PART FOUR

Metabolism and Nutrition

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Bone Cytokines and Renal Osteodystrophy in Peritoneal Dialysis Patients

Bone turnover is regulated by local concentrations of cytokines such as osteoprotegerin (OPG) and receptor activator of nuclear factor κB ligand (RANKL). It is not known whether these cytokines can predict renal osteodystrophy in peritoneal dialysis (PD) patients.

We measured serum levels of OPG, RANKL, intact parathyroid hormone (iPTH), calcium, phosphorus, and biologic parameters of bone turnover [carboxy-terminal propeptide of type I procollagen (PICP) and β-crosslaps (βCL)] in 21 PD patients and 42 healthy subjects matched for age and sex, who served as controls. Bone mineral density (BMD) was also evaluated (Z-scores) in the PD patients.

Circulating levels of OPG were significantly higher in PD patients than in healthy subjects (p < 0.001). Mean levels of RANKL did not differ from normal. However, RANKL levels were increased in the group of patients with iPTH levels above 322 pg/mL. Biologic parameters of bone turnover (PICP and βCL) were significantly increased in PD patients (p < 0.001). We found a positive correlation between serum levels of βCL and iPTH. At several skeletal sites, βCL also correlated with the BMD Z-score. No correlations were observed between OPG, RANKL, PICP, βCL, Ca×P, or time on dialysis.

Circulating levels of OPG and RANKL do not reflect bone status in PD patients. The value of βCL is a good marker of bone resorption that correlates with iPTH and BMD.

Key words
OPG, RANKL, bone densitometry, bone turnover markers

Introduction
Renal osteodystrophy (ROD) is a common complication in dialysis patients. It results from a reduction in

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bone mass and an alteration in the micro-architecture of bone, and it leads to pain, disability, and skeletal fractures (1).

Disturbances in the control of parathyroid hormone (PTH) secretion, hyperplasia of the parathyroid glands, and alteration of vitamin D metabolism are key elements in the pathogenesis of ROD (2). Available data indicate that PTH is the major regulator of bone remodeling and skeletal turnover in patients with ROD. Parathyroid hormone promotes the recruitment and differentiation of osteoclasts, the cells that are primarily responsible for the dissolution of bone mineral and the degradation of bone collagen during bone resorption (3). Because osteoclasts do not express receptors for PTH, the action of PTH on osteoclasts is mediated indirectly by the release of various cytokines and growth factors from cells of osteoblastic lineage and other cells within the microenvironment of bone and adjacent bone marrow. The recent identification of osteoprotegerin (OPG), the receptor activator of nuclear factor κB (RANK), and RANK ligand (RANKL) provides important new insights into the molecular signaling that accounts for the close coordination of osteoclast-mediated bone resorption and osteoblast-mediated bone formation during skeletal remodeling (4–6).

In the present study, we evaluated OPG and RANKL serum levels in patients on peritoneal dialysis (PD), and we assessed whether the levels of those cytokines were predictors of ROD. We also studied the relationships of those cytokines with bone turnover rate, estimated by biologic markers of bone formation [carboxy-terminal propeptide of type I procollagen (PICP)], of bone resorption [β-crosslaps (βCL)], and of bone density.

Patients and methods
We studied 10 men and 11 women with a median age of 54 years (range: 33 – 84 years) who had been stable on PD for more than 2 months (mean: 20 months;

range: 2.5 – 66 months) and 42 healthy control subjects (20 men and 22 women) with a median age of 52 years (range: 27 – 80 years) matched for age and sex. Renal failure in PD patients was attributable to various causes, including diabetic nephropathy ($n = 8$), HIV nephropathy ($n = 4$), chronic glomerulonephritis ($n = 4$), nephroangiosclerosis ($n = 4$), and tuberous sclerosis ($n = 1$). We excluded patients who had cancer or myeloma, and those on corticosteroid therapy.

We measured serum albumin, calcium, and phosphate in the PD patients during one of their monthly clinic visits. We also measured serum intact PTH (iPTH) by immunoradiometric assay (kit: DiaSorin, Stillwater, MN, U.S.A.), RANKL and OPG by ELISA (kit: Biomedica, Vienna, Austria; protocol described by R&D Systems Europe, Abingdon, U.K.), βCL by ELISA (kit: Nordic Bioscience Diagnostics, Hovedgade, Denmark), and PICP by radioimmunoassay (kit: Orion Diagnostica, Espoo, Finland). Bone densitometry was performed by dual-energy X-ray absorptiometry (Hologic QDR 4500: Hologic, Bedford, MA, U.S.A.) at the lumbar spine, left and right hip, and right forearm. Bone mineral density (BMD) scores were determined according to age and sex (Z-score).

Statistical analysis

All results are expressed as median and range. The nonparametric Mann–Whitney test was used to compare differences between groups. The Kruskal–Wallis test was used for nonparametric analysis of variance. The Spearman and Pearson coefficients were used to describe correlations between non normal and Gaussian-distributed variables respectively.

Results

All results are presented in Tables I – III and Figures 1 and 2.

Characteristics of the PD population

Most patients had high iPTH levels and hyperphosphatemia; their mean Ca×P product was also slightly increased.

Serum levels of OPG and RANKL

As compared with levels in control subjects, OPG levels were higher in PD patients ($p < 0.001$). No difference was observed between the sexes in either group.

TABLE I   Baseline patient ($n = 21$) characteristics

	Mean±SD	*Normal values*
Ca corrected (mg/dL)	9.31±0.85	9.1–10.2
P (mg/dL)	5.16±1.4	2.4–4.4
Ca×P (mg²/dL²)	45.7±15	<45
iPTH (pg/mL)	326±234	10–55

SD = standard deviation; iPTH = intact parathyroid hormone.

TABLE II   Distribution [median (range)] of serum levels of osteoprotegerin (OPG), receptor activator of nuclear factor κB ligand (RANKL), β-crosslaps (βCL), and carboxy-terminal propeptide of type I procollagen (PICP) in peritoneal dialysis patients (PDPs) and control subjects (Controls)

	*Controls (*n=42*)*	*PDPs (*n=21*)*	p *Value*
OPG (ng/mL)	1.8 (0.8–3.4)	4.3 (2.6–8.7)	<0.001
RANKL (pg/mL)	0.05 (0–5.7)	0.05 (0.05–5.8)	NS
RANKL/OPG	0.05 (0–3.1)	0.01 (0.006–1.1)	<0.001
βCL (ng/mL)	0.33 (0.01–1.6)	3.1 (0.8–16.0)	<0.001
PICP (μg/L)	101 (31–206)	204 (88–495)	<0.001

TABLE III   Correlation between bone mineral density (BMD) Z-scores at lumbar, femoral, and radial sites and β-crosslaps (βCL) serum levels

BMD site	*Correlation coefficient*	p *Value*
Femoral	–0.83	0.008
Lumbar spine	–0.73	0.031
Radius	–0.80	0.013

No difference in RANKL levels was observed between PD patients and controls. The RANKL/OPG ratio was significantly lower in PD patients than in control subjects ($p < 0.05$ and $p < 0.01$).

Serum levels of bone turnover markers

Serum levels of both PICP and βCL were higher in PD patients than in control subjects.

Correlation between bone cytokines, bone turnover markers, and iPTH serum levels

Although iPTH correlated well with βCL ($p < 0.01$, Figure 1), we found no correlation between iPTH serum level and OPG, RANKL, RANKL/OPG ratio, or PICP. However, levels of RANKL varied significantly between iPTH tertiles and was significantly higher in patients with the highest iPTH levels (Figure 2).
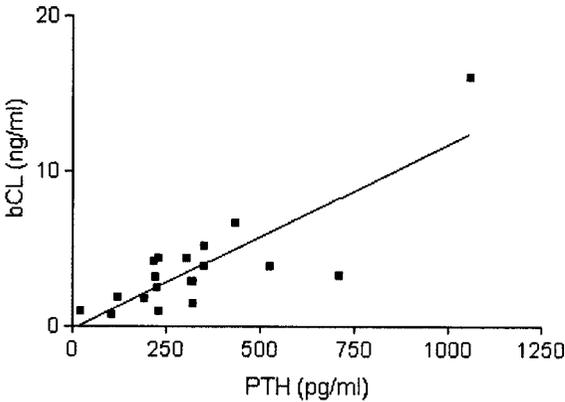
FIGURE 1   Correlation between β-crosslaps (βCL) and serum levels of intact parathyroid hormone (iPTH).

FIGURE 2   Serum levels of receptor activator of nuclear factor κB ligand (RANKL) by intact parathyroid hormone (iPTH) tertile.

Determinants of OPG, RANKL, and markers of bone turnover

In PD patients and control subjects alike, OPG correlated with age. No such correlation was observed for RANKL, RANKL/OPG ratio, or βCL and PICP. We observed no correlation between levels of OPG, RANKL, RANKL/OPG ratio, PICP, or βCL and dialysis duration or Ca×P product, nor between OPG, RANKL, RANKL/OPG ratio and PICP or βCL.

Correlation between BMD and biochemical measurements

In PD patients, we observed a correlation between the serum level of βCL and BMD Z-scores at the lumbar spine, femoral neck, distal third of the radius, and total radius. We found no significant correlation between Z-scores and iPTH, OPG, RANKL, RANKL/OPG ratio, or PICP.

Results for diabetic and non diabetic PD patients

The levels of PTH, OPG, RANKL, OPG/RANKL ratio, βCL, and PICP were not statistically different between patients with and without diabetes.

Discussion

Our results show that circulating OPG is increased in PD patients and that RANKL increases significantly across iPTH tertiles. Those findings point to a possible stimulating effect of PTH on RANKL synthesis. Circulating levels of RANKL may weakly reflect the degree of secondary hyp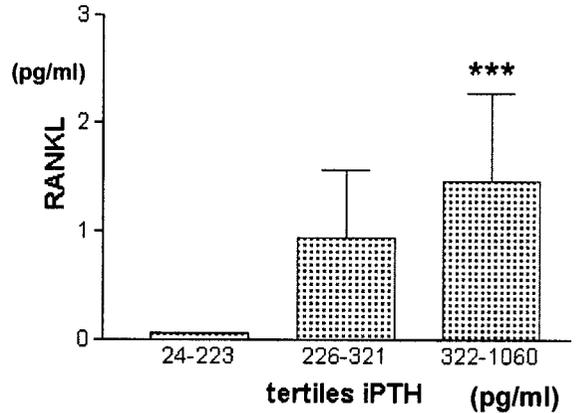erparathyroidism. However, because circulating OPG is high in PD patients, the RANKL/OPG ratio in serum is low, and neither cytokine is related to parameters of bone resorption or BMD.

In a recent study, significantly higher levels of RANKL were found in patients on hemodialysis than in healthy age-matched control subjects (7). However, in our study, no correlation was found between RANKL and iPTH serum levels. There may be several reasons for those results:

- Both cytokines are also produced by extraskeletal tissue (such as lymphocytes and endothelia). High levels of OPG have been observed in both normal and uremic patients with coronary and other vascular calcifications (8–11).
- Serum concentrations of RANKL and OPG depend not only on production, but also on catabolism and excretion, which may be altered in PD patients. No information is available in the literature regarding RANKL and OPG catabolism and excretion.
- The laws that govern the passage of locally produced RANKL and OPG in bone toward the circulation are not yet fully understood.
- The action of RANKL on bone cells depends on the membrane-bound molecule more than on the soluble fraction.

Conclusions

As is well known, PD patients show varying degrees of hyperparathyroidism as demonstrated by their

iPTH and BMD levels. Serum βCL seems to be a good marker of bone resorption that correlates well with serum iPTH and BMD levels. On the other hand, serum OPG and RANKL are only weakly related to the degree of hyperparathyroidism; they are not predictors of bone disease.

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