NDT Plus (2008) 1 [Suppl 3]: iii54–iii58
doi: 10.1093/ndtplus/sfn088

NDT PLUS
Nephrology Dialysis Transplantation

Reversed whole PTH/intact PTH ratio as an indicator of marked parathyroid enlargement: five case studies and a literature review

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Abstract

Parathyroid hormone (PTH) levels detected by intact PTH assays are generally higher than those detected by the whole PTH assay because the latter does not detect non-(1–84) PTH fragments, mainly PTH (7–84). Rare exceptions to this rule have been reported in patients with severe primary or secondary hyperparathyroidism and parathyroid carcinoma. Overproduction of an N-form of PTH other than PTH (1–84) has been observed in the sera of these patients. We report five additional cases with the reversed whole PTH/intact PTH ratio associated with severe hyperparathyroidism in haemodialysis patients. Three patients demonstrated enlargement of a single hypervascular gland, whereas the other two had undergone surgical parathyroidectomy and later showed recurrent hyperparathyroidism due to progressive autograft hyperplasia. In the case of a single enlarged gland, the pathological pattern and heterogeneous expression of parathyroid adenomatosis 1/cyclin D1 suggested it to be a single nodule of uraemic hyperparathyroidism rather than sporadic primary adenoma. These cases suggested that the reversed whole PTH/intact PTH ratio could be an indicator of marked parathyroid enlargement. Further studies are required to elucidate the clinical significance of the reversed whole PTH/intact PTH ratio in haemodialysis patients.

Keywords: intact PTH; N-PTH; secondary hyperparathyroidism; single nodule; whole PTH

Introduction

Disorder of mineral and bone metabolism is one of the most prevalent and serious abnormalities in dialysis patients. Second-generation parathyroid hormone (PTH) assays, also called intact PTH assays, have been widely used for optimal management of parathyroid function and bone remodelling [1–3]. Until recently, these assays were believed to react only with full-length PTH (1–84); however, they also detect large C-terminal fragments with a partially preserved N-terminal structure, mainly PTH (7–84) [4,5]. This is especially relevant in dialysis patients because reduced clearance from the kidneys may result in increased levels of these fragments [6]. Moreover, PTH (7–84) has been shown to antagonize the calcaemic and bone-resorbing effects of PTH (1–84) [7, 8].

Third-generation PTH assays, such as the whole PTH assay or bio-intact PTH assay, have recently been developed to overcome such complex methodological and biological problems. These assays are more sensitive and specific when measuring bioactive PTH (1–84) [9,10]. PTH values obtained from intact PTH assays are generally higher than those obtained from the whole PTH assay because the latter does not detect non-(1–84) fragments [10–13]. Rare exceptions to this rule have been reported in patients with severe primary and secondary hyperparathyroidism and parathyroid cancer [14–19]. Moreover, a new molecular form of N-PTH, distinct from PTH (1–84), has been identified in the circulation after HPLC fractionation of serum [14–16,18].

We present a series of cases with progressive hyperparathyroidism in whom whole PTH levels were paradoxically higher than intact PTH levels. We then reviewed the recent literature and summarized the clinical features of published cases with the reversed whole PTH/intact PTH ratio, which suggested that this reversed ratio could be a marker for the severity of hyperparathyroidism.

Case reports

Case 1

A 55-year-old male had been receiving haemodialysis since February 2003 for end-stage renal disease (ESRD) due to diabetic nephropathy. In May 2006, on detection of high intact PTH levels (327 pg/ml; Elecsys PTH; Roche Diagnostics, Mannheim, Germany) the patient was treated with intravenous calcitriol (1.0 μg/week). However, serum intact PTH levels progressively increased to 528 pg/ml and

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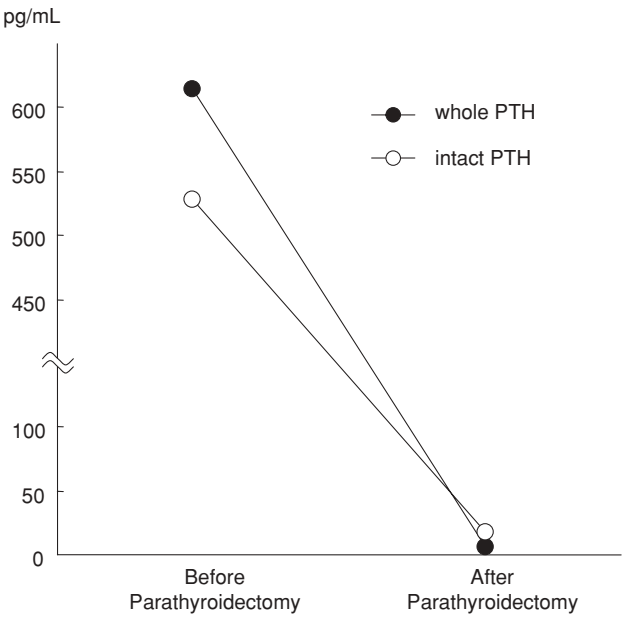

Fig. 1. Serum PTH levels before and after parathyroidectomy. Reversed whole PTH/intact PTH ratio normalized after surgery, as is usual in uraemic patients.

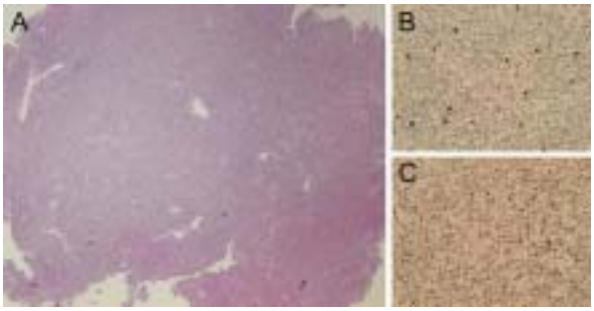

Fig. 2. (**A**) Histological findings of the resected parathyroid gland. Note the single nodular hyperplasia of the parathyroid cells without the normal rim. Carcinomatous changes are not evident. (**B**) Immunohistochemical findings of Ki-67 indicate accelerated cell growth progression (labelling index 28/1000). (**C**) Heterogeneous expression of parathyroid adenomatosis 1/cyclin D1 (labelling index 360/1000) suggesting a single nodule of uraemic hyperparathyroidism rather than a sporadic primary adenoma.

abnormally elevated levels of whole PTH (614 pg/ml; Whole PTH; Scantibodies Laboratories, Santee, CA, USA) were found. Serum bone alkaline phosphatase levels increased to 46 U/l. Doppler ultrasonography showed a single enlarged hypervascular parathyroid gland (14 mm × 11 mm × 7 mm). Surgical parathyroidectomy was performed, and the intact and whole PTH levels decreased to 17 and 5.9 pg/ml, respectively, with normalization of the reversed whole PTH/intact PTH ratio (Figure 1).

The resected gland showed hyperplasia of the parathyroid cells without the normal rim, supporting the diagnosis of secondary hyperparathyroidism (Figure 2A). However, the complication of sporadic primary adenoma remains a possibility in dialysis patients, especially those with a single enlarged gland. Thus, we performed immunohistochemical

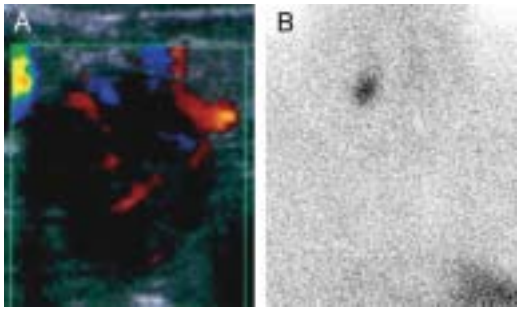

Fig. 3. (**A**) Power Doppler ultrasonography showing a single enlarged hypervascular parathyroid gland (18 mm × 14 mm × 14 mm). (**B**) ^{99m}Tc methoxyisobutyl-isonitrile scintigraphy demonstrating a hot spot in the right inferior parathyroid gland.

analysis, which is known to be helpful when differentiating these two disorders, as previously reported by Tominaga *et al*. [20]. The immunostaining pattern of Ki-67 indicated accelerated cell growth progression (labelling index 28/1000) (Figure 2B), while heterogeneous expression of parathyroid adenomatosis 1/cyclin D1 was not consistent with primary adenoma (labelling index 360/1000) (Figure 2C). Thus, the gland was diagnosed as a single nodule, the most advanced type of parathyroid hyperplasia in chronic uraemic patients.

Case 2

A 58-year-old female had been receiving haemodialysis since May 1996 for ESRD due to IgA nephropathy. In February 2007, despite maintenance of intact PTH levels (178 pg/ml; Elecsys PTH) and whole PTH levels (134 pg/ml), routine Doppler ultrasonography revealed a hypervascular enlarged gland (14 mm × 12 mm × 12 mm). With the intention of preventing the progression of secondary hyperparathyroidism, oral falecalcitriol (0.15 μg/day) was started; however, after 10 months of treatment, serum intact and whole PTH levels progressively increased to 685 and 704 pg/ml, respectively, with the reversed whole PTH/intact PTH ratio. Re-examination by ultrasonography showed a progressive enlargement of the parathyroid gland (18 mm × 14 mm × 14 mm) (Figure 3A), and a hot spot was detected at the same location by ^{99m}Tc methoxyisobutyl-isonitrile scintigraphy (Figure 3B). Serum bone alkaline phosphatase levels increased up to 102 U/l, indicating extremely high bone turnover. Accordingly, the patient was scheduled for parathyroidectomy.

Case 3

A 60-year-old male had been receiving haemodialysis since November 2001 for ESRD due to diabetic nephropathy. For several years, serum intact PTH levels were maintained between 200 and 300 pg/ml (Elecsys PTH). However, in June 2006, despite ideal control of parathyroid function, a routine neck ultrasonography examination revealed an enlarged hypervascular parathyroid gland (17 mm × 14 mm × 15 mm). Subsequently, secondary hyperparathyroidism rapidly advanced, and in December 2007, serum intact

and whole PTH levels increased to 612 and 801 pg/ml, respectively, with the reversed whole PTH/intact PTH ratio. Significantly progressive enlargement of the parathyroid gland (23 mm × 21 mm × 21 mm) was shown by ultrasonography. Serum bone alkaline phosphatase levels increased to 54 U/l. Accordingly, the patient was scheduled for parathyroidectomy.

Case 4

A 54-year-old male had been receiving haemodialysis since 1974 for ESRD due to chronic glomerulonephritis. Since 1981, he was found to have extremely high carboxyl-terminal PTH (C-PTH) levels between 20 and 30 ng/ml (normal range, 0.2–1.0 ng/ml). Total parathyroidectomy with forearm autograft was performed in 1986, and the C-PTH levels effectively decreased to 0.5 ng/ml. The resected gland showed parathyroid hyperplasia. For about two decades after surgery, secondary hyperparathyroidism, which was evaluated by intact PTH assays, had been managed well; however, in November 2007, abnormally higher whole PTH levels (358 pg/ml) than intact PTH levels (278 pg/ml; total PTH; Scantibodies Laboratories) were found. Ultrasonography of the forearm showed an enlarged parathyroid tissue (17 mm × 7 mm × 3 mm). After informed consent was obtained, we performed a simplified Casanova test (total ischaemic blockade of the graft-bearing arm), as previously described [21]. Following temporary ischaemic autograftectomy, the intact and whole PTH levels significantly decreased to 127 and 117 pg/ml, respectively, with normalization of the reversed whole PTH/intact PTH ratio, suggesting that the autografted parathyroid tissue was the cause of the reversed ratio. The patient was then scheduled for surgical removal of the autografted gland.

Case 5

A 39-year-old female had been receiving haemodialysis since 1979 for ESRD due to chronic glomerulonephritis. In 1993, total parathyroidectomy with forearm autograft was performed for secondary hyperparathyroidism, which was then maintained for several years. However, in January 2007, elevated levels of intact PTH (396 pg/ml; Elecsys PTH), whole PTH (263 pg/ml) and bone alkaline phosphatase activity (63 U/l) were found. Ultrasonography of the graft-bearing arm showed an enlargement of the parathyroid tissue (17 mm × 16 mm × 5 mm). When PTH levels were measured in the sera obtained from the graft-bearing arm, extremely elevated levels of intact and whole PTH levels were found (3600 and 4490 pg/ml, respectively), with the reversed whole PTH/intact PTH ratio. Surgical removal of the autografted gland was performed under local anaesthesia. After surgery, the intact and whole PTH levels measured in the sera obtained from the graft-bearing arm decreased to 27 and 39 pg/ml, respectively, but without normalization of the reversed ratio. It was then suggested that small fragments of the parathyroid tissue (i.e. the probable cause of the reversed whole PTH/intact PTH ratio) remained in the forearm. As expected, recurrent hyperparathyroidism soon developed and further treatment was required.

Discussion

We report five exceptional cases with the reversed whole PTH/intact PTH ratio associated with progressive hyperparathyroidism, seen in relatively rapid succession in clinical practice. Three patients demonstrated an enlargement of a single hypervascular gland, and the pathological pattern and heterogeneous expression of parathyroid adenomatosis 1/cyclin D1 suggested it to be a single nodule of uraemic hyperparathyroidism in case 1. The other two patients had undergone surgical parathyroidectomy and subsequently showed recurrent hyperparathyroidism due to progressive autograft hyperplasia.

Abnormally higher whole PTH levels than intact PTH levels have been reported in a minority of patients with severe hyperparathyroidism and parathyroid cancer [14–19]. D'Amour *et al*. analysed serum samples from these patients by HPLC and were the first to identify a new molecular form of PTH with an intact N-terminal, distinct from (1–84) PTH [14–16]. More recently, we also revealed the overproduction of N-PTH in a haemodialysis patient with a single nodule of uraemic hyperparathyroidism [18]. This N-form of PTH is detectable by the whole PTH assay, but is less reactive in intact PTH assays [14–16,22]. Hence, the reversed whole PTH/intact PTH ratio strongly suggests the existence of N-PTH. This new molecular form is not produced during the peripheral metabolism of PTH (1–84) [23], while we have recently shown that the source of excess N-PTH is associated with the pathological parathyroid tissue [18].

To date, our group has reported three haemodialysis patients with severe hyperparathyroidism associated with the reversed whole PTH/intact PTH ratio [17–19]. The first [17] and second [18] cases showed a single nodule of uraemic hyperparathyroidism for which surgical parathyroidectomy was performed, and the reversed PTH ratio normalized after surgery. The third case showed excessive growth of parathyroid gland that outstripped vascular supply, and spontaneous remission due to autoinfarction of the parathyroid gland resulted in normalization of the reversed whole PTH/intact PTH ratio [19]. The clinical features of the present cases and previously published cases with the reversed whole PTH/intact PTH ratio are summarized in Table 1. All cases with the reversed whole PTH/intact PTH ratio showed progressive enlargement of the parathyroid gland. These findings are in accordance with previous cases of the reversed whole PTH/intact PTH ratio associated with clinically worse parathyroid disease [14–16]. Taken together, it is suggested that overproduction of N-PTH, represented by the reversed whole PTH/intact PTH ratio, could be an indicator of progressive hyperparathyroidism.

Differential diagnosis between primary and secondary hyperparathyroidism is very difficult in dialysis patients with a single enlarged gland [20], especially those receiving haemodialysis for a long time. In this study, we performed immunohistochemical analysis to differentiate these two disorders. Consequently, the advanced type of uraemic hyperparathyroidism was corroborated by the heterogeneous expression of parathyroid adenomatosis 1/cyclin D1, one of the genetic abnormalities responsible for tumorigenesis in primary adenoma (case 1). Thus, the reversed whole

Table 1. Clinical features of haemodialysis patients with the reversed whole PTH/intact PTH ratio: previous and present reports

Author (year)	Age	Sex	Dialysis vintage (years)	Whole PTH/Intact PTH (pg/ml)	Size of the largest gland (mm)	Characteristics
Tanaka *et al.* (2005) [17]	67	M	8	840/770	18 × 16	Single enlarged gland
Arakawa *et al.* (2006) [18]	61	F	32	648/270	20	Recurrent HPT due to ectopic parathyroid gland in the mediastinum
Komaba *et al*. (2008) [19]	59	M	2	1010/792	23 × 17 × 15	Spontaneous remission due to autoinfarction of the single enlarged gland
Case 1 (PR)	55	M	3	614/528	14 × 11 × 7	Single enlarged gland
Case 2 (PR)	58	F	12	704/685	18 × 14 × 14	Single enlarged gland
Case 3 (PR)	60	M	6	801/612	23 × 21 × 21	Single enlarged gland
Case 4 (PR)	54	M	33	358/278	17 × 7 × 3	Recurrent HPT due to autograft hyperplasia
Case 5 (PR)	39	F	18	4490/3600[a]	17 × 16 × 5	Recurrent HPT due to autograft hyperplasia

HPT, hyperparathyroidism; PR, present report.
[a]Whole and intact PTH levels were measured in the sera obtained from the graft-bearing arm.

PTH/intact PTH ratio is also a distinct possibility in severe type of secondary hyperparathyroidism. Surgical parathyroidectomy may be required for such patients with the reversed whole PTH/intact PTH ratio and enlarged parathyroid gland.

The physiological role of N-PTH is controversial. Recent clinical data suggest that this new form of PTH has a significant biological effect on bone turnover [18,19]. The present cases also showed relatively high serum levels of bone alkaline phosphatase compared with intact PTH levels. In clinical practice, we should remember that intact PTH assays usually overestimate the real activity of PTH on bone, as previously shown [24], but they also occasionally underestimate its activity, as the present cases. With regard to its structure, this molecular form reacts poorly in intact PTH assays, having early 12–18 or 12–24 epitopes, but normally in the Roche Elecsys assay with a 26–32 epitope, suggesting a modification in the region 15–20 [14–16,22]. The only post-translational modification ever described is phosphorylation in the N-terminal region [25]. Thus, several investigators postulated that N-PTH could be phosphorylated on Ser17 [14,16]. In our patients, however, the values of the whole PTH assay were abnormally higher than those of the Elecsys assay with a 26–32 epitope. The precise reason for this finding is unknown, but one possible explanation is diversity in the mode of modification of this molecule.

In conclusion, we report a series of five exceptional cases with the reversed whole PTH/intact PTH ratio. All cases demonstrated progressive enlargement of the parathyroid gland. It is speculated that the enlarged parathyroid gland overproduced and secreted a novel form of N-PTH. These cases suggested that the reversed whole PTH/intact PTH ratio or the overproduction of N-PTH is associated with progressive hyperparathyroidism. Moreover, the abnormally elevated whole PTH/intact PTH ratio, even if it is not reversed, might indicate progressive hyperparathyroidism [26]. Further studies are required to elucidate the clinical significance of the reversed whole PTH/intact PTH ratio in haemodialysis patients.

Acknowledgements. The authors acknowledge Dr Toyonori Tsuzuki (Department of Pathology, Nagoya Second Red Cross Hospital, Nagoya, Japan) for performing immunohistochemical analysis and Mr Yasushi Shimizu (Motomachi HD Clinic, Kobe, Japan) for his expert technical assistance.

Conflict of interest statement. None declared.

References

1. Goodman WG, Juppner H, Salusky IB *et al*. Parathyroid hormone (PTH), PTH-derived peptides, and new PTH assays in renal osteodystrophy. *Kidney Int* 2003; 63: 1–11.
2. Brown RC, Aston JP, Weeks I *et al*. Circulating intact parathyroid hormone measured by a two-site immunochemiluminometric assay. *J Clin Endocrinol Metab* 1987; 65: 407–414
3. Blind E, Schmidt-Gayk H, Scharla S *et al*. Two-site assay of intact parathyroid hormone in the investigation of primary hyperparathyroidism and other disorders of calcium metabolism compared with a midregion assay. *J Clin Endocrinol Metab* 1988; 67: 353–360
4. Brossard JH, Cloutier M, Roy L *et al*. Accumulation of a non-(1–84) molecular form of parathyroid hormone (PTH) detected by intact PTH assay in renal failure: importance in the interpretation of PTH values. *J Clin Endocrinol Metab* 1996; 81: 3923–3929
5. Lepage R, Roy L, Rousseau L *et al*. A non-(1–84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples. *Clin Chem* 1998; 44: 805–809
6. Brossard JH, Cardinal H, Roy L *et al*. Influence of glomerular filtration rate on intact parathyroid hormone levels in renal failure patients: role of non-(1–84) PTH detected by intact PTH assays. *Clin Chem* 2000; 46: 697–703
7. Divieti P, John MR, Juppner H *et al*. Human PTH-(7–84) inhibits bone resorption *in vitro* via actions independent of the type 1 PTH/PTHrP receptor. *Endocrinology* 2002; 143: 171–176
8. Langub MC, Monier-Faugere MC, Wang G *et al*. Administration of PTH-(7–84) antagonizes the effects of PTH-(1–84) on bone in rats with moderate renal failure. *Endocrinology* 2003; 144: 1135–1138
9. John MR, Goodman WG, Gao P *et al*. A novel immunoradiometric assay detects full-length human PTH but not amino-terminally truncated fragments: implications for PTH measurements in renal failure. *J Clin Endocrinol Metab* 1999; 84: 4287–4290
10. Gao P, Scheibel S, D'Amour P *et al*. Development of a novel immunoradiometric assay exclusively for biologically active whole parathyroid

hormone (1–84): implication for improvement of accurate assessment of parathyroid function. *J Bone Miner Res* 2001; 16: 605–614
11. Nakanishi S, Kazama JJ, Shigematsu T *et al*. Comparison of intact PTH assay and whole PTH assay in long-term dialysis patients. *Am J Kidney Dis* 2001; 38(Suppl 1): S172–S174
12. Inaba M, Nakatsuka K, Imanishi Y *et al*. Technical and clinical characterization of the Bio-PTH(1–84) immunochemiluminometric assay and comparison with a second-generation assay for parathyroid hormone. *Clin Chem* 2004; 50: 385–390
13. Souberbielle JC, Boutten A, Carlier MC *et al*. Inter-method variability in PTH measurement: implication for the care of CKD patients. *Kidney Int* 2006; 70: 345–350
14. D'Amour P, Brossard JH, Rousseau L *et al*. Amino-terminal form of parathyroid hormone (PTH) with immunologic similarities to hPTH(1–84) is overproduced in primary and secondary hyperparathyroidism. *Clin Chem* 2003; 49: 2037–2044
15. Rakel A, Brossard JH, Patenaude JV *et al*. Overproduction of an amino-terminal form of PTH distinct from human PTH(1–84) in a case of severe primary hyperparathyroidism: influence of medical treatment and surgery. *Clin Endocrinol* 2005; 62: 721–727
16. Rubin MR, Silverberg SJ, D'Amour P *et al*. An N-terminal molecular form of parathyroid hormone (PTH) distinct from hPTH(1–84) is overproduced in parathyroid carcinoma. *Clin Chem* 2007; 53: 1470–1476
17. Tanaka M, Itoh K, Matsushita K *et al*. Normalization of reversed bio-intact-PTH(1–84)/intact-PTH ratio after parathyroidectomy in a patient with severe secondary hyperparathyroidism. *Clin Nephrol* 2005; 64: 69–72
18. Arakawa T, D'Amour P, Rousseau L *et al*. Overproduction and secretion of a novel amino-terminal form of parathyroid hormone from a severe type of parathyroid hyperplasia in uremia. *Clin J Am Soc Nephrol* 2006; 1: 525–531
19. Komaba H, Takeda Y, Abe T *et al*. Spontaneous remission of severe hyperparathyroidism with normalization of the reversed whole PTH/intact PTH ratio in a haemodialysis patient. *Nephrol Dial Transplant* 2008; 23: 1760–1762
20. Tominaga Y, Tsuzuki T, Uchida K *et al*. Expression of PRAD1/cyclin D1, retinoblastoma gene products and Ki67 in parathyroid hyperplasia caused by chronic renal failure versus primary adenoma. *Kidney Int* 1999; 55: 1375–1383
21. Schlosser K, Sitter H, Rothmund M *et al*. Assessing the site of recurrence in patients with secondary hyperparathyroidism by a simplified Casanova autograftectomy test. *World J Surg* 2004; 28: 583–588
22. D'Amour P, Brossard JH, Rakel A *et al*. Evidence that the amino-terminal composition of non-(1–84) parathyroid hormone fragments starts before position 19. *Clin Chem* 2005; 51: 169–176
23. D'Amour P. Circulating PTH molecular forms: what we know and what we don't. *Kidney Int* 2006; 70(Suppl 102): S29–S33
24. Quarles LD, Lobaugh B, Murphy G. Intact parathyroid hormone overestimates the presence and severity of parathyroid-mediated osseous abnormalities in uremia. *J Clin Endocrinol Metab* 1992; 75: 145–150
25. Rabbani SA, Kremer R, Bennett HP *et al*. Phosphorylation of parathyroid hormone by human and bovine parathyroid glands. *J Biol Chem* 1984; 259: 2949–2955
26. Tanaka M, Komaba H, Itoh K *et al*. The whole-PTH/intact-PTH ratio is a useful predictor of severity of secondary hyperparathyroidism. *NDT Plus* 2008; Suppl 3: iii59–62

Received for publication: 5.2.08
Accepted in revised form: 29.2.08