

# IMPLICATIONS OF OSTEOPROTEGERIN IN POSTMENOPAUSAL OSTEOPOROSIS

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## ABSTRACT

**Objectives:** To examine the association between circulating levels of osteoprotegerin (OPG) and ageing, estradiol, bone turnover, bone density in women with postmenopausal osteoporosis.

**Material and methods:** The study was performed on a group of 107 women with postmenopausal osteoporosis and 28 controls. Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry. The study protocol consisted in baseline evaluation of bone mineral density, serum levels of OPG, estradiol, and of the biochemical markers of bone turnover (DPD, osteocalcin).

**Results:** We obtained a significant positive correlation between serum OPG and age. There was a significant negative correlation between the serum OPG levels, BMD and T score in postmenopausal women with osteoporosis. There was a significantly positive correlation between OPG levels and biochemical markers of bone turnover in postmenopausal women with osteoporosis. The correlation between serum OPG levels and estradiol was positive but statistically insignificant.

**Conclusions:** Serum OPG levels increase with age and are higher in postmenopausal women with an increased rate of bone loss. Our study has proved a significant negative correlation between OPG levels and BMD and a positive correlation between OPG and biochemical markers of bone turnover in women with postmenopausal osteoporosis.

**Key Words:** osteoprotegerin, osteoporosis, estradiol, bone, postmenopause

## INTRODUCTION

It is well known that estrogen deficiency is a major contributory factor to postmenopausal osteoporosis. There is a dramatic decrease in the level of circulating estradiol after menopause in all women, although there remains some considerable variation in residual endogenous estradiol levels. Since the introduction of more sensitive and reproducible assays, it became possible to measure the low levels of circulating estradiol in postmenopausal women.

The mechanism of action of estrogen on bone is still not fully understood. There is evidence suggesting that estrogen may suppress the production of

proinflammatory cytokines.<sup>1</sup> Recent in vitro studies have shown that estradiol increases the production of the novel peptide osteoprotegerin (OPG) in human osteoblast-like cells.<sup>2</sup> This may be an important signaling pathway for the action of estrogen on bone.

The discovery of osteoprotegerin in 1997 has enhanced the understanding of the way in which the processes of bone remodeling are regulated.<sup>3,4</sup>

Osteoprotegerin (OPG) is a secreted soluble member of the tumor necrosis factor receptor superfamily (TNFR), also known as osteoclastogenesis inhibitory factor (OCIF).<sup>3,4</sup> The specificity of OPG/OCIF function for inhibiting osteoclast differentiation was illustrated by the phenotype of OPG/OCIF-deficient mice. These mice develop severe osteoporosis due to the increased numbers of osteoclasts.<sup>5</sup>

OPG acts by binding and inactivating a recently discovered ligand of the TNF family, receptor activator of nuclear factor- $\kappa$ B (NF- $\kappa$ B) ligand (RANKL), also known as TNF-related activation-induced cytokine (TRANCE), osteoprotegerin ligand (OPGL), or osteoclast differentiation factor (ODF).<sup>6</sup> RANKL is expressed on osteoblastic and stromal cells, and has been found to be an essential requirement for osteoclast differentiation and activation.<sup>6</sup>

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RANKL binds to a membrane bound receptor in the TNF receptor family on osteoclast precursors and osteoclasts to induce differentiation and activation, respectively. This receptor was originally isolated in dendritic cells and is known as RANK. RANK is identical to the receptor on osteoclasts that binds RANKL and also called osteoclast differentiation and activation receptor (ODAR).<sup>7</sup>

OPG, which can bind to RANKL, acts as a decoy receptor, blocking the interaction between osteoblasts/stromal cells and the osteoclasts precursors, thereby inhibiting osteoclast formation.<sup>8</sup>

The RANKL/RANK/OPG system is involved in various skeletal diseases characterized by increased bone resorption and bone loss, including postmenopausal osteoporosis.

There have been several studies designed to assess the importance of OPG to the skeleton in postmenopausal women with osteoporosis. The results of these studies have been conflicting. In one study, women with osteoporosis were shown to have higher circulating levels of OPG than controls.<sup>9</sup> Another study, has shown no difference between serum OPG levels in osteoporotic versus healthy postmenopausal women.<sup>10</sup> The relationship between serum concentrations of endogenous OPG and bone turnover is uncertain, with different studies yielding different results. A relationship between circulating OPG and estradiol has not yet been established.

## **OBJECTIVES**

To examine the association between circulating levels of OPG and ageing, estradiol, bone turnover, bone density in women with postmenopausal osteoporosis.

## **MATERIAL AND METHODS**

The patients selected for this study have been evaluated at the National Centre of Clinical and Biological Research in Osteoporosis from the C.I. Parhon Endocrinological Institute in Bucharest.

The study was performed on a group of 107 women with postmenopausal osteoporosis and 28 postmenopausal controls. From the 107 women with postmenopausal osteoporosis, 31 presented severe osteoporosis with fractures.

The study protocol consisted in baseline evaluation of bone mineral density, serum levels of OPG, estradiol, and of the biochemical markers of bone turnover, standard radiographs centered on the lumbar and dorsal spine for the evaluation of the vertebral fractures and deformities in women without any antiresorptive treatments.

Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DEXA) using LUNAR DPX-L (USA) at the antero-posterior lumbar spine (L1-L4). At the spine level we took as reference the values registered for the L2-L4 vertebrae.

The instruments were calibrated on a monthly basis, and control tests were performed daily. The variabilities of the measurements did not exceed the level of 0.5%. The results were expressed as bone mineral density (BMD) in grams of mineral per unit area scanned ( $\text{g}/\text{cm}^2$ ), T and Z scores.

The osteoporosis diagnoses were established according to the recommendations of the World Health Organization (WHO):

- Normal – T score  $> -1$  DS;
- Osteopenia – T score between  $-1$  and  $-2,5$  DS;
- Osteoporosis – T score  $< -2,5$  DS;
- Severe osteoporosis – T score  $< -2,5$  DS and

the presence of one or more osteoporotic fragility fractures.

DS = standard deviation

The fractures diagnosis was established on the basis of the anamnestic data for wrist, upper arm, pelvis and hip.

Serum osteoprotegerin was measured with an ELISA test in “sandwich” system. The normal range was 40-625 pg/ml. The intra- and interassay variabilities were below 10%. This procedure measures dimeric forms of osteoprotegerin.

Serum estradiol was measured by ELISA DSL kit.

Serum osteocalcin was measured with an N-MID™ Osteocalcin One Step ELISA kit with the use of two highly specific monoclonal antibodies against human osteocalcin. This procedure measures both the intact fragments and N-terminal-Mid fragment of osteocalcin. The normal range in postmenopausal women was 9.5-48.3 ng/ml.

The free urinary deoxypyridinols (DPD) were measured by chemoluminescence in automat system (Chyron, ACS). The results were expressed as a ratio of the deoxypyridinols measured per mmol urinary creatinine to avoid the errors due to the variations of urine concentration. The measurement was performed on the second sample of the morning urine to avoid the diurnal variations of this marker (up to 30% during the day). The normal range for premenopausal women was 5-40 nmol/mmol urinary creatinine and for postmenopausal women 10-60 nmol/mmol urinary creatinine.

## **RESULTS**

Table 1 shows descriptive statistics for the subjects taking part in the study.

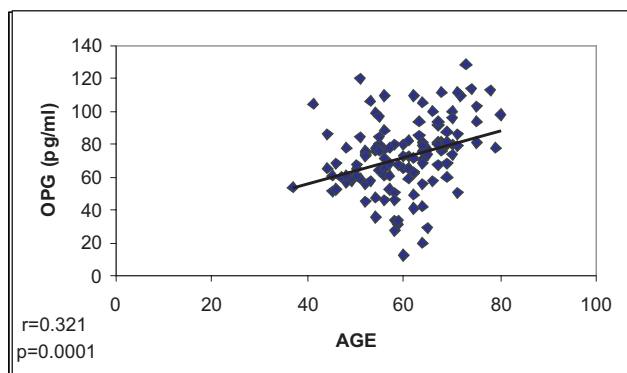
**Table 1.** Characteristics of subjects chosen for the study

Variable	Controls (n=28)	Osteoporosis (n=76)	Fractures (n=31)
Age	55±7	59±8	64±8
BMD(g/cm <sup>2</sup> )	1.184±0.941	0.777±0.115	0.734±0.100
T score	-0.13±0.113	-3.34±0.86	-3.70±0.85
OPG (pg/ml)	65.13±17.29	69.47±22.66	79.94±20.34
Estradiol (pg/ml)	37.98±26.17	27.86±19.53	26.31±15.77
Osteocalcin (ng/ml)	16.54±9.94	30.17±16.45	23.14±10.70
DPD (nmol/mmol creat)	7.05±1.84	8.58±3.94	8.65±3.65

We found different levels of serum OPG between the three groups, being statistically significant between the group with osteoporosis and the group with fractures ( $p=0.02$ ) and between the controls and the group with fractures ( $p=0.004$ ). (Table 1)

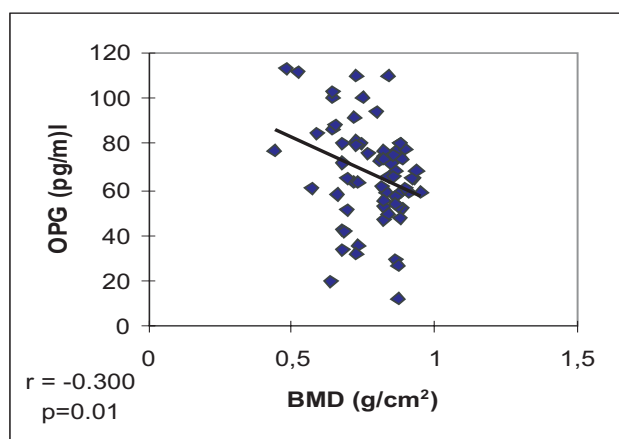
The association between serum OPG levels and age, estradiol, bone turnover and bone density was estimated using linear regression models.

We obtained a significant positive association between serum OPG and age in all the three groups (Fig 1).



**Figure 1.** Correlation between OPG and age in all the studied groups

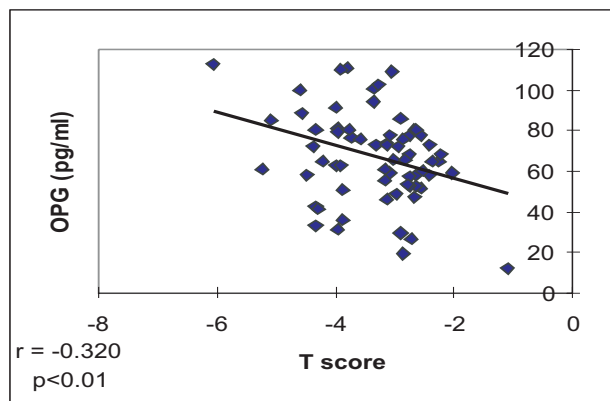
In the osteoporosis group there was proved a significant negative correlation between the serum OPG levels, BMD and T score (Fig. 2, 3).



**Figure 2.** Correlation between OPG and BMD in the osteoporosis group

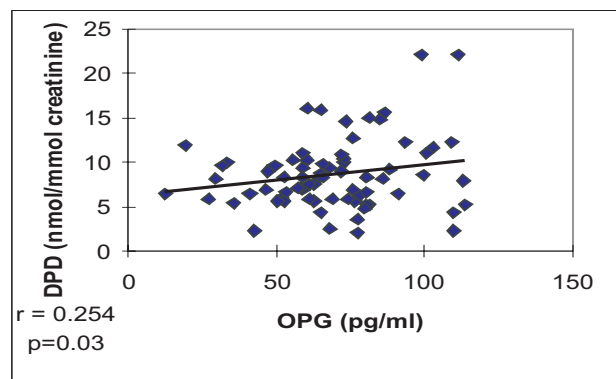
In the osteoporosis group we obtained a significant positive correlation between OPG levels and bioche-

mical markers of bone turnover (DPD, osteocalcin) (Fig. 4, 5).

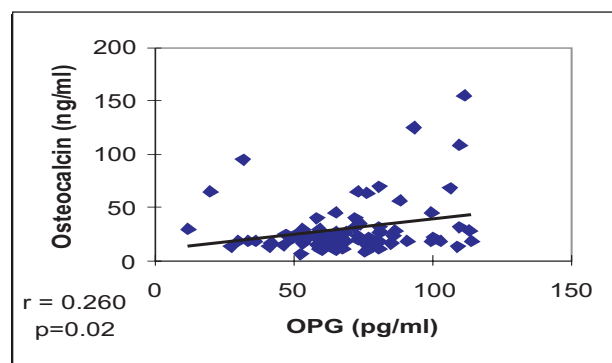


**Figure 3.** Correlation between OPG and T score in the osteoporosis group

The correlation between serum OPG levels and estradiol was positive but statistically insignificant in the three studies groups.



**Figure 4.** Correlation between OPG and DPD in the osteoporosis group



**Figure 5.** Correlation between OPG and osteocalcin in the osteoporosis group

## DISCUSSION

It has been suggested that circulating OPG levels are higher in osteoporotic women compared with controls, and that this occurs as a protective mechanism to slow down the increased bone resorption and subsequent bone loss seen in osteoporosis.<sup>9,11</sup> Our results do not appear to endorse this observation.

Serum levels of OPG have been shown to increase with age.<sup>9,12</sup> Rogers et al. found no correlation between age and OPG concentrations.<sup>13</sup> In our study, we obtained a significant increase of OPG levels with ageing in all the three studied groups.

In a study by Yano et al., OPG serum levels were negatively correlated with bone mineral density (BMD) at various sites (lumbar spine, femoral neck and total body) and positively correlated with biochemical markers of bone turnover.<sup>9</sup> Another group which employed a similar design (but a different OPG ELISA system) could not detect a correlation between OPG serum levels and biochemical markers of bone turnover but confirmed the negative correlation of OPG serum concentrations and BMD in postmenopausal women.<sup>11,14</sup> Our study has proved a significant negative correlation between OPG levels and BMD and a positive correlation between OPG and biochemical markers of bone turnover in the group of women with postmenopausal osteoporosis.

There is evidence from *in vitro* studies that estradiol stimulates the expression of OPG.<sup>2</sup> In this study and in the study by Rogers et al. a positive relationship between OPG and serum estradiol levels was observed.<sup>2, 13</sup> In our study, the positive relation between the serum OPG levels and estradiol levels was statistically insignificant. This suggests that estradiol may inhibit osteoclastogenesis by pathways independent of OPG/RANKL.

## CONCLUSIONS

Serum OPG levels increase with age and are higher in postmenopausal women who have an increased rate of bone loss, thus supporting the hypothesis of a counter-regulatory function of OPG in order to prevent further bone loss.

Our study has proved a significant negative correlation between OPG levels and BMD and a positive correlation between OPG and biochemical markers of bone turnover in the group of women with postmenopausal osteoporosis.

Circulating OPG levels may not fully reflect the activity of OPG within the bone microenvironment. OPG is synthesized by both skeletal and nonskeletal cell types and is regulated by a variety of hormones

and cytokines.<sup>15</sup> It is also likely that the biological activity of OPG is dependent on the relative levels of both OPG and its ligand (RANKL). Measurements of the ratio of OPG and RANKL may thus provide more evidence of the significance of circulating OPG concentrations in postmenopausal osteoporosis.

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