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Critical issues of PTH assays in CKD

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ABSTRACT

Measurement of bioactive parathyroid hormone (PTH) is essential for the optimal management of secondary hyperparathyroidism and its associated bone disorders in chronic kidney disease (CKD) patients. For this purpose, three generations of increasingly specific PTH assays have been developed over the last 4 decades. To date, however, only second-generation PTH assays are most widely used, although these have been shown to cross-react with large PTH fragments having a partially preserved N-structure, mostly PTH(7-84). The newly developed third-generation PTH assays are believed to be the most specific means of measuring PTH (1-84), but their clinical utility remains debatable. More recently, these latter assays have also been shown to react with a new N-form of PTH, which has been identified in patients with severe hyperparathyroidism and parathyroid carcinoma. Progressive research in this area has advanced our understanding considerably regarding the circulating molecular forms of PTH and their pathophysiological roles in bone abnormalities associated with CKD. However, developing an ideal PTH assay continues to be difficult because of key issues such as the reliability of PTH as a surrogate marker for bone turnover, practicality of employing third-generation PTH assays, and unknown biological implications of N-PTH and other PTH fragments. Further research exploring these issues is mandatory to understand and optimally manage parathyroid disorders and bone abnormalities in CKD patients.

Introduction

Parathyroid hormone (PTH), a polypeptide comprising 84 amino acids, is secreted by the chief cells of the parathyroid gland [1]. It plays a critical role in regulating various processes involved in skeletal and mineral homeostasis, including bone remodeling, renal calcium and phosphate handling, and renal calcitriol production. Binding of the N-structure to the type 1 PTH/PTHrP receptor is responsible for the classical biological effects of PTH on the bone and kidney [2,3]. The C-terminal region of PTH binds to a different C-PTH receptor and exerts biological actions that are opposite to the classical effects of PTH [4,5]. Circulating PTH released from parathyroid glands is rapidly metabolized in peripheral tissues. Having a half-life of only 2 to 4 min, it is degraded to form various C-terminal fragments. The half-life of these fragments is five to ten times longer than that of full-length PTH(1-84), and they constitute 80% of the total circulating PTH molecules in normal individuals [6–9].

In patients with chronic kidney disease (CKD), several factors such as phosphate retention, hypocalcemia, and calcitriol deficiency stimulate PTH secretion and lead to secondary hyperparathyroidism [10–13]. In addition, it has recently been suggested that fibroblast growth factor 23 (FGF23) plays a crucial role in the development of secondary hyperparathyroidism. In patients with CKD, a progressive increase in FGF23 secretion promotes phosphaturia as the kidney function declines, but this occurs at the expense of suppressed calcitriol synthesis. In advanced CKD, however, the net amount of phosphate excretion does not increase despite high FGF23 levels. Subsequently, high serum phosphate levels and decreased calcitriol levels further stimulate PTH secretion, resulting in the development of secondary hyperparathyroidism [14–16].

The pathological consequences of persistent hyperparathyroidism and its associated mineral disorders have a significant impact on bone abnormalities, cardiovascular calcification, and mortality [17–19]. Accurate measurement of bioactive PTH is therefore essential for the optimal management of secondary hyperparathyroidism [20]. However, a major problem is the difficulty in specifically measuring bioactive PTH(1-84), without confusing them with C-terminal fragments that constitute 95% of the total circulating PTH molecules in renal failure [8,9,21]. To achieve this goal, increasingly specific assays have been developed to measure full-length PTH(1-84).

Evolution of assays for accurate assessment of PTH

Advances in chemical and molecular biology have led to the development of three generations of PTH assays over the last 4 decades [22,23]. First-generation PTH assays were first implemented in 1963 [24]. They used a single antibody with epitopes in the mid- and C-terminal regions of PTH in radioimmunoassay procedures. However, since these assays detected numerous PTH fragments along with PTH (1-84), they had limited clinical value.

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Second-generation PTH assays, also called "intact" PTH assays, have been developed since 1987 [25]. These assays use two different antibodies. The first antibody is a solid-phase capture antibody against the C-terminal region of PTH (amino acids 39–84), and the second is a revealing antibody directed against the N-terminal region (amino acids 13–34 as in the Allegro Intact PTH assay from Nichols Institute Diagnostics or 12–18 as in the Total Intact PTH assay from Scantibodies Laboratory Inc.). These newer assays were purported to detect only the intact PTH molecule and have been widely used for the management of parathyroid and bone abnormalities in CKD [20]. These second-generation assays, however, were eventually proven to react with large PTH fragments having a partially preserved N-structure, mostly PTH(7-84) (Fig. 1)[21]. This is of particular relevance in dialysis patients in whom such fragments accumulate and account for up to 45–50% of all circulating PTH molecular forms [8,9,21,26]. Moreover, PTH(7-84) is not an inert compound since it has been shown to antagonize the calcemic and bone-resorbing effects of PTH(1-84) [27,28].

To overcome these shortcomings, the development of third-generation PTH assays has been underway since 1999 [29,30]. These "whole" PTH or "bio-intact" PTH assays use the same first antibody as that used in second-generation PTH assays, but the second antibody is specific for the region 1–4 or 1–5. These assays have proven to be more specific than intact PTH assays for measuring full-length PTH(1-84). However, despite their greater specificity, PTH measurements obtained with third-generation PTH assays have been shown to have a high correlation with those obtained with second-generation PTH assays in several studies [31,32]. Therefore, whether third-generation PTH assays are actually superior in the clinical setting compared to second-generation assays has been a point of great contention.

Inter-assay variability between the numerous PTH assays

The target serum PTH levels recommended in the K/DOQI guidelines were based on the Nichols Allegro Intact PTH assay [20]. However, this assay is no longer commercially available and has now been replaced by several other intact PTH assays. These assays are widely used to evaluate parathyroid function and bone turnover in dialysis patients. However, several recent studies have reported a marked inter-method variability in the evaluation of circulating PTH levels measured using these assay kits. This is primarily due to both assay-specific standardization regimens and detecting antibodies that recognize not only PTH(1-84) but also the other molecular fragments of PTH [33,34]. These findings imply that we may under- or overestimate parathyroid function depending on the PTH assay chosen. To further complicate matters, PTH results differ depending on whether the samples are measured in serum, EDTA plasma, or citrate plasma [34–36]. These facts can potentially influence clinical decisions, and clinicians should keep these issues in mind when evaluating parathyroid function with these kits.

Second- versus third-generation PTH assays in CKD patients

Surrogate marker for bone disease in CKD

Until recently, second-generation PTH assays have been used as a surrogate marker of bone turnover in patients with CKD. The use of these assays for this purpose was reinforced by the K/DOQI guidelines [20], which set target intact PTH values with supposedly normal bone turnover based on bone histomorphometric studies [37–39].

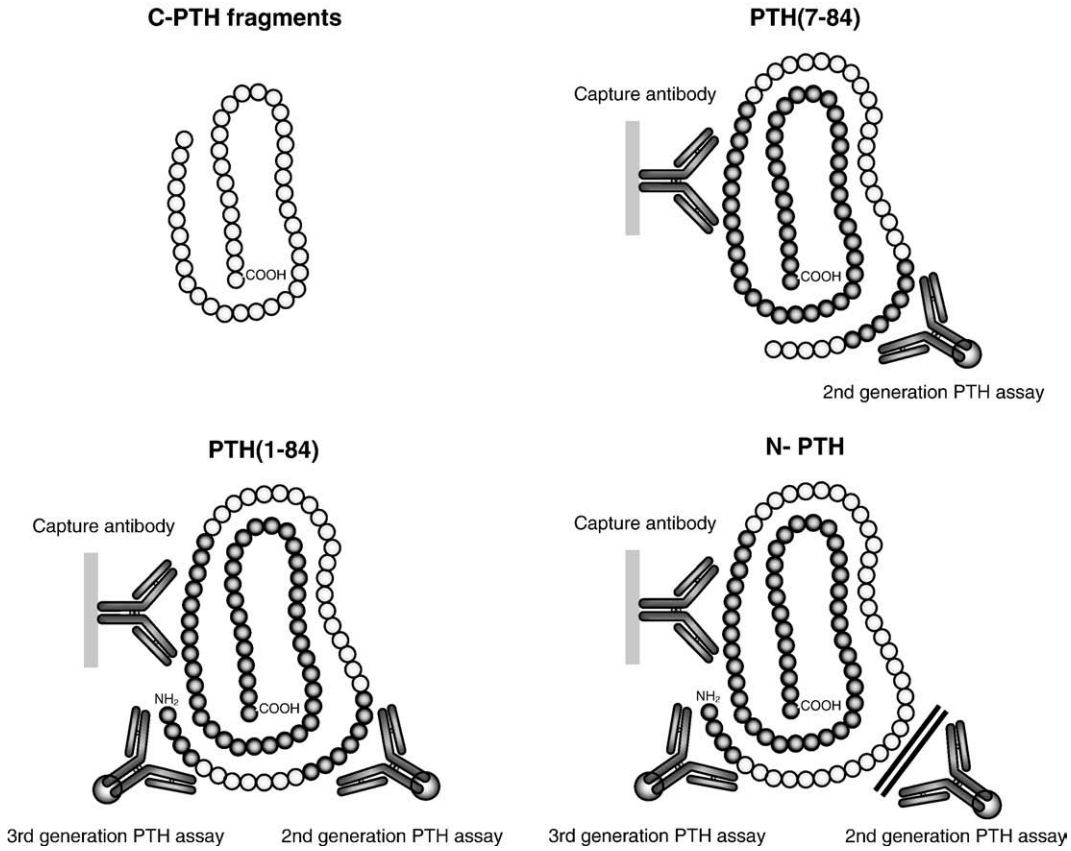

Fig. 1. Detection of various molecular forms of PTH by second- and third-generation PTH assays. C-PTH fragments, starting from residues 34, 37, 41, and 43, can be measured only with first-generation PTH assays such as mid- or C-terminal PTH assays. Second- and third-generation PTH assays do not react with these fragments. PTH(7-84), a non-PTH(1-84) fragment having a partially preserved N-structure, cross-reacts with second-generation PTH assays, resulting in an overestimation of intact PTH levels. PTH(1-84) is a full-length, bioactive form of PTH and can be detected with each generation of PTH assay. Third-generation PTH assays, however, can most specifically measure PTH(1-84) levels. N-PTH is a new molecular form of PTH, which is overproduced in rare cases of severe hyperparathyroidism. This molecule is also detected by third-generation PTH assays, but is less reactive with second-generation PTH assays. This is presumably due to a post-translational modification in the region 15–20.