et al. Suppression of bone resorption by madindoline A, a novel nonpeptide antagonist to gp130. Proc Natl Acad Sci USA 2002; 12: 14728–14733.
10. Mysliwiec J, Zbucki R, Winnicka M et al. A crucial role of interleukin-6 in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in mice. Hormon Metab Res 2007; 39: 1–5.
11. Montero-Julian FA. The soluble IL-6 receptors: serum levels and biological function. Cell Mol Biol 2001; 47: 583.
12. Karga H, Papapetrou PD, Korakovouni A et al. Bone mineral density in hyperthyroidism. Clin Endocrinol (Oxf) 2004; 61: 466–472.
13. Vestergaard P, Mosekilde L. Fractures in patients with hyperthyroidism and hypothyroidism: a nationwide follow-up study in 16249 patients. Thyroid 2002; 12: 411–419.
14. Baldini M, Gallazzi M, Orsatti A et al. Treatment of benign nodular goitre with mildly suppressive doses of L-thyroxine: effects on bone mineral density and on nodule size. J Intern Med 2002; 251: 407–414.
15. Fujikawa M, Kamihira K, Sato K et al. Elevated bone resorption markers in a patient with hypercalcemia associated with post-partum thyrotoxicosis and hypoadrenocorticism due to pituitary failure. J Endocrinol Invest 2004; 27: 782–787.
16. Pantazi H, Papapetrou PD. Changes in parameters of bone and mineral metabolism during therapy for hyperthyroidism. J Clin Endocrinol Metab 2000; 85: 1099–1106.
17. Varga F, Spitzer S, Rumpler M, Klaushofer K. 1,25-Dihydroxyvitamin D3 inhibits thyroid hormone-induced osteocalcin expression in mouse osteoblast-like cells via a thyroid hormone response element. J Mol Endocrinol 2003; 30: 49–57.
18. Kousteni S, Chen JR, Bellido T et al. Reversal of bone loss in mice by nongenotropic signaling of sex steroids. Science 2002; 298: 843–846.
19. Siddiqi A, Monson JP, Wood DF, Besser GM, Burrin JM. Serum cytokines in thyrotoxicosis. J Clin Endocrinol Metab 1999; 84: 435–439.
20. Takai S, Tokuda H, Hanai Y, Kozawa O. Phosphatidylinositol 3-kinase/Akt plays a part in Tumor Necrosis Factor induced interleukin-6 synthesis in osteoblasts. Horm Metab Res 2006; 38: 563–569.
21. Siddiqi A, Burrin JM, Wood DF, Monson JP. Tri-iodothyronine regulates the production of interleukin-6 and interleukin-8 in human bone marrow stromal and osteoblast-like cells. J Endocrinol 1998; 157: 453–461.
22. Kim CH, Kim HK, Shong YK. Thyroid hormone stimulates basal and interleukin (IL)-1-induced IL-6 production in human bone marrow stromal cells: a possible mediator of thyroid hormone-induced bone loss. J Endocrinol 1999; 160: 97–102.
23. Lakatos P, Foldes J, Horvath C et al. Serum interleukin-6 and bone metabolism in patients with thyroid function disorders. J Clin Endocrinol Metab 1997; 82: 78–81.
24. Akalin A, Colak O, Alatas O, Efe B. Bone remodelling markers and serum cytokines in patients with hyperthyroidism. Clin Endocrinol (Oxf) 2002; 57: 125–129.
25. O'Brien CA, Jilka RL, Fu Q, Stewart S, Weinstein RS, Manolagas SC. IL-6 is not required for parathyroid hormone stimulation of RANKL expression, osteoclast formation, and bone loss in mice. Am J Physiol Endocrinol Metab 2005; 289: E784–E793.
26. Sims NA, Jenkins BJ, Quinn JM et al. Glycoprotein 130 regulates bone turnover and bone size by distinct downstream signaling pathways. J Clin Invest 2004; 113: 379–389.
27. O'Brien CA, Gubrij I, Lin SC, Saylors RL, Manolagas SC. STAT3 activation in stromal osteoblastic cells is required for induction of the receptor activator of NF-B ligand and stimulation of osteoclastogenesis by gp130-utilizing cytokines or interleukin-1 but not 1,25-dihydroxyvitamin D3 or parathyroid hormone. J Biol Chem 1999; 274: 19301–19308.
28. Liu XH, Kirschenbaum A, Yao S, Levine AC. Interactive effect of interleukin-6 and prostaglandin E2 on osteoclastogenesis via the OPG/RANKL/RANK system. Ann N Y Acad Sci 2006; 1068: 225–233.
29. Mysliwiec J, Zbucki R, Nikolajuk A et al. Estrogens modulate RANKL-RANK/ /osteoprotegerin mediated Interleukin-6 effect on thyrotoxicosis-related bone turnover in mice. Horm Metab Res 2011; 43: 236–240.