Serum osteoprotegerin correlates with age and bone mass in postmenopausal, but not in fertile age women

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Summary

Purpose of investigation: Osteoprotegerin (OPG) and receptor activator of nuclear factor κB ligand (RANKL) are bone turnover modulators expressed by osteoblasts. The aim of this study was to assess the relationship between the circulating OPG/RANKL system, age and bone mass, in fertile age and postmenopausal women. *Methods*: In this cross-sectional observational study on 48 patients (fertile age, n = 22; postmenopause, n = 26), we investigated the correlation between serum OPG and RANKL, age and bone mineral density (BMD). Serum concentrations of OPG and RANKL were determined by enzyme-linked immunosorbent assay (ELISA); estimate BMD evaluation was performed with heel quantitative ultrasonometry (QUS). *Results:* Serum OPG significantly increased ($p = 0.003$) and serum RANKL significantly decreased ($p = 0.002$), in the postmenopausal group compared to fertile age women. A significant correlation of serum OPG with age ($r_S = 0.39$, $p = 0.047$) and BMD ($r_S = 0.45$, $p = 0.023$) in postmenopausal women, and between RANKL and BMD ($r_S = 0.48$, $p = 0.024$) in fertile age was found. *Conclusion:* These data demonstrate in vivo that the OPG/RANKL system is significantly associated with menopausal status and could play a role in postmenopausal osteoporosis.

Key words: Osteoprotegerin; RANKL; Menopause; Osteoporosis.

Introduction

The decrease in estrogen circulating levels during menopausal hormonal switch represents the most important cause of bone loss, with a predominance of osteoclast mediated resorption and high incidence of osteoporosis [1, 2].

The bone mass balance is under estrogen influence because of double activity: one, direct, mediated by receptors present on the bone cell [3-5], the other, indirect and delayed, modulating calcium metabolism at the level of the intestine, kidneys and parathyroids [6]. Nevertheless, estrogens prevent bone loss also by regulating several cytokines that modulate osteoclastic bone resorption, including interleukin-1β (IL-1β), interleukin-6 (IL-6), osteoprotegerin (OPG), and receptor activator of nuclear factor κB (RANK) ligand (RANKL) by cells of osteoblastic lineage [7].

OPG is a soluble glycoprotein, expressed by osteoblasts, that acts as a decoy receptor for RANKL blocking the process of osteoclast differentiation and modulating the apoptosis process in these cells; moreover, OPG is a negative regulator of osteoclast mediated bone resorption [8, 9]. The role of the OPG/RANKL system in physiological bone remodelling has been well characterised [10-12], and in vitro and in vivo studies have demonstrated that estrogens and raloxifene prevent bone loss also by stimulating OPG production by osteoblasts [4, 5, 13, 14]. On the other hand, the most recent literature review reports conflicting results on the correlation of serum levels of OPG and RANKL with age, menopausal status and bone mineral density (BMD) [15-17].

The aim of this study was to assess the relationship, if one exists, between serum OPG and RANKL levels, age and bone mass in fertile age and postmenopausal women to have a better definition of the role of the OPG/RANKL system in postmenopausal osteoporosis.

Patients and Methods

Study groups. A cohort of consecutive women (n = 48) referred to the Fondazione IRCCS SDN of Naples were enrolled for this cross-sectional study and divided into two groups: group F (fertile age women, n = 22), group M (postmenopausal women, n = 26).

The patients were selected according to the following inclusion criteria: age ≥ 30 years for group F, clinical and hormonal diagnosis of postmenopause for group M (serum estradiol levels < 110 pmol/l, serum FSH levels > 30 IU/l). Exclusion criteria were: body mass index (BMI) ≥ 30, bone disorders except osteoporosis, use of hormone replacement therapy (HRT) or other bone-active agents less than six months before enrollment.

Biochemical measurements. Serum levels of OPG and RANKL and BMD were measured on the entire study population. OPG/RANKL ratio was also calculated.

Serum OPG and RANKL evaluation was performed with a blood sample obtained by venipuncture, immediately separated and stored at –20°C. Serum levels were measured by enzyme-linked immunosorbent assay (ELISA) as pmol/l (Human Osteoprotegerin ELISA, Human sRANKL ELISA; Biovendor, Brno, Czech Republic): detection limit 0.13 pmol/l, intra- and interassay coefficients of variation (CVs) 4.5% and 5.5%, respectively, for OPG; detection limit 0.2 pmol/l, intra- and interassay CVs 8.70% and 9.19%, respectively, for RANKL.

BMD estimate was performed with a heel quantitative ultrasonometry (QUS) (Sahara; Hologic, Bedford, USA) measured on the nondominant foot. Ultrasound frequency of 0.6 MHz was used to measure estimated BMD as g/cm^2 and speed of sound (SoS) as m/s: CVs 3% for BMD (absolute precision 0.014 g/cm^2) and 0.22% for SoS (absolute precision 3.4 m/s).

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Statistical analysis. All values are depicted as the mean values ± standard deviation (SD). Due to the nonparametric data and small population, the statistical significance was calculated by the Mann-Whitney test and the correlation analysis was performed using the Spearman correlation test (coefficient r_s). The value $p < 0.05$ was regarded as significant.

Results

The mean age in group F was 43.3 ± 7.2 years and the BMI 25.4 ± 4.5 kg/m^2; in group M, the mean age was 60.6 ± 6.7 years and the BMI 28.0 ± 3.6 kg/m^2 ($p < 0.05$).

Serum levels of OPG and RANKL achieved the detection level in all assays with the following mean values: serum OPG was 3.66 ± 1.57 pmo/l in group F and significantly increased to 4.60 ± 1.19 pmo/l in group M (p = 0.003); serum RANKL was 317.07 ± 177.64 pmo/l in group F and significantly decreased to 181.78 ± 88.41 pmo/l in group M (p = 0.002). Concerning bone assessment, the QUS estimated BMD was 0.555 ± 0.127 g/cm^2 and 0.449 ± 0.135 g/cm^2 in group F and M, respectively (p = 0.002). A complete description of characteristics of the subjects and clinical findings was reported in Table 1.

Correlations between OPG and RANKL serum levels, age and BMD are fully reported in Tables 2 and 3: a significant correlation of serum OPG with age (r_s = 0.39, p = 0.047) and BMD (r_s = 0.45, p = 0.023) in postmenopausal women (Figures 1 and 2), and between RANKL and BMD (r_s = 0.48, p = 0.024) in fertile age was found. No other significance was achieved in the correlation between circulating OPG and RANKL, age and BMD in the two study groups (Tables 2-3).

Table 1. — *Characteristics of the subjects and clinical findings by study group (mean ± S.D.).*

	Group F (n = 22)	Group M (n = 26)	p value
Ages (year)	43.3 ± 7.2	60.6 ± 6.7	< 0.001
BMI (kg/m^2)	25.4 ± 4.5	28.0 ± 3.6	0.012
Age of menopause (years)	–	49.2 ± 3.8	–
Duration of menopause (years)	–	11.5 ± 8.8	–
Serum OPG (pmol/l)	3.66 ± 1.57	4.60 ± 1.19	0.003
Serum RANKL (pmol/l)	317.07 ± 177.64	181.78 ± 88.41	0.002
OPG / RANKL Ratio	0.015 ± 0.010	0.033 ± 0.026	< 0.001
QUS estimate BMD (g/cm^2)	0.555 ± 0.127	0.449 ± 0.135	0.002
QUS SoS (m/s)	1556.1 ± 36.5	1528.0 ± 34.1	0.002

BMI, body mass index; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor κB ligand; QUS, quantitative ultrasonometry; BMD, bone mineral density; SoS, speed of sound.

Table 2. — *Spearman correlation between OPG and RANKL serum levels and age by study group.*

	Group F (n = 22)		Group M (n = 26)	
	r value	p value	r value	p value
Serum OPG	+0.31	0.159	+0.39	0.047
Serum RANKL	–0.07	0.766	–0.00	0.995
OPG / RANKL ratio	+0.21	0.340	+0.17	0.395

OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor κB ligand.

Table 3. — *Spearman correlation between OPG and RANKL serum levels and estimate BMD by study group.*

	Group F (n = 22)		Group M (n = 26)	
	r value	p value	r value	p value
Serum OPG	+0.10	0.658	+0.45	0.023
Serum RANKL	+0.48	0.024	+0.13	0.522
OPG / RANKL ratio	–0.32	0.146	+0.09	0.649

OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor κB ligand; QUS, quantitative ultrasonometry; BMD, bone mineral density.

Discussion

An increase in active osteoclast pool size, with increased bone resorption and decreased bone mass, occurs in many osteopathic disorders, including postmenopausal osteoporosis. Estrogen decrease of menopausal transition inhibits mature osteoblasts and osteocytes and promotes osteoblastic apoptosis [18]. Moreover, this hormonal deficit interferes with osteoclastic lineage increasing its recruitment and activation due to the stimulation of osteoblast cytokines release: IL-1, IL-6, TNF-α [19, 20]. Interference of osteoclastic activity acts also through a reduction of TGF-β and OPG production, and the identification in the mid to late 1990s of the OPG/RANKL system clarified the role played by osteoblasts in osteoclastogenesis [21-26].

The importance of OPG in bone metabolism is suggested by the positive correlation between gene production of OPG [9] and bone mass increase in animal models [1, 27]. In humans, OPG seems to be implicated in the pathogenesis of postmenopausal osteoporosis [28] and other metabolic diseases characterized by bone loss [29], but the link between OPG levels, menopausal status and BMD is still under debate.

There is general agreement that OPG levels increase after menopause [10, 30-34], even if other studies showed no significant differences among premenopausal, postmenopausal, or estrogen-replete postmenopausal women [35].

Some authors state that circulating OPG levels reflect the antiresorptive activity in bone [10], and serum OPG levels have been found to be positively related to BMD and negatively related with bone resorption in postmenopausal women [32, 36, 37], even if increases in conditions of postmenopausal osteoporosis have been reported with a negative correlation to BMD [28, 30, 33, 38, 39]; unrelated to BMD for other authors [31, 40]. In 382 healthy postmenopausal women, circulating levels of OPG were significantly associated with age, and, at multivariate analysis, age and OPG were the independent determinants for BMD [41]. In a population-based study of 2,134 women, BMD was negatively associated with OPG at baseline, and in postmenopausal women not on hormone replacement therapy, bone loss increased with increasing OPG, whereas no relationship was found in men, premenopausal women, or postmenopausal women on HRT [39].

Serum RANKL levels have also been investigated in postmenopausal women and reported not to be associated with menopausal status, HRT or BMD [32, 35, 42].

Fig. 1

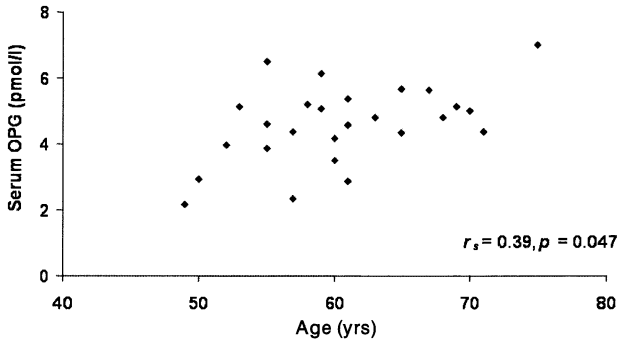

Fig. 2

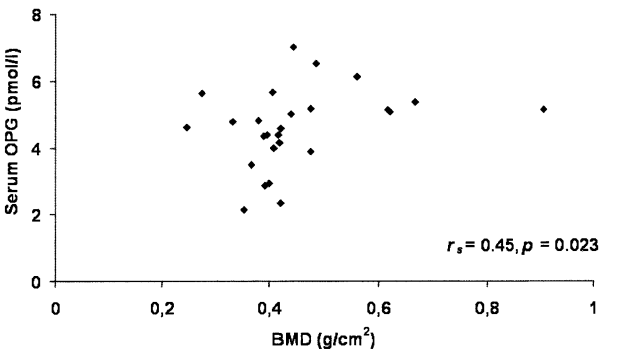

Figure 1. — Spearman correlation between OPG serum levels and age in the postmenopausal group (n = 26).
Figure 2. — Spearman correlation between OPG serum levels and estimate BMD in the postmenopausal group (n = 26).

These contradictory findings may relate to differences in study design, methodology, or other aspects influencing the measurements of these factors. Moreover, circulating OPG levels are dependent on several factors and paracrine mechanisms involved in osteoporosis genesis and on non-skeletal sources [19, 30, 43].

In our experience, the comparison of a postmenopausal group with a control group of fertile age women showed a significant variation in OPG (increase) and RANKL (decrease) serum levels (p = 0.003 and p = 0.002, respectively), according to other evidence [10, 30, 34] and to the hypothesis that elevation of serum OPG along with age might be a compensatory mechanism against accelerated bone resorption, while decreased levels of serum RANKL might be helpful for restoring reduced bone formation [44]. Moreover, our data revealed a significant correlation of serum OPG with age (p = 0.047) and BMD (p = 0.023) in postmenopausal women, and between RANKL and BMD (p = 0.024) in fertile age, while no other significance was achieved in the correlation between circulating OPG and RANKL, age and BMD in either study group. Then, although limited by a small population, this comparative study shows that serum OPG correlates with age and bone mass in postmenopausal women compared to the fertile age group; these findings are in accord with other studies about correlation with age [10, 30, 43, 45] and BMD [32, 36, 37], although in other experiences a significant association between serum OPG and bone mass was not found [30, 43, 45].

BMD has been measured with an ultrasound method, heel QUS measured on the nondominant foot [46-49], not considered as the gold standard in the diagnosis of postmenopausal osteoporosis, but able to estimate bone density decreases and predict the risk of fractures in respect to the conventional DXA technique [50-55].

Conclusion

In conclusion, our results confirm the hypothesis that the OPG/RANKL system plays a role in bone resorption and menopausal status probably modulates this role. In particular, these findings might improve the definition of the role of OPG/RANKL system in postmenopausal osteoporosis, opening a therapeutic awareness and application of OPG-modulating drugs [56]: OPG-inducing drugs, as estrogens [57], raloxifene [14], bisphosphonates [58], strontium ranelate [59], teriparatide [60]; OPG-mimicking drugs (anti-RANKL drugs), as recombinant OPG [61] and monoclonal antibody denosumab [62].

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