Clinical Report

Decreased expression of calcium-sensing receptor and parafibromin in secondary hyperparathyroidism with an abnormal whole PTH/intact PTH ratio

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Abstract
A 34-year-old female hemodialysis patient with an abnormal whole parathyroid hormone (PTH)/intact PTH ratio (ratio = 1.14) underwent parathyroidectomy for advanced secondary hyperparathyroidism. The abnormal PTH ratio indicated a relative increase in the serum N-terminus PTH, a new molecular form of PTH. The abnormal ratio normalized immediately after the largest parathyroid gland (PTG) was removed, proving the largest PTG as causative. An immunohistochemical analysis revealed extremely decreased expression of the calcium-sensing receptor and parafibromin in the largest PTG in comparison with the other PTGs. This case indicated the possible involvement of decreased calcium-sensing receptor and parafibromin signaling in the development of the abnormal PTH ratio.

Keywords: calcium-sensing receptor; N-terminus PTH; parafibromin; secondary hyperparathyroidism

Introduction
The accurate measurement and appropriate control of the serum parathyroid hormone (PTH) concentration is essential in the management of uremic secondary hyperparathyroidism (SHPT) [1]. The so-called third generation PTH assay that can exclusively detect PTH (1–84), a full-length PTH fragment, has become widely available [2]. The value measured by the second generation assay is usually higher than that measured by the third generation assay, because the former assay can detect both PTH (1–84) and PTH (7–84), [3]. As a result, the whole PTH/intact PTH ratio is theoretically <1.0.

Several investigators have recently reported that there are quite a few patients with hyperparathyroidism or parathyroid cancer, whose whole PTH/intact PTH ratio is >1.0 [4–7]. The relative increase of N-terminus PTH (N-PTH) is thought to be the cause of the abnormal PTH ratio in these rare cases. N-PTH, a full-length PTH (1–84) first identified through high-performance liquid chromatography fractionation by D’Amour et al. [8], is reported to have post-translational modification at the site where the first antibody to the intact PTH assay recognizes [7, 9]. The whole PTH assay can detect N-PTH, but the intact PTH assay cannot because of the structural modification of N-PTH and the difference in the recognition sites between intact PTH assay and whole PTH assay [8]. Thus, the abnormal PTH ratio can theoretically occur in cases with high serum N-PTH value compared with serum PTH (7–84). However, whether there are any specific biological and histological features in the causative parathyroid gland (PTG) showing the abnormal PTH ratio remains undetermined.

In this report, we describe a hemodialysis patient with SHPT whose abnormal whole PTH/intact PTH ratio became normalized immediately after the largest PTG was surgically removed. We also discuss the possible underlying mechanism of the abnormal whole PTH/intact PTH ratio by comparing the immunohistochemical features of the causative PTG with those of the other PTGs in the patient.

Case
A 34-year-old woman under 5 years of hemodialysis therapy was hospitalized for the treatment of uncontrollable SHPT. Total parathyroidectomy with forearm autotransplantation was scheduled, because intravenous calcitriol therapy over 4 years failed to control SHPT. The corrected serum calcium (Ca) at the time of admission was 3.1 mmol/L, phosphorus 2.0 mmol/L, alkaline phosphatase (ALP) 345 U/L, bone-specific ALP 35.6 U/mL, intact PTH 84.9 pmol/L (801 pg/mL) (Elecsys PTH; Roche Diagnostics), whole PTH 96.6 pmol/L (901 pg/mL) (whole PTH; Scantibodies Laboratories); the whole PTH/intact PTH ratio was 1.14. Neck ultrasonography revealed four PTGs,
while no ectopic PTGs were detected by $^{99m}$Tc-methoxyisobutyrylisonitrile scintigraphy. Bone scintigraphy disclosed markedly increased bone turnover in the systemic bone.

Total parathyroidectomy resected all the four PTGs in the order of PTG4, PTG3, PTG2 and PTG1: the smallest gland (PTG1) was auto-transplanted in the right forearm (Figure 1A). The patient’s serum was obtained 5 min after each PTG was removed; each PTG was resected at an ∼5 min interval. The weight and macroscopic photographs of each PTG are presented in Figure 1A. The serial changes in the serum whole PTH, intact PTH and the whole PTH/intact PTH ratio are shown in Figure 1B. The PTH ratio decreased immediately after the left lower PTG (PTG4) was removed, and the ratio was maintained at <1.0 after all the four PTGs were resected (ratio: 1.14 → 0.41). The serum whole PTH and intact PTH was 3.0 pmol/L (29.7 pg/mL) and 6.1 pmol/L (58 pg/mL), respectively, 3 years after surgery; the PTH ratio was 0.48.

Histopathologic examination of the four resected PTGs was performed. Light microscopy with hematoxylin–eosin staining showed that the smallest PTG (PTG1) was diffuse hyperplastic, and the remaining three PTGs were nodular hyperplastic (Figure 2A). Immunohistochemistry was performed for the following PTG-related proteins: Ca-sensing receptor (CaSR), vitamin D receptor, Ki-67, parafosbin and galectin-3. The number of Ki-67-positive cells was greater and the galectin-3 positive areas were larger in PTG3 and PTG4 than those observed in PTG1 and PTG2 (Supplementary Figure S1A and B). As for the vitamin D receptor, the expression of VDR in PTG3 and PTG4 was decreased compared with that observed in PTG1 and PTG2 (Supplementary Figure S1C). However, these markers could not be used to differentiate between PTG3 and PTG4. As shown in Figure 2B and C, the expression of CaSR and parafosbin in PTG4 was extremely diminished in comparison with the other three PTGs, distinguishing PTG4 from other PTGs and indicating the possible involvement of CaSR and parafosbin in the pathogenesis of the abnormal PTH ratio in this patient.

Discussion

N-PTH is constitutively produced and secreted into the serum in the normal and proliferative PTGs, although the amount of N-PTH production is relatively small [10]; the proportion of N-PTH among all forms of serum PTH fragments is usually low, and the whole PTH/intact PTH is <1.0 [11]. N-PTH can only be detected by the third-generation assay, not by the second-generation assay [6] because 10–20 amino acid sites from the N-terminus are reported to be modified by unknown mechanisms [7, 9]. Therefore, the amount of N-PTH is theoretically higher than that of PTH (7–84) in patients with an abnormal whole PTH/intact PTH ratio. Although the current study did not directly demonstrate the increase of N-PTH by high-performance liquid chromatography, the normalization of the whole PTH/intact PTH ratio after removal of the largest PTG strongly suggests that the largest PTG was secreting a relatively large amount of N-PTH, thus leading to the abnormal PTH ratio.

The possible mechanisms of the abnormal whole PTH/intact PTH ratio can be theoretically due to either hypersecreetion of N-PTH in the PTG or decreased clearance of serum N-PTH. The present case demonstrated that the whole PTH/intact PTH ratio was normalized after the removal of largest PTG, indicating that the clearance of N-PTH from serum did not contribute to the abnormal PTH ratio. D’Amour et al. [10] reported in their in vitro study that low Ca concentration in the medium increases the proportion of N-PTH and decreases the proportion of PTH (1–84), followed by the increase of the whole PTH/intact PTH ratio. In addition, Komaba et al. [6] reported that cinacalcet hydrochloride, an allosteric modulator of CaSR, lowers the whole PTH/intact PTH ratio, and the ratio returns to an abnormal level after the discontinuation of cinacalcet treatment, thereby indicating the involvement of CaSR signaling in the modulation of the whole PTH/intact PTH ratio. The expression of CaSR as determined by immunohistochemistry was extremely reduced in the largest PTG in the current case, suggesting that CaSR signaling could have been decreased in the same PTG, leading to the increase of the whole PTH/intact PTH ratio. Although it is undetermined how CaSR signaling is involved in the regulation of the whole PTH/intact PTH ratio, one possibility is that CaSR signaling plays a crucial role in the degradation of PTH fragment or in the stabilization of PTH mRNA.

The diminished expression of parafosbin can partially explain the mechanism of the abnormal whole PTH/intact PTH ratio. Parafosbin is a protein that regulates the cell cycle by suppressing the expression of Cyclin D1 and other cell cycle-dependent proteins [12]. The loss of parafosbin is also reported to be involved in the development of sporadic parathyroid carcinoma and primary hyperparathyroidism [13, 14]. In some cases with parathyroid carcinoma, a diminished expression of parafosbin in the glands with the abnormal whole PTH/intact PTH ratio was

Fig. 1. (A) A schematic presentation of the four parathyroid glands and their macroscopic photographs and weights measured after parathyroidectomy. (B) The serial changes in the intact PTH, whole PTH and whole PTH/intact PTH ratio during surgery. PTH, parathyroid hormone; PTG, parathyroid gland.
reported, indicating the possible involvement of parafibromin in the pathogenesis of the abnormal PTH ratio. Furthermore, the loss of parafibromin is also highly associated with downregulation of CaSR in parathyroid carcinoma [15]. In addition, a mouse model of Cyclin D1 overexpression in the PTG demonstrated CaSR downregulation in the PTG [16]. These results suggest that a diminished expression of parafibromin in the PTG decreases CaSR signaling via Cyclin D1 upregulation, leading to a decrease in N-PTH degradation and a relative increase in the N-PTH fraction and the whole PTH/intact PTH ratio.

Why only limited cases exhibit abnormal PTH levels remains unclear, although the CaSR on the PTG is often observed to decrease in patients with severe SHPT. One possible factor is that the pattern of the whole PTH/intact PTH ratio in SHPT patients has not been fully investigated. Indeed, recent clinical studies have shown that many SHPT patients exhibit an inverse whole PTH/intact PTH ratio [17]. Another possible explanation is that a decrease in the CaSR protein expression is only a necessary condition, not a sufficient condition. Hence, we speculate that parafibromin is a possible factor that is directly or indirectly involved in the pathogenesis of the abnormal whole PTH/intact PTH ratios in SHPT patients.

Finally, the biologic features of N-PTH remain to be elucidated. Previous reports show that patients with an abnormal PTH ratio tend to show relatively high-bone turnover with severe hyperparathyroidism [5, 6]. The bone turnover as demonstrated by the serum bone-specific ALP level and bone scintigraphy seemed high in the present case. Although there has been no experimental data that examined the biologic activity of N-PTH, given that PTH (7–84) was historically identified as having antagonistic action against the PTH receptor [18], it is plausible that N-PTH has an unknown biologic effect apart from PTH (1–84).

The present study is associated with several limitations. First, we did not measure the ionized Ca level during PTX. Because the ionized Ca level can influence the whole PTH/intact PTH ratio, a further evaluation of the effects of ionized Ca on serial changes in the PTH ratio during PTX should be conducted. Secondly, we cannot explain why calcimimetics do not affect the whole PTH/intact PTH ratio in patients with parathyroid carcinoma [7]. Finally, this study consisted of only one patient with an inverse PTH ratio. Therefore, the present immunohistochemical results cannot be easily generalized to other patients with the inverse whole PTH/intact PTH ratios, and causal inference remains in the range of speculation. Further studies are needed to clearly determine the effects of CaSR and parafibromin in the pathogenesis of the abnormal whole PTH/intact PTH ratios in dialysis patients.

In summary, this report presented the case of an SHPT patient with an abnormal whole PTH/intact PTH ratio, which normalized immediately after the largest PTG was surgically resected. An immunohistochemical analysis revealed that the expression of the CaSR and parafibromin were extremely decreased in the most advanced form of PTG. This case supports the hypothesis that a diminished expression of the CaSR and parafibromin in the advanced

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**Fig. 2.** Histologic and immunohistochemical characteristics of the four resected parathyroid glands. (A) Hematoxylin-eosin staining (original magnification ×1). Immunohistochemical staining of (B) the calcium-sensing receptor (original magnification ×200) and (C) parafibromin (original magnification ×100, ×200). PTG, parathyroid gland.
form of PTG is involved in the pathogenesis of an abnormal whole PTH/intact PTH ratio in severe SHPT patients.

Supplementary data

Supplementary data is available online at http://ckj.oxfordjournals.org.

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Conflict of interest statement. None declared.

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