Using the Ratio of Serum Osteoprotegerin Ligand to Osteoprotegerin to Evaluate Renal Osteodystrophy in Dialysis Patients

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Osteoclast function is important in the development of renal osteodystrophy (ROD). Osteoclast activity is modulated by the osteoprotegerin ligand–osteoprotegerin (OPGL/OPG) system. In the present study, we checked levels of serum OPG and soluble OPGL in dialyzed patients and correlated those levels with routinely measured parameters of bone metabolism. The study was carried out in 39 patients on hemodialysis (HD) and 29 on peritoneal dialysis (PD). The control group included 13 healthy volunteers.

Patients on HD had lower OPGL (p = 0.027) and higher OPG (p = 0.000) levels than control subjects did (OPGL: 0.6 pmol/L (median) and 0.0 – 10.0 pmol/L (range) vs. 1.9 pmol/L (median) and 0.0 – 10.5 pmol/L (range); OPG: 7.7 pmol/L (median) and 0.9 – 16.5 pmol/L (range) vs. 2.2 pmol/L (median) and 1.0 – 3.9 pmol/L (range)). Patients on PD differed from controls only in OPG level [4.0 pmol/L (median) and 2.1 – 13.4 pmol/L (range), p = 0.043]. Patients on HD and on PD both had a lower OPGL/OPG ratio than did the control subjects [HD: 0.09 (median) and 0.00 – 1.45 (range), p = 0.000; PD: 0.35 (median) and 0.00 – 3.89 (range), p = 0.018; controls: 1.07 (median) and 0.00 – 5.14 (range)]. Patients on HD did not differ from patients on PD in levels of OPGL and OPGL/OPG, but they had a higher OPG level (p = 0.001). Patients on HD also showed significantly higher total alkaline phosphatase (ALP) activity and higher inorganic phosphate (iP), but lower total calcium and blood pH. In PD patients, OPGL and OPG both correlated with pH (OPGL positively and OPG negatively). In HD patients, OPGL showed a positive correlation with ALP and a negative correlation with calcium; OPG correlated positively with iP. In 36 patients on HD (92.3%) and 15 patients on PD (51.7%), OPG was elevated above the normal value.

Differences in serum OPG and OPGL/OPG ratio between groups of dialyzed patients and of control subjects indicate that ROD is more advanced in HD patients than in PD patients. Higher serum OPG and lower serum OPGL in the HD group is probably an effect of higher osteoclast activity. In about 50% of PD patients, osteoclast function is also disturbed, as indicated by elevated OPG levels.

Key words
Osteoprotegerin, osteoprotegerin ligand, renal osteodystrophy

Introduction
Renal osteodystrophy (ROD) is still one of the major complications of chronic renal failure and is associated with increasing morbidity over time. During the last few years, some new information about the pathogenesis of ROD has appeared; however, the precise mechanisms of the various types of ROD are not fully understood. Recently, it was suggested that the osteoprotegerin ligand/osteoprotegerin (OPGL/OPG) system is involved in the pathogenesis of ROD (1,2).

Osteoprotegerin is a member of the tumor necrosis factor (TNF) receptor superfamily. Its gene is located on chromosome 8q23-24 (3), and OPG mRNA has wide tissue distribution that is not restricted to bone or immune tissues (3–5). Human OPG is synthesized as a 401-amino-acid peptide, from which the signal peptide is cleaved to generate the mature peptide consisting of 380 amino acids (3,4). In contrast to all other members of the TNF receptor superfamily, OPG lacks transmembrane and cytoplasmic domains and is secreted as soluble protein (3–5).
Synthesis of OPG in osteoblastic lineage cells is increased by cytokines such as interleukin-1α (IL-1α), IL-1β, TNFα, TNFβ, 1α,25-(OH)2D3, and bone morphogenetic protein 2 (6), and by the antiresorptive agents estrogen (7) and transforming growth factor beta (TGFβ) (8). The level of OPG protein is reduced by glucocorticoids (9), prostaglandin E2 (PGE2) (10), and pure estrogen receptor antagonist ICI 182,780 (7).

Osteoprotegerin acts as a soluble secreted receptor for OPGL. It prevents OPGL from binding to and activating the osteoclast differentiation and activation receptor on the osteoclast surface. Thus, the biological effect of OPG on bone cells is the opposite of that of OPGL, including inhibition of the terminal stages of osteoclast differentiation (4,5,11), suppression of the activation of mature osteoclasts (5,12), and induction of apoptosis (13). Osteoprotegerin also inhibits osteoclastic pit formation in mature osteoclasts (5,12) and antagonizes the induction of bone resorption by 1α,25-(OH)2D3, PGE2, parathormone (PTH), and IL-1α (5,14), in addition to OPGL (14).

Osteoprotegerin ligand is a member of TNF ligand family, whose gene is located on chromosome 13q14 (15). The human form is a 317-amino-acid protein that lacks a signal peptide and that has cytoplasmic and transmembrane domains and an extracellular region at the C-terminus that contains the active ligand site (15–17). Osteoprotegerin ligand exists in two biologically active forms: a cellular, membrane-bound form and a soluble form (16). In osteoblastic lineage cells, the level of OPGL is upregulated by various calcitropic hormones and cytokines including dexamethasone, 1α,25-(OH)2D3, IL-1β, IL-11, TNFα, PTH, and PGE2 (17,18); TGFβ suppresses its synthesis (18). In bone, OPGL stimulates osteoclast differentiation, enhances the activity of mature osteoclasts, and inhibits osteoclast apoptosis (14,16).

Together, OPG and OPGL constitute a complex mediator system involved in the regulation and resorption processes in bone—a system that is probably responsible for the homeostatic mechanism of bone turnover. Alteration in that system may be involved in the pathogenesis of ROD (1,2). Bodily levels of interleukins, growth factors, and cytokines are well known to be involved in the process of bone turnover and in mediating the effect of PTH (19,20). Levels of those interleukins, growth factors, and cytokines are abnormal in dialysis patients and may influence synthesis of OPG and OPGL.

In the present study, we checked concentrations of OPG and soluble OPGL in serum from peritoneal dialysis (PD) and hemodialysis (HD) patients, and correlated those levels with routinely measured parameters of bone metabolism.

**Patients and methods**

Our study was performed in 29 PD patients [mean age: 55.1 ± 14.5 years; median PD duration: 11.4 months (range: 0.1 – 57.4 months)] and 39 HD patients [mean age: 63.0 ± 10.6 years; median HD duration: 24.1 months (range: 1.1 – 186.3 months)]. A control group included 13 healthy volunteers. The groups did not differ significantly in age or in dialysis duration. The causes of end-stage renal disease in the PD patients included diabetic nephropathy (9 patients), chronic glomerulonephritis (6 patients), tubulointerstitial nephropathy (6 patients), polycystic kidney disease (3 patients), and hypertensive nephropathy (2 patients). In HD patients, end-stage renal disease was caused by diabetic nephropathy (10 patients), tubulointerstitial nephropathy (9 patients), chronic glomerulonephritis (6 patients), polycystic kidney disease (6 patients), obstructive nephropathy (3 patients), and ischemic nephropathy (1 patient). In 3 PD patients and 4 HD patients, the causes of end-stage renal disease were unknown. The distribution of the frequency of end-stage renal disease in the two groups did not reach statistical significance.

Serum OPG and OPGL concentrations were measured by enzyme immunoassay (kit: Biomedica, Vienna, Austria) using specific biotinylated OPG or OPGL detection antibodies. In the first step, the substance to be detected combines with the precoated capture anti-OPG antibody or the recombinant OPGL and forms a sandwich. In the last step, the OPG or OPGL is quantitated by an enzyme-catalyzed color change detectable on a standard ELISA reader. The intensity of the color that develops is directly proportional to the amount of the measured substance.

We measured the serum concentration of intact PTH (iPTH) by immunoassay (DuopTH: BioRépair, Sinsheim, Germany). Other parameters such as inorganic phosphates (iP), total calcium, total alkaline phosphatase (ALP) activity, and blood pH were simultaneously measured by routine methods.

All results are expressed as mean ± standard deviation or median and range, as appropriate. Analysis of variance for nonparametric data was used to
elucidate differences between the HD, PD, and control groups. Either the Student $t$-test for paired data or the Mann–Whitney $U$-test was used to check differences between the PD and HD patients. Correlations between non normally distributed values were described by the Spearman coefficient, and normally distributed variables, by the Pearson coefficient. A value of $p < 0.05$ was considered statistically significant.

**Results**

Dialyzed patients had serum OPG levels significantly higher than the levels seen in control subjects. In PD patients, the OPG levels were lower than the levels seen in HD patients (Figure 1). The concentration of OPG was elevated above normal in 15 PD patients (51.7%) and in 36 HD patients (92.3%).

Serum levels of OPGL were not significantly different in PD patients as compared with HD patients or control subjects, but HD patients showed lower OPGL levels than the control subjects did (Figure 2).

The OPGL/OPG ratio did not vary significantly between the dialyzed patient groups, but HD and PD patients both had lower ratios than the ratios that were seen in control subjects (Figure 3).

The only significant difference between the groups in terms of serum concentration of iPTH was the difference between the HD patients and control subjects [195.4 pmol/mL (median) and 10.3 – 1266.9 pmol/mL (range) vs. 34.9 pmol/mL (median) and 18.9 – 76.8 pmol/mL (range)]. As compared with the HD patients, PD patients showed lower total ALP activity [78.0 U/L (median) and 34.0–583.0 U/L (range) vs. 114.5 U/L (median) and 59.0 – 577.0 U/L (range), $p = 0.000$] and a lower serum concentration of iP (1.5 ± 0.4 mmol/L vs. 3.1 ± 2.3 mmol/L, $p = 0.001$), but a higher serum level of total calcium (2.4 ± 0.2 mmol/L vs. 2.2 ± 0.3 mmol/L, $p = 0.003$) and a higher blood pH (7.412 ± 0.051 vs. 7.326 ± 0.043, $p = 0.000$).

In PD patients, soluble OPGL and OPG both correlated with pH: OPGL correlated positively ($r = 0.503, p = 0.010$), and OPG correlated negatively ($r = –0.417, p = 0.038$).

In HD patients, soluble OPGL showed positive correlations with OPG ($r = 0.337, p = 0.039$) and ALP ($r = 0.403, p = 0.018$) and a negative correlation with
FIGURE 2  Soluble osteoprotegerin ligand (OPGL) concentration in hemodialysis (HD) patients, peritoneal dialysis (PD) patients, and control subjects.

FIGURE 3  Soluble osteoprotegerin-ligand/osteoprotegerin (OPGL/OPG) ratio in hemodialysis (HD) patients, peritoneal dialysis (PD) patients, and control subjects.
calcium \((r = -0.431, p = 0.010)\). The level of OPG correlated positively with iP \((r = 0.548, p = 0.000)\).

**Discussion**

Our results indicate that the OPGL/OPG system is disturbed in dialyzed patients, which confirms earlier reports on elevated serum OPG in dialyzed patients \((1,2,21)\). Our findings also show a significantly lower serum OPGL concentration in PD patients than in HD patients. Additionally, both groups of dialyzed patients had lower OPGL/OPG ratios than were found in control subjects. The OPGL/OPG system was probably less disturbed in PD patients because, in that group, the concentration of OPG was lower than that found in the HD group, and also because the level of soluble OPGL did not differ between the PD patients and the control subjects, whereas the OPGL concentration was significantly lower in HD patients than in healthy subjects.

Whether elevated serum OPG is connected with adynamic bone disease (ABD) or high turnover bone disease is not clear \((1,2)\). We lean toward the hypothesis that a lower serum OPGL level is connected to lower osteoclast activity, which appears in ABD \((2)\), with less compensatory production of OPG. Coen et al. \((2)\) found an inverse correlation between serum OPG and histomorphometric parameters of bone resorption. That situation may pertain in the case of PD patients, who are more predisposed to ABD \((22)\). A negative correlation between serum levels of OPG and blood pH in PD patients and a positive correlation between OPG and iP in HD patients seem to confirm a relationship between lower serum OPGL concentrations and lower osteoclast activity \((2)\).

To continue with the hypothesis, lower soluble OPGL concentrations should illustrate the same compensating reaction that causes an increase in OPG concentrations. Such a decrease is clearly marked in HD patients, but less so in PD patients, whose levels of OPGL did not achieve statistical significance in our study. However, in the HD group, OPGL concentration correlates positively with OPG concentration and ALP activity and negatively with total calcium. In PD patients, a positive correlation of OPGL with blood pH is seen. The mechanisms leading to these correlations are not so clear, especially given that administration of OPGL to mice causes acute hypercalcemia \((16)\). That finding, plus data showing that OPG causes a reduction in serum calcium concentration \((23)\), may explain the higher serum calcium level found in PD patients. Perhaps these contradictory facts can be explained by hypothesis that soluble OPGL reflects osteoblast function, which may be confirmed by the positive correlation of soluble OPGL with total ALP activity and the significantly higher ALP activity in the HD group. On the other hand, Mesquita et al. \((21)\) concluded that circulating OPGL and OPG do not predict bone disease in PD patients, because they do not correlate with serum levels of carboxy-terminal propeptide of type I procollagen, betacellulin, or bone density measured at the spine, hip, and radius.

**Conclusions**

According to our findings, the differences in serum OPG and OPGL/OPG ratio between dialyzed groups and controls indicate that ROD is more advanced in HD patients than in PD patients. Higher serum OPG and lower serum OPGL concentrations are probably related to greater osteoclast activity in the HD group than in the PD group. In about 50% of PD patients, osteoclast function is also disturbed, as indicated by elevated OPG. The correlation between OPGL and OPG shows a strong relationship between osteoblast and osteoclast activity. Correlations of OPG and OPGL with ALP activity, calcium, and iP reflect their influence on bone metabolism, which is modified by blood pH.

**References**

5. Kwon BS, Wang S, Udagawa N, et al. TR1, a new member of the tumor necrosis factor receptor family,


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