Ratio of cyclase activating and cyclase inactive parathormone (CAP/CIP) in dialysis patients: correlations with other markers of bone disease

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Abstract

Purpose: **We checked correlation of CAP/CIP with osteoprotegrin (OPG), its soluble ligand (OPGL) and routinely measured parameters of bone turnover in patients treated with peritoneal dialysis (PD) and hemodialysis (HD).**

Material & methods: **In 30 patients (22 HD, 8 PD) we determined serum concentrations of intact parathormone (iPTH), CAP, OPG, OPGL, total Ca, inorganic phosphates (Pi), creatinine, urea, total alkaline phosphatase (AP) and blood pH. CIP was calculated by subtraction of CAP from iPTH. Controls (Cs) included 9 healthy persons in whom iPTH, CAP, OPG and OPGL were measured as well as CIP, CAP/CIP and OPGL/OPG were calculated.**

Results: **Differences between HD and PD patients included dialysis duration, OPGL, OPGL/OPG, AP, Pi, Ca and pH. After adjustment to dialysis duration differences in OPGL/OPG, Pi, Ca and pH remained significant. HD patients differed from Cs in terms of iPTH, CAP, CIP, OPGL, OPG and OPGL/OPG. In whole group of patients iPTH, CAP, CIP but not CAP/CIP correlated negatively with OPGL and OPGL/OPG as well as positively with dialysis duration, OPG and AP.**

Conclusions: **Despite more advanced uremic bone disease in longer dialyzed HD patients than in shorter dialyzed PD ones, CAP/CIP is not different neither between these groups nor Cs persons. CAP/CIP does not seem to be more powerful tool in noninvasive diagnosis of bone disease than iPTH or CAP and CIP alone.**

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Introduction

1-84 parathormone (PTH) – cyclase activating PTH (CAP) and 7-84 PTH – cyclase inactive PTH (CIP) are secreted by the parathyroid glands. CAP is a whole PTH molecule, which consists of 84 amino acids, but only the first 34 are essential in keeping of mineral homeostasis. CIP is a C-terminal fragment of 1-84 PTH and has been demonstrated to be an antagonist of CAP with inverse biological activities as it was shown in thyroparathyroidectomized and nephrectomized rats [1]. CAP operates through the type 1 PTH/PTH related peptide (rP) receptor and stimulates synthesis of cyclic adenosinomonophosphoran (cAMP) [2]. Human CIP inhibits bone resorption in vitro via actions independent of the type 1 PTH/PTHrP receptor [3]. CIP appears to operate through a C terminal PTH receptor [4]. CAP is hypercalcemic and increases bone turnover, whereas CIP has been demonstrated to be hypocalcemic and able to lower bone turnover through an inhibition of osteoclast formation and differentiation resulting in an overall inhibition of bone resorption. CIP does not increase synthesis of cAMP [2].

The 2nd generation "intact" PTH (iPTH) assays measure sum of CAP and CIP. Thus, estimation of iPTH, if considered as "active" PTH, provides PTH activity over 30% higher due to detection of CIP additionally to CAP [5]. Such high PTH levels may result in over treatment with D-analogues, leading to adynamic bone disease and soft tissue calcifications. Advanced assay 3rd generation methodology anables to measure biologically active CAP [5,6].

CAP/CIP (the optimal range 1.5-1.8) is recently considered as useful in assessment of bone turnover in dialysis patients [4, 6]. It has been demonstrated to predict bone turnover with a histologically determined 93% predictability. CAP/CIP below unity indicates in dialysis patients adynamic low bone turover (87.5%) [7,8]. More difficult task is to answer the question which value

of CAP/CIP is characteristic for high bone turnover, because among patients with CAP/CIP of 2.0 or higher there are 60% of patients with normal bone turnover [9]. For clinical practice, it is adviced to use value of 1.4 as separating dialysis patients with low bone turnover (CAP/CIP < 1.4) and normal or high bone turnover (CAP/CIP > 1.4) [9].

In this study we checked correlation of CAP/CIP with osteoprotegrin (OPG), its soluble ligand (OPGL) and routinely measured parameters of bone turnover in patients treated with peritoneal dialysis (PD) or hemodialysis (HD).

Material and methods

The study was performed in 30 patients (22 were treated with HD, 8 – with PD). Mean patients' age was 61.6 (36.7 - 79.6) years. Patients were treated with dialysis for 15.2 (1.3 - 186.3) months. Dialysis duration was significantly longer in HD patients than in PD ones (p = 0.014; 26.7, 3.5 - 186.3 months for HD vs 5.0, 1.3 - 26.7 months for PD).

In dialyzed patients serum concentrations of iPTH and CAP were measured by Duo PTH (BioRepair, Sinsheim, Germany). OPG and OPGL were measured by ELISA (Biomedica, Vienna, Austria). Other measured parameters included serum total alkaline phosphatase (AP) activity, serum total calcium (Ca) and inorganic phosphate (Pi) concentrations, blood pH and serum creatinine and urea levels. All the parameters were measured by routinely used methods.

CIP was calculated by subtraction of CAP from iPTH. CAP/CIP and OPGL/OPG were also calculated in examined patients.

Control group (Cs) included 9 healthy persons in whom serum levels of iPTH, CAP, OPG and OPGL were measured as well as CIP, CAP/CIP and OPGL/OPG were calculated.

All results are expressed as median and range. ANOVA for nonparametric data was used to elucidate differences between HD, PD and Cs. Either Student's t-test for non-paired data or Mann-Whitney U test was used to check differences between PD and HD patients. Spearman's and Pearson's coefficients were respectively used to describe correlations between non-normal and normal distributed variables. Results were also adjusted to dialysis duration. The p level less than 0.05 was considered as significant.

Results

There was no statistically significant difference in terms of iPTH, CAP, CIP and CAP/CIP between HD and PD patients. Only HD patients differed from Cs in terms of iPTH, CAP and CIP, but not in CAP/CIP (*Tab. 1*).

Significant differences were shown between HD patients and Cs for OPG (p = 0.004; 7.8, 1.5 - 15.8 pmol/L for HD vs 2.2, 1.0 - 3.9 pmol/L for Cs), OPGL (p = 0.035; 0.6, 0.0 - 10.0 pmol/L for HD vs 3.4, 0.4 - 10.5 pmol/L for Cs) and OPGL/OPG (p = 0.000; 0.10, 0.00 - 1.45 for HD vs 1.23, 0.18 - 5.4 for Cs) as well as between HD patients and PD ones for OPGL (p = 0.016; 2.1, 0.0 - 5.3 pmol/L for PD) and OPGL/OPG (p = 0.003; 0.73, 0.00 - 1.60 for PD). After adjustment to dialysis duration differences in OPGL/OPG between HD patients and PD ones remained significant.

Table 1. **Serum parathormone levels in hemodialysis (HD) patients, peritoneal dialysis (PD) patients and controls**

	HD patients	PD patients	Controls
iPTH (pg/ml)	199.9 (10.3-1266.9)	109.2 (13.7-334.9)	37.4 (18.9-76.8)[a]
CAP (pg/ml)	126.7 (6.5-887.9)	79.7 (9.3-238.6)	23.5 (11.2-42.6)[b]
CIP (pg/ml)	70.4 (3.3-398.9)	48.4 (2.4-129.0)	13.5 (0.9-34.2)[c]
CAP/CIP ratio	1.73 (0.84-4.21)	1.97 (0.90-4.67)	1.45 (0.92-3.61)

No significant changes between HD patients and PD ones
Changes between HD patients and controls:
[a] p = 0.016 [b] p = 0.018 [c] p = 0.019

Significant differences between HD patients and PD ones also included standard parameters related to bone turnover: serum concentrations of Ca (p = 0.004; 2.2, 0.8 - 2.8 mmol/L for HD vs 2.6, 2.2 - 2.6 mmol/L for PD) and Pi (p = 0.003; 2.5, 1.2 - 7.3 mmol/L for HD vs 1.2, 0.99 - 2.1 mmol/L for PD), total AP activity in serum (p = 0.000; 122, 74 - 577 U/L for HD vs 64, 60 - 98 U/L for PD) and blood pH (p = 0.003; 7.31, 7.28 - 7.35 for HD vs 7.43, 7.32 - 7.50 for PD). After adjustment to dialysis duration all these parameters except total AP remained significantly different between both groups of patients.

In whole group of examined patients iPTH, CAP, CIP but not CAP/CIP correlated negatively with OPGL (iPTH: r = -0.377, p = 0.018; CAP: r = -0.356, p = 0.026; CIP: r = -0.383, p = 0.016), OPGL/OPG (iPTH: r = -0.435, p = 0.006; CAP: r = -0.414, p = 0.009; CIP: r = -0.440, p = 0.005) and positively with dialysis duration (iPTH: r = 0.415, p = 0.025; CAP: r = 0.420, p = 0.023; CIP: r = 0.392, p = 0.035), OPG (iPTH: r = 0. 374, p = 0.019; CAP: r = 0.366, p = 0.022; CIP: r = 0.406, p = 0.010) and AP (iPTH: r = 0.606, p = 0.007; CAP: r = 0.577, p = 0.002; CIP: r = 0.617, p = 0.001). iPTH additionally showed correlation with pH (r = -0.514, p = 0.042).

In PD patients correlation of CAP/CIP with serum urea concentration (r = -0.717, p = 0.030) was shown. In HD patients correlation between AP and iPTH (r = 0.619, p = 0.005), CAP (r = 0.605, p = 0.006) and CIP (r = 0.657, p = 0.002) was shown, and between iPTH and serum urea level (r = 0.633, p = 0.036).

Discussion

Comparisons of iPTH, CAP, CIP, CAP/CIP and total AP indicate that secondary hyperparathyroidism is more advanced in longer dialyzed HD patients than in shorter dialyzed PD ones. The later group, if small, may not differ from controls in terms of serum PTH concentrations. CAP/CIP is not able to differentiate HD patients, PD patients, and controls. It suggests

that regulatory system preserving normal proportions between serum CAP and CIP concentrations is not disturbed in dialyzed patients. According to Gao et al. [5], CAP as percent of total PTH is lower and serum CIP concentration is higher in uremic patients. Both these abnormalities acting together or separately may explain a resistance to active PTH in uremia, causing an effect that 3-5 times higher iPTH concentration is needed to keep normal bone turnover [10].

OPG acts as a soluble secreted receptor for OPGL that prevents it from binding to and activating osteoclast differentiation an activation receptor on the osteoclast surface. The biological effects of OPG on bone cells are the opposite of that of OPGL, including inhibition of terminal stages of osteoclast differentiation [11-13], suppression of the activation of mature osteoclasts [12,14], and induction of apoptosis [15]. OPG also inhibits osteoclastic pit formation of mature osteoclasts [12,14] and antagonizes the induction of bone resorption by $1\alpha,25\text{-}(OH)_2D_3$, PGE_2, PTH, IL-1α [12,16], as well as OPGL [16].

Increased OPG level was already shown in HD patients [17, 18]. However, reports on diagnostic value of OPG estimations in serum are controversial. Coen et al. [17] have demonstrated lower OPG concentration in patients with adynamic bone disease than in patients with high bone turnover. Haas et al. [18] reported both OPG and PTH as markers of high turnover osteodystrophy and decreased bone mineralisation in HD patients. We lean towards hypothesis that lower serum OPG level is connected with lower activity of osteoclasts, what appears in adynamic bone disease, and with less compensating production of OPG. When serum PTH increases, OPG also rises to prevent bone destruction associated with PTH action.

CAP/CIP did not show significant correlations with other markers of bone turnover, dialysis duration or blood pH. Such correlations were observed for iPTH, CAP and CIP. It is worthy to notice that in our study in dialyzed patients both CAP and CIP showed a significant positive correlation with AP as it was earlier demonstrated in kidney transplant patients [19]. This may suggest that changes in CAP/CIP can be hardly used to show relations between parameters of bone turnover. Additionally, there were no significant correlations in our study between serum concentrations of total Ca and CAP/CIP. According to the earlier studies of the other authors [4], CAP/CIP decreases in dialysis patients when serum calcium concentration increases and vice versa. In PD patients, lower serum OPG level may contribute to higher serum calcium level because OPG causes a decrease in serum calcium concentration [20].

Our studies indicate that despite more advanced uremic bone disease in longer dialyzed HD patients than in shorter dialyzed PD ones, CAP/CIP is not different neither between these groups nor Cs persons. We conclude that CAP/CIP does not seem to be more powerful tool in noninvasive diagnosis of bone disease than iPTH, CAP and CIP alone, OPGL, OPGL/ /OPG or even AP.

References

1. Faugere MC, Langub M, Malluche HH. The effects of PTH-(1--84) on bone turnover are antagonized by PTH-(7-84) in thyroparathyroidectomized and nephrectomized rats. Abstr. J Am Soc Nephrol, 2001; 12: 764A.
2. Nguyen-Yamamoto L, Rousseau L, Brossard JH, Lepage R, D'Amur P. Synthetic carboxyl-terminal fragments of parathyroid hormone (PTH) decrease ionised calcium concentration in rats by acting on receptor different from the PTH/PTH-related peptide receptor. Endocrinology, 2001; 142: 1386–92.
3. Divietti P, John MR, Juppner H, Bringhurst FR. Human PTH-(7-84) inhibits bone resorption in vitro via actions independent of the type 1 PTH/PTHrP receptor. Endocrinology, 2002; 143: 171-6.
4. Cantor T. The clinical application of cyclase activating PTH assay and the inhibitor ratio. Proc. 23rd Annual Conf. Perit. Dial. Seattle. 2003, p. 51-5.
5. Gao P, Scheibel S, D'Amur P, John MR, Rao SD, Schmidt-Gayk H, Cantor TL. Development of the novel immunoradiometric assay exclusively for biologically active whole parathyroid hormone 1-84: implications for improvement of accurate assessment of parathyroid function. J Bone Miner Res, 2001; 16: 605–14.
6. Amerling R. What is the 3rd generation PTH assay and how do 3rd generation assays differ? Proc. 24th Annual Conf. Perit. Dial. San Antonio. 2004, pp. 443-58.
7. Monier-Faugere MC, Geng Z, Mawad H, Friedler RM, Gao P, Cantor TL, Malluche HH. Improved assessment of bone turnover by the PTH- (1-84) / large C-PTH fragments ratio in ESRD patients. Kidney Int, 2001; 60: 1460–8.
8. Faugere MC, Geng Z, Mawad H, Friedler RM, Gao P, Cantor T, Malluche HH. Improved assesssment of bone turnover by the PTH 1-84/large C-PTH fragments ratio in ESRD patients. Kidney Int, 2001; 60: 1460-8.
9. Cantor T. What to look in assessing the value of an existing or new PTH test. Proc. 24th Annual Conf. Perit. Dial. San Antonio. 2004, p. 459-70.
10. Slatopolsky E, Finch J, Clay P, Martin D, Sicard G, Singer G, Gao P, Cantor T, Dusso A. A novel mechanism for skeletal resistance in uremia. Kidney Int, 2000, 58; 753–61.
11. Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Soto Y, Goto M, Yamaguschi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegrin (OPG): A mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology, 1998; 39: 1329-37.
12. Kown BS, Wang S, Udagawa N, Haridas V, Lee ZH, Kim KK, Oh KO, Greene J, Li Y, Su J, Gentz R, Aggarwal BB, Ni J. TR1, a new member of the tumor necrosis factor receptor family, induces fibroblast proliferation and inhibits osteoclastogenesis and bobe resorption. FASEB J, 1998; 12: 845-54.
13. Tan HL, Van G, Scully S, Shimamoto G, Kelley M, Boyle B, Dunstan C, Lacey D. Recombinant osteoprotegrin (OPG), a novel TNF-receptor family member, inhibits in vitro murine osteoclast formation form bone marrow precursors (abstract P213). J. Bone Miner. Res. 1997; 12(Suppl 1): S155.
14. Hakeda Y, Kobayashi Y, Yamaguchi K, Yasuda H, Tsuda E, Higashio K, Miyata T, Kumegawa M. Osteoclastogenesis inhibitory factor (OCIF) directly inhibits bone-resorbing activity of isolated mature osteoclasts. Biochem Biophys Res Com, 1998; 251: 796-801.
15. Akatsu T, Murakami T, Nishikawa M, Ono K, Shinomiya N, Tsuda E, Mochizuki SI, Yamaguchi K, Kinosaki M, Higashio K, Yamamoto M, Motoyoshi K, Nagata N. Osteoclastogenesis-inhibitory factor supresses osteoclast survival by interfering in the interaction of stromal cells with osteoclast. Biochem Biophys Res Com, 1998; 250: 229-34.
16. Fuller K, Wong B, Fox S, Choi Y, Chambers TJ. TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. J Exp Med, 1998; 188: 997-1001.
17. Coen G, Ballanti P, Balducci A, Calabria S, Fischer MS, Jancovic L, Manni M, Morosetti M, Moscaritolo E, Sardella D, Bonucci E. Serum osteoprotegrin and renal osteodystrophy. Nephrol Dial Transplant, 2002, 17; 233–8.
18. Haas M, Leko-Mohr Z, Roschager P, Kletzmayr J, Schwartz C, Domenig C, Zsontsich T, Klaushofer K, Delling G, Oberbauer R. Osteoprotegrin and parathyroid hormone as markers of high-turnover osteodystrophy and decreased bone mineralization in hemodialysis patients. Am J Kidney Dis, 2002; 39: 580–6.
19. Spiechowicz U, Kokot F, Więcek A. Function of parathyroid glands in kidney transplant patients – diagnostic value of CAP and CIP. Abstr. The 2nd Poland – Korea Seminar on Renal Replacement Therapy. Seoul. 2004, p. 21.
20. Morony S, Capparelli C, Lee R, Shimamoto G, Lacey DL, Dunstan CR. A chimeric form of osteoprotegrin inhibits hypercalcemia and bone resorption induced by IL-1β, TNF-α, PTH, PTHrP, and $1,25(OH)_2D_3$. J Med Bone Miner Res, 1999; 14: 1478-85.