ORIGINAL ARTICLE

Full-length versus intact PTH concentrations in pseudohypoparathyroidism type 1 and primary hyperparathyroidism: clinical evaluation of immunoassays in individuals from China

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Abstract

Purpose The application of the third-generation parathyroid hormone (PTH) assay [PTH(1–84) assay] for evaluating PTH levels in patients with pseudohypoparathyroidism type-1 (PHP1) is less popular than the second-generation assay. Therefore, we aimed at examining the conformity between the PTH(1–84) assay and the intact PTH (iPTH) assay, specifically examining their performance in individuals with PHP1 versus individuals with primary hyperparathyroidism (PHPT), compared to healthy controls.

Methods PTH(1–84) and iPTH assay were performed in patients with PHP1, patients with PHPT, and healthy volunteers. ΔPTH%, PTH(1–84)/iPTH ($3^{rd}/2^{nd}$ ratio), iPTH/upper limit of normal (ULN), and PTH (1–84)/ULN of each group were calculated for comparison. Linear regression, Kappa conformity test, and Bland–Altman analysis of ΔPTH/mean of iPTH and PTH(1–84) (percent bias) plotted against the mean of iPTH and PTH(1–84) were performed to determine the conformance of PTH(1–84) assay with iPTH assay.

Results A total of 54 patients with PHP1, 127 patients with PHPT, and 65 healthy volunteers were enrolled in this study. All the three groups showed strong linear relationship between iPTH and PTH (1–84) ($r^2 = 0.9661$, 0.7733, and 0.9575, respectively). No significant differences were noted in $3^{rd}/2^{nd}$ ratio (median 0.76 vs. 0.72) between the PHP1 and PHPT groups ($p > 0.05$). Conformity examination showed the Kappa value was 0.778 and 0.395 for PHP1 and PHPT groups respectively. No difference in the Kappa values was found between PHP1A and PHP1B subgroups. Bland–Altman plot demonstrated that the proportion of data points that were plotted within mean ± 1.96 SD in PHP1, PHPT and normal control groups were 96.3%, 93.7%, and 98.5%, respectively. The mean percent bias of the three groups were 26.1%, 31.2%, and 17.0%, respectively. The range of mean ± 1.96 SD of percent bias of the three groups were 2.2%–50.0%, −14.3%–76.6%, and 6.7%–27.2%, respectively.

Conclusion Although iPTH and PTH(1–84) values were both lower in the present PHP1 cohort than in the PHPT cohort, there appear to be differences in the relative agreement between both immunoassays, and in the relationship between the two values, especially in comparison to healthy controls. Whether these differences are due to differential accumulation of C-terminal fragments or other factors requires further study.

Keywords Pseudohypoparathyroidism · PTH assay · Third-generation PTH assay

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Background

Parathyroid hormone (PTH) secreted by the chief cell of the parathyroid is an important regulator that maintains the homeostasis of calcium and phosphate, and its concentration in circulation is mainly regulated by extracellular calcium. The full-length PTH (1–84) that is the biologically active PTH is detectable, along with various fragments such as the C-terminal fragment [1, 2]. The proportion of fragments in total detectable PTH and the ratio to full-length PTH varies depending on the different physical and pathological statuses.

After the establishment of the immunoassay of PTH, detection methods have advanced over time. The second-generation immunoassay (intact PTH [iPTH] assay) uses two groups of antibodies against the C-terminal and N-terminal of PTH. Because the cross-reactivity with smaller fragments is lower compared with that in the first-generation assay, the specificity of the iPTH assay is greatly improved. The N-terminal antibody is not directed to the first six amino acids; therefore, the results of the iPTH assay can be considered to detect the total amount of full-length PTH and some large C-terminal fragments including PTH(7–84) as the main source [3]. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is another promising technique. It is more specific but less sensitive compared with other immunoassays [4]. The more recent third-generation assay of PTH retains the C-terminal antibody used in the second-generation assay, but the N-terminal antibody is coupled with the epitope of the first few amino acids, which eliminates the interference of PTH fragments and makes the detection of only full-length PTH (1–84) possible [5].

The effectiveness and sensitivity of different PTH assays have been reported in many clinical trials and investigations, mostly in chronic renal disease and primary hyperparathyroidism [6]. Primary hyperparathyroidism (PHPT) is a disorder featured by autonomous secretion of PTH by parathyroid adenoma or hyperplastic parathyroid. Both intact and full-length PTH assays have been used for the diagnosis of the disease, though opinions varied on which assay is better at evaluating increased PTH levels. Based on the results of four clinical studies [5–8], the international workshop on diagnosing asymptomatic PHPT suggested that both PTH assays are good at performance because they showed similar sensitivity and suggested using assay-specific reference ranges for evaluation [9]. However, one study using high-performance liquid chromatography (HPLC) recommended the full-length PTH assay because patients with PHPT had a higher proportion of PTH fragments [5]. Patients with pseudohypoparathyroidism type 1 (PHP1) also present with increased serum PTH levels; however, the main difference between the two diseases is that PHP1 patients bear a significantly lower level of serum calcium, which is the result of resistance in PTH receptors on proximal renal tubule. Different pathogenesis suggested a different PTH secretion pattern between the two diseases but data on using a different PTH assay in patients with PHP1 is limited [10]. An excelling assay for PTH concentration evaluation should be selected.

In this study, the levels of iPTH and PTH (1–84) were tested simultaneously in a relatively large population of Chinese patients with PHP1. The results were compared with those in patients with PHPT and healthy volunteers, which provided more evidence on the way to assess PTH levels under different circumstances for clinicians.

Method

Patients and materials

PHP1 and PHPT patients were recruited at the outpatient division of the Endocrinology Department, Peking Union Medical College Hospital. All clinical investigations and management were conducted in accordance with the standard clinical procedures at our center. The clinical diagnosis of PHP1 was established upon hypocalcemia, elevated PTH level, and typical clinical manifestations such as epilepsy, tetany, ectopic ossification, and short metacarpals. The exclusion criteria of PHP1 patients included the following: patients with idiopathic or secondary hypoparathyroidism, secondary hyperparathyroidism, abnormal renal function, severe liver failure, and/or on medications that could affect calcium/phosphorus metabolism, except for calcium and vitamin D. All of the PHP1 patients had received treatment of vitamin D agents combined with calcium to relieve their hypocalcemia symptoms. PHPT was diagnosed based on hypercalcemia (serum calcium >2.70 mmol/L or ionized calcium >1.28 mmol/L) with an uninhibited PTH level. The exclusion criteria of PHPT patients included patients with secondary hyperparathyroidism, post-parathyroidectomy patients, severe liver failure, with an estimated glomerular filtration rate (eGFR) <60 mL/(min·1.73 m^2), and/or the usage of medications that could affect calcium/phosphorus metabolism, except for calcium and vitamin D. The normal control group was recruited from healthy volunteers who had visited our hospital for routine physical examination in a gender-matching pattern with the PHP1 group. Volunteers with metabolic bone disease, severe liver or renal failure, cardiovascular disease, diabetes mellitus, or obesity were excluded. All patients were informed about the sample collection as well as the genetic analysis for PHP1 patients to be conducted in this study. Written informed consent was obtained from the participants or their guardians for genetic analysis. This study was approved and supervised by the local ethics committee of PUMCH.

Serum samples from the peripheral blood of PHP1 and PHPT patients were collected in silicon-coated tubes (367812, BD Vacutainer® Blood Collection Tubes, BD, US). The PTH(1–84) assay was applied in a clinical routine test at our center on November 7, 2020. Before that, the serum samples were collected and stored under −80 °C to be tested simultaneously in the same batch for iPTH and PTH(1–84). After November 7, 2020, the PHP1 and PHPT patients had their iPTH and PTH(1–84) levels tested simultaneously immediately after sample collection.

The peripheral blood samples were collected from PHP1 patients (367856, BD Vacutainer® Blood Collection Tubes, BD, US), from which DNA samples were extracted using a commercial kit (D3494, E.Z.N.A. Blood DNA Midi Kit, Omega Bio-tek, US). Confirmation and subtyping of PHP1 were conducted using DNA samples by conducting methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) and *GNAS* analysis (as described elsewhere [11, 12]). Patients of PHP1 with clear molecular subtyping were included in our research, and their serum samples were analyzed under simultaneous PTH level evaluation with two different assays.

Measurements

The serum PTH concentration was measured with second-generation (iPTH) (Kit No. 07251068500, Roche Diagnostics, Germany) and third-generation [PTH(1–84)] (Kit No. 07027745190, Roche Diagnostics) assays. For the iPTH assay, a biotinylated monoclonal anti-PTH antibody (mouse) was used against PTH amino acid regions 26–32 and 37–42, and a monoclonal anti-PTH antibody (mouse) labeled with ruthenium was used against the antibody. For PTH(1–84) assay, a biotinylated monoclonal anti-PTH antibody (mouse) was used against PTH amino acid regions 1–5 and 54–59. Measurements were conducted using Cobas e601 analyzer (Roche Diagnostics). The intra-assay and inter-assay coefficients of variation in the two methods were 1.0%–2.6%, 1.8%–3.1%, and 1.6%–3.1%, 3.6%–7.9% respectively. The measuring range for the two assays was 40–4000 pg/mL and 5.5–2300 pg/mL, respectively.

Serum calcium (Ca) (reference range: 2.13–2.70 mmol/L), phosphate (P) (reference range: 0.81–1.45 mmol/L), and creatinine (Cr) (reference range: 45–84 μmol/L) were measured by using a multichannel automatic biochemical analyzer (AU5800; Beckman Coulter, Mishima, Japan). The eGFR level was calculated according to the 2009 CKD-EPI formula.

The concentration of PTH was examined by two different methods—iPTH and PTH(1–84)—as described earlier. To evaluate the differences in the two measurements, ΔPTH, ΔPTH%, $3^{rd}/2^{nd}$ ratio, as well as iPTH/upper-limit of normal (ULN) and PTH(1–84)/ULN were calculated using the following formulas:

$$\Delta PTH = iPTH - PTH(1-84)$$

$$\Delta PTH\% = \frac{\Delta PTH}{iPTH} \times 100\%$$

$$3^{rd}/2^{nd}\ ratio = \frac{PTH(1-84)}{iPTH}$$

$$iPTH/ULN = \frac{iPTH}{ULN\ for\ iPTH\ assay}$$

$$PTH(1-84)/ULN = \frac{PTH(1-84)}{ULN\ for\ PTH(1-84)assay}$$

Statistical analysis

The results were analyzed by SPSS24. All data that were normally distributed were described by the mean and standard deviation. Non-normally distributed data were described as the median (Q1, Q3). Non-continuous variables were described by the number of cases (%). Categorical variables were described by the number of cases (%). A student *t*-test was applied to examine the differences in the groups of normally distributed data, and a non-parametric test was applied to examine the differences between the groups of non-normally distributed data. Simple linear regression was performed to evaluate the correlation between different factors, and a linear regression chart was prepared with SPSS24. Conformity of iPTH and PTH(1–84) for PHP1 and PHPT were examined by the Kappa conformance test and the Bland–Altman analysis; for normal control, conformity was tested by Bland–Altman analysis alone. Bland–Altman analysis was conducted with MedCalc. The Bland–Altman plots were created for each cohort by plotting percent bias [ΔPTH/mean of iPTH and PTH(1–84), vertical axis] against the mean of iPTH and PTH(1–84) (horizontal axis). The proportion of data points within mean ± 1.96 SD of percent bias of the three groups were calculated, the mean and the mean ± 1.96 SD were also analyzed. $P < 0.05$ was considered to indicate statistical significance.

Results

Clinical characteristics of all subjects

A total of 54 patients with PHP1 (28 males and 26 females), 127 patients with PHPT (28 males and 99 females), and 65

Table 1 Basic demographic and biochemical information

	PHP1 ($n = 54$)	PHPT ($n = 127$)	Normal Control ($n = 65$)	P
Age (years)	26.0 ± 11.1	53.8 ± 13.5	$37.0(30.0, 50.0)$	<0.001
Gender (M/F)	28/26	28/99	33/32	<0.001
Serum Ca (mmol/L)	2.18 ± 0.28	$2.67(2.59, 2.80)$	2.65 ± 0.07	<0.001
Serum P (mmol/L)	1.58 ± 0.40	0.90 ± 0.17	1.18 ± 0.12	<0.001
Serum Cr (μmol/L)	68.9 ± 17.4	$59.0(52.0, 69.0)$	72.5 ± 14.5	0.033
eGFR [mL/(min·1.73 m^2)]	119.00 ± 19.49	98.29 ± 17.13	101.80 ± 12.68	<0.001

Serum Ca serum calcium, *reference range* 2.13–2.7 mmol/L, *serum P* serum phosphate, *reference range* 0.97–1.87 mmol/L (<11 years), 0.81–1.53 mmol/L (11–18 years), 0.81–1.45 mmol/L (>18 years), *serum Cr* serum creatinine, *reference range* 45–84 μmol/L, *eGFR* estimated glomerular filtration rate, calculated according to the 2009 CKD-EPI formula

P value indicates the significance of the index between the PHP and PHPT groups. *P* value of <0.05 indicated significant differences between the PHP1 and PHPT groups

Table 2 PTH levels and the differences evaluated by two methods

		PHP1 ($n = 54$)	PHPT ($n = 127$)	Normal control ($n = 65$)
PTH levels	iPTH (pg/ml)	$79.3 (41.9, 141.8)^{a,b}$	$96.3 (71.8, 133.0)^c$	38.8 ± 11.0
	PTH(1–84) (pg/ml)	$57.6 (34.4, 97.4)^{a,b}$	$70.0 (50.9, 95.5)^c$	33.0 ± 9.1
	P_1	<0.001	<0.001	<0.001
PTH/ULN	iPTH/ULN	$1.22 (0.64, 2.18)^{a,b}$	$1.48 (1.11, 2.05)^c$	0.60 ± 0.17
	PTH(1–84)/ULN	$1.11 (0.60, 1.71)^{a,b}$	$1.23 (0.90, 1.68)^c$	0.58 ± 0.16
	P_2	<0.001	<0.001	<0.001
ΔPTH (pg/ml)		$19.4 (7.8, 45.3)^{a,b}$	$24.0 (15.0, 38.8)^c$	$5.6 (4.1, 7.5)$
ΔPTH%		$24.4\% \pm 9.2\%^a$	$27.9\% (18.8\%, 33.8\%)^c$	$15.5\% \pm 17.0\%$
3rd/2nd ratio		0.76 ± 0.09^a	$0.72 (0.66, 0.81)^c$	0.84 ± 0.05

iPTH the level of iPTH. *PTH(1–84)* the level of PTH(1–84). *ΔPTH* the differences between iPTH and PTH(1–84). *iPTH/ULN* iPTH/upper-limit of normal for iPTH assay (65 pg/mL). *PTH(1–84)/ULN* PTH(1–84)/upper-limit of normal for PTH(1–84) assay (56.9 pg/mL). ΔPTH = iPTH − PTH(1–84)

ΔPTH% = (ΔPTH/iPTH) × 100%

Reference ranges: iPTH: 15.0–65.0 pg/mL; PTH(1–84): 14.9–56.9 pg/mL

P_1: comparison of iPTH with PTH(1–84) within PHP1, PHPT, and normal control groups

P_2: comparison of iPTH/ULN with PTH(1–84)/ULN within PHP1, PHPT, and normal control groups

P value of <0.05 was considered to indicate a statistically significant difference

[a]PHP1 group comparing against normal control, $p < 0.05$

[b]PHP1 group comparing against PHPT, $p < 0.05$

[c]PHPT group comparing against normal control, $p < 0.05$

healthy volunteers (33 males and 32 females) were enrolled in this study. The molecular analysis showed that 16 patients with PHP1 had *GNAS* mutation with the subtype of PHP1A. The remaining individuals had methylation alterations in one or more *GNAS* TSS:DMRs; therefore, these 38 individuals were patients with PHP1B.

The distribution of gender, age, biochemical indexes, and eGFR are shown in Table 1. Age and gender ratios were significantly different between the PHP1 and PHPT groups (for both indexes, $p < 0.001$). The gender ratio between PHP1 and normal control groups was similar as the participants in the normal control group were recruited in a gender-matching pattern ($p = 0.809$), whereas a significant difference was found in the age distribution between the two groups ($p < 0.001$). All the participants in our study possessed a normal renal function of eGFR ≥ 60.00 mL/(min·1.73 m^2). Patients with PHP1 had higher levels of serum Cr and calculated eGFR than patients with PHPT ($p = 0.028$ and $p < 0.001$, respectively). Compared with the normal control group, patients with PHP1 had a significantly higher level of serum Cr and eGFR value ($p = 0.033$ and $p < 0.001$, respectively), but the calculated eGFR levels in patients with PHPT were similar to those of the normal control group, even though the Cr level was
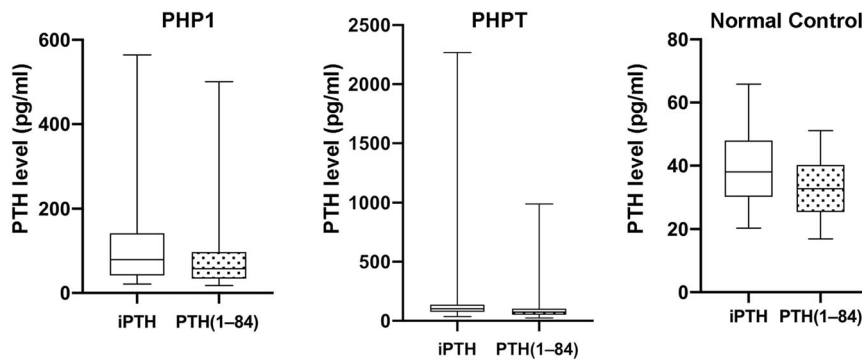
Fig. 1 Box plot of PTH levels under two analyses of the samples of the 3 cohorts. Hollow squares in each diagram indicate the level of iPTH, while the ones filled with black spots indicate the level of PTH(1–84)

lower in the PHPT group ($p = 0.072$ and $p < 0.001$, respectively, not shown in Table).

PTH levels measured by the two assays in the three groups

The results of iPTH and PTH(1–84) levels are shown in Table 2 and Fig. 1.

The iPTH and PTH(1–84) levels of patients with PHP1 were significantly lower than that of patients with PHPT ($p = 0.030$ and $p = 0.027$ respectively, not shown in the Table). As expected, the iPTH and PTH(1–84) levels of both PHP1 and PHPT were significantly higher than the normal control group. The simple linear regression indicated a strong linear relationship between iPTH and PTH (1–84) in the PHP1, PHPT, and normal control groups (r^2 were 0.9661, 0.7733, and 0.9575, respectively; $p < 0.001$ for all three groups). (Regression equations are marked in Fig. 2).

Serum iPTH levels were significantly higher than PTH(1–84) levels in all the three different groups ($p < 0.001$ for all), and the medium calculated ΔPTH% were 24.3%, 27.9%, and 15.0% for PHP1, PHPT, and the normal control group, respectively. Even though ΔPTH% and 3rd/2nd ratio of PHP1 and PHPT were similar, the indexes of PHP1 and PHPT groups were significantly higher than the normal control group. Notably, a total of nine cases of PHPT patients were present with a 3rd/2nd ratio over 1.0, which indicated a higher level of PTH (1–84) than iPTH in these nine patients.

Subgroup comparisons were also performed between the 16 patients with PHP1A and 38 patients with PHP1B. PHP1A and PHP1B groups showed similar iPTH and PTH (1–84) levels [medium iPTH level were 103.6 pg/mL and 74.6 pg/mL, and medium PTH (1–84) level was 77.2 pg/mL and 54.3 pg/mL, $p = 0.495$ and 0.437, respectively]. The calculated ΔPTH and ΔPTH% did not show any significant difference between the two subgroups (medium ΔPTH was 21.7 pg/mL and 19.4 pg/mL, and average ΔPTH% were 24.7% and 24.3%, $p = 0.684$ and 0.886, respectively). The

average 3rd/2nd ratios were 0.75 and 0.76 for patients with PHP1A and PHP1B, respectively, which showed no significant difference ($p = 0.887$). Both iPTH/ULN and PTH (1–84)/ULN values were similar between the two subgroups ($p = 0.495$ and 0.437, respectively). Together, the two subgroups presented with similar levels of iPTH, PTH (1–84), ΔPTH, ΔPTH%, and 3rd/2nd ratio, iPTH/ULN, and PTH(1–84)/ULN compared with each other and with the entire PHP1 group, respectively (all $p > 0.05$, data not shown in Table).

Among the patients, 49 out of 54 patients with PHP1 and 89 out of 127 patients with PHPT had their serum sample stored under −80 °C for a median of 379 days (317.5, 473.5) and 90 days (72, 385), respectively. The medium level of iPTH at the time of serum collection was 129.2 pg/mL and 145.0 pg/mL for the two samples, respectively, which suggested a distinct declination of iPTH in both groups during storage. The calculated decay rate was $38.2 \pm 18.2\%$ in the PHP1 group and $39.8 \pm 19.8\%$ in the PHP1 group. The levels of iPTH at the time of sample collection and the decay rate did not differ significantly between the PHP1 and PHPT groups (p value were 0.091 and 0.631 respectively). Moreover, no significant differences were observed in the comparisons of ΔPTH%, 3rd/2nd ratio, iPTH/ULN, and PTH (1–84)/ULN between the stored sample and fresh samples (data not shown).

Test of conformity between PTH (1–84) and iPTH in PHP1 and PHPT patients

The results of the conformity test between PTH (1–84) and iPTH for evaluating PTH concentrations in PHP1 and PHPT patients are shown in Table 3.

Among patients with PHP1, 27 of them (50.0%) presented with increased PTH levels under both iPTH and PTH (1–84) assays, whereas 21 patients (38.9%) had normal PTH levels under both assays (Table 3A). The Kappa conformity test showed that in patients with PHP1, the

Table 3 Conformance of PTH(1–84) and iPTH in PHP1 (A) and PHPT (B)

A

Testing of conformance of PTH(1–84) and iPTH in PHP1

Parameter		iPTH		Kappa value	P
		Hyper-PTH	Normal PTH		
PTH(1–84)	Hyper-PTH	27 (50%)	0 (0%)	0.778	<0.001
	Normal PTH	6 (11.1%)	21 (38.9%)		

B

Testing of conformance of PTH(1–84) and iPTH in PHPT

Parameter		iPTH		Kappa value	P
		Hyper-PTH	Normal PTH		
PTH(1–84)	Hyper-PTH	85 (66.9%)	1 (0.8%)	0.395	<0.001
	Normal PTH	27 (21.3%)	14 (11.0%)		

Reference ranges: iPTH: 15.0–65.0 pg/mL; PTH(1–84): 14.9–56.9 pg/mL

$P < 0.05$ indicates significant conformity

Kappa value of PTH (1–84) assay and iPTH assay were 0.778 ($p < 0.001$). The number of patients with PHPT with increased PTH levels under both assays was 85 (66.9%), whereas 14 patients (11.0%) had a PTH level that was within the normal range of both assays (Table 3B). In patients with PHPT, the Kappa value of the two methods was 0.395 ($p < 0.001$), which indicated relatively poor conformity.

The conformity test was also performed in different subtypes of PHP1 (Table 4). In the 16 patients with PHP1A, the two assays showed good conformity with a Kappa value of 0.738 ($p = 0.002$, Table 4A), whereas the Kappa value of the test in the 38 patients with PHP1B was 0.791 ($p < 0.001$, Table 4B). Both conformity tests indicated that iPTH and PTH(1–84) agreed well with each other in evaluating PTH concentration in patients with PHP1.

For further conformity analysis, Bland–Altman plot was prepared. In PHP1 patients, the Bland–Altman plot showed that 96.3% of the data points were within Mean ± 1.96 SD (Fig. 2B). The mean of percent bias of PHP1 group was 26.1%, and the range of Mean ± 1.96 SD of percent bias was 2.2%–50.0% (Table 5). The proportion of data points within Mean ± 1.96 SD was 93.7% and 98.5% in the PHPT group and the normal control group, respectively (Fig. 2D, F). The mean of percent bias of these two groups were 31.2% and 17.0%, respectively, and the range of Mean ± 1.96 SD were −14.3%–76.6% and 6.7%–27.2%, respectively (Table 5).

Discussion

In the present study, we performed PTH(1–84) measurement in a relatively large group of patients with PHP1 and compared the conformity of the PTH(1–84) assay with the iPTH assay. The results showed that similar to that in the normal control group, the PTH(1–84) assay correlated well with iPTH in the PHP1 and PHPT groups. Bland–Altman plot indicated that iPTH and PTH(1–84) had good conformity in evaluating the PTH level in the normal control group, and the Kappa conformity test showed that the two methods had good conformity in the judgment of elevated PTH levels in PHP1 group, but relatively poor conformity in PHPT group. Moreover, the two assays showed similar power in evaluating increased PTH levels in different subtypes of the patients with PHP1.

Since the establishment of the PTH(1–84) assay, its reliability and potential usefulness in estimating parathyroid function have been tested and confirmed by various researchers [8, 13, 14]. The validity of the PTH(1–84) assay in the present study was supported by its good correlation with the iPTH assay and a similar ability to determine a higher-than-normal PTH level compared with that of the iPTH assay. However, the differences between the two assays should not be neglected. According to the different epitopes of antibodies used in the two assays, the PTH fragments of various lengths in circulation were responsible for the difference between them. In the present study, the three indices, ΔPTH%, and the $3^{rd}/2^{nd}$ ratio showed that approximately a quarter of iPTH concentration in the patients with PHP1 and PHPT consisted of PTH fragments, which was significantly higher than that in the normal controls (about 15%). Previous studies had shown that the $3^{rd}/2^{nd}$ ratio differed among diseases, though most of them focused on one disease group at one time. This ratio was 0.60–0.62 in patients with CKD in different studies [15–17], whereas the ratio in patients with PHPT was

Table 4 Conformance of PTH(1–84) and iPTH in PHP1A (A) and PHP1B (B)

A					
Testing of conformance of PTH(1–84) and iPTH in PHP1A					
Parameter		iPTH		Kappa value	P
		Hyper-PTH	Normal PTH		
PTH(1–84)	Hyper-PTH	9 (56.3%)	0 (0%)	0.738	0.002
	Normal PTH	2 (12.5%)	5 (31.3%)		
B					
Testing of conformance of PTH(1–84) and iPTH in PHP1B					
Parameter		iPTH		Kappa value	P
		Hyper-PTH	Normal PTH		
PTH(1–84)	Hyper-PTH	18 (47.4%)	0 (0%)	0.791	<0.001
	Normal PTH	4 (10.5%)	16 (42.1%)		

Reference ranges: iPTH: 15.0–65.0 pg/mL; PTH(1–84): 14.9–56.9 pg/ml

P value of <0.05 means significant conformity

0.60–0.64 [8, 18]. Only in one single-center observational study, these two assays were performed among different patient groups, showing that the medium $3^{rd}/2^{nd}$ ratio for hemodialysis patients, renal transplantation recipients, patients with PHPT, patients with parathyroid carcinoma (PC), and healthy elderly people (median age was 72.6 years) was 0.74, 0.77, 0.76, 1.16, and 0.80, respectively [19]. Further analysis of the comparison of these two assays is necessary for the in-depth knowledge of the $3^{rd}/2^{nd}$ ratio and the differences between these assays.

Due to the rarity of PHP1, the studies on the comparison of these two assays in such patients are limited. Hatakeyama et al. performed the two assays on seven newly diagnosed patients with PHP1 and healthy volunteers, and they found that the patients with PHP1 presented with a significantly higher proportion of PTH(7–84)-like fragments when compared with the normal control group, and the $3^{rd}/2^{nd}$ ratio was lower in the PHP1 group than in the normal control group (the average $3^{rd}/2^{nd}$ ratio was 0.64 and 0.77, respectively, $p < 0.01$) [10]. In this study, we measured iPTH and PTH(1–84) simultaneously in a larger group of patients with PHP1 with clear molecular subtyping who had received calcium and calcitriol treatment. We observed a lower $3^{rd}/2^{nd}$ ratio in the patients with PHP1 than that in the normal controls; however, the ratios were relatively higher in both groups (0.76 and 0.84, respectively) than those reported in a previous study. The discrepancy in the results may be because of differences in sample sizes and assay kits. Decay during sample storing as well as calcium and calcitriol treatment that all of our patients were receiving may also contribute to the difference between these two assays [10]. We further compared the $3^{rd}/2^{nd}$ ratio and conformity of the two assays in two subgroups of PHP1A and PHP1B classified by molecular analysis and found no significant differences, suggesting inactivating mutation

Table 5 Parameters of bland–altman plot of PTH(1–84) and iPTH in PHP1, PHPT and normal control

	PHP1	PHPT	Normal Control
Mean (%)	26.1	31.2	17.0
Mean ± 1.96 SD (%)	2.2–50.0	−14.3–76.6	6.7–27.2
Number of points within Mean ± 1.96 SD	52 (96.3%)	119 (93.7%)	64 (98.5%)

Mean the mean of percent bias

(PHP1A) and methylation alteration (PHP1B) in *GNAS* had not led to the difference in the proportion of PTH fragments in circulation. Studies with more fresh serum samples and patients with different treatment statuses are needed to thoroughly evaluate the clinical application of PTH(1–84) in patients with PHP1.

Considering the difference in the mechanisms of PTH increase in PHP1 (PTH resistance with preserved PTH responsiveness to serum calcium change) [20, 21] and PHPT (the autonomous production and secretion of PTH by the parathyroid adenoma), a difference in PTH degradation leading to changes in PTH fragments in circulation between these two clinical entities was speculated. We have noticed a better performance in the conformity test of the two assays in the analysis of increased PTH levels and higher r^2 in linear regression in the patients with PHP1 than those in the patients with PHPT. However, the calculated ΔPTH% and the $3^{rd}/2^{nd}$ ratio showed no statistically significant differences between the two groups, indicating that the patients with PHP1 and PHPT possessed a relatively similar proportion of PTH fragments in circulation. Previous studies showed that PTH fragments of various lengths were mostly from the liver, whereas a part of them was secreted directly by the parathyroid gland [1, 2], and their clearance relied on
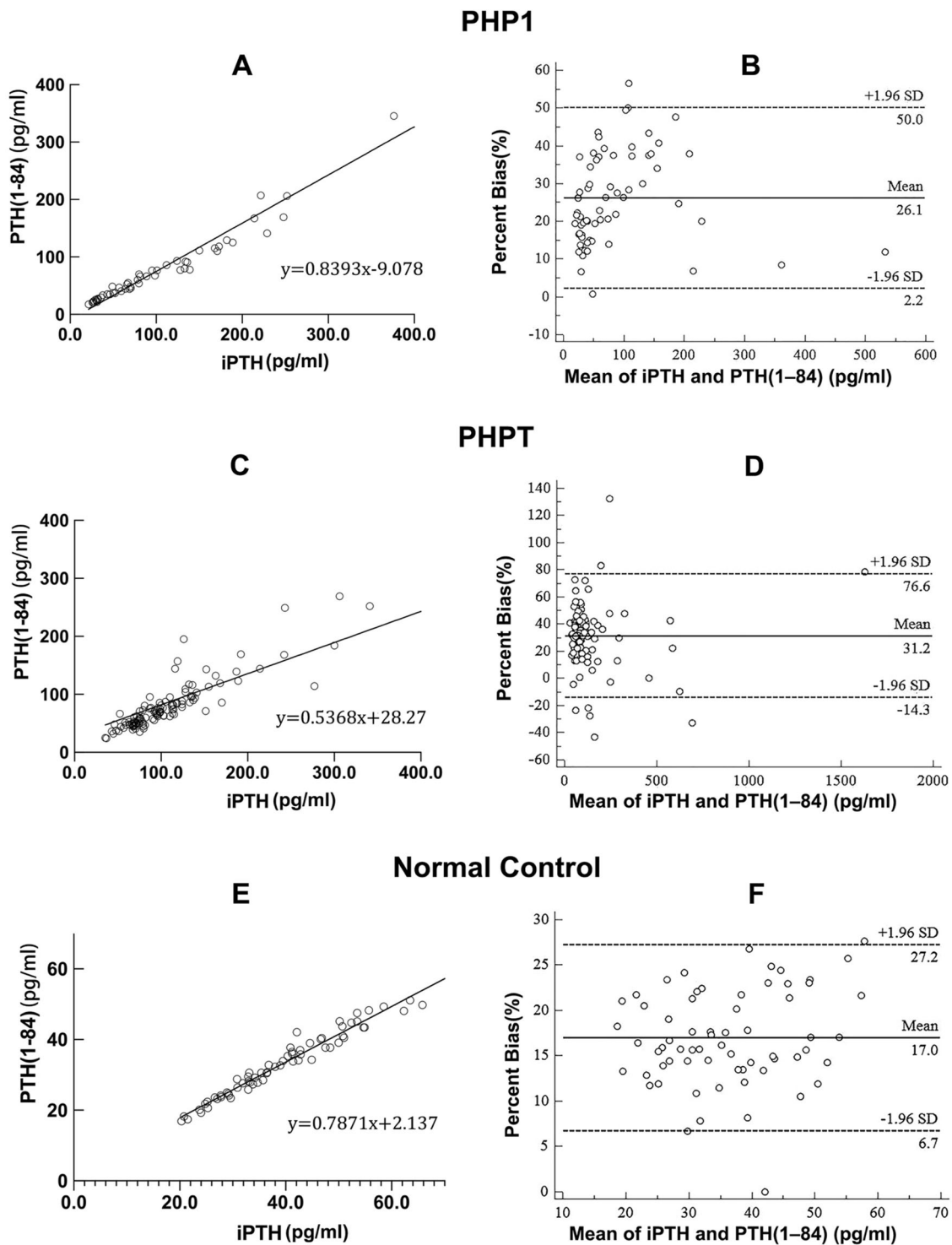
Fig. 2 Linear regression and Bland–Altman plots of iPTH and PTH(1–84) in PHP1, PHPT, and normal control groups. Percent Bias(%): ΔPTH/ mean of iPTH and PTH(1–84). In each square, 2 figures of PHP1, PHPT, and normal control, respectively, are shown. **A**, **C**, **E** linear regression figures of PHP1, PHPT, and normal control, respectively, along with regression equations. The horizontal axis indicates the measured iPTH level, while the vertical axis indicates the measured PTH(1–84) level. R^2 for linear regression were 0.9661, 0.7733, and 0.9575 for PHP1, PHPT, and normal control group, respectively. **B**, **D**, **F** Bland–Altman plots of Percent Bias (vertical axis) over Mean of iPTH and PTH(1–84) (horizontal axis) of PHP1, PHPT, and normal control, respectively. Mean is indicated with a solid line, while the Mean ± 1.96 SD are shown in the dashed line in the Figure

renal function. A recent study involving predialysis patients with CKD showed that differences between iPTH and PTH(1–84) increased along with the degree of renal function impairment which was found in about 30% of these patients [22]. Though eGFR levels were different between the groups, all patients enrolled in the study showed normal renal functions, which would be a reason for the similar ΔPTH% and the 3rd/2nd ratio between the PHP1 and PHPT groups. Although with similar means of calculating ΔPTH% and the 3rd/2nd ratio, a greater variation was observed in these two indices in the patients with PHPT, which may have caused the differences in the conformity test. The differences in the severity or pathology (such as PC) of the disease may be responsible for the relatively poor conformity in the PHPT group, though further investigations are needed to support the hypothesis.

In the present study, the PTH(1–84) values were higher than the iPTH values in nine patients with PHPT. Medical history confirmation revealed that among them, six patients were diagnosed with PC, and one had atypical adenoma. Another patient later presented with hypergastrinemia and pancreatic occupation, which indicated the diagnosis of multiple endocrine neoplasia type 1 (MEN1). One patient did not undergo surgery. Our findings were consistent with those of a previous study using an automated PTH evaluation platform, which featured PC with an average 3rd/2nd ratio >1, whereas the ratio was <1 in their control group consisting of individuals with benign parathyroid adenoma or hyperplasia [19]. A higher 3rd/2nd ratio and a greater proportion of patients with a 3rd/2nd ratio >1 have been shown in several groups previously [23–25]. Another study showed that the overproduced N-terminal molecular PTH in patients with PC was responsible for the relatively higher PTH(1–84) value [18]. Though investigations showed that the N-terminal fragments were also concentrated in patients with CKD [26], the normal eGFR level of the six patients with PC in the present study suggested that the specific pathology rather than the declination of renal function was more likely to be the reason. The potential mechanism of the similar 3rd/2nd ratio that the patient with MEN1 presented in this study is still unknown; hence, studying more cases of MEN1 is necessary to explore the exact mechanism.

To the best of our knowledge, this was the first study that compared the conformity of PTH(1–84) with iPTH among patients with PHP1. The two assays were performed using clinically verified diagnostic kits and an automated platform in succession, thus making the results highly reliable. Another strength was that the study was performed on a relatively large population of Chinese patients with PHP1. However, the declination of post-storing iPTH compared with pre-storing iPTH indicated that samples stored for an extended period should be analyzed carefully. The declination of PTH(1–84) between pre-storing and post-storing

stages as well as the conformity test between the PTH(1–84) and iPTH assays before storing could have been performed if our center had been equipped with the PTH(1–84) assay setup at the time of sample collection. Considering the rarity of the disease and the effect of the COVID-19 pandemic on patients' visiting, the sample size of patients with PHP1 was rather small. Another limitation of this study was that all patients with PHP1 were undergoing calcium and calcitriol treatment. Further recruitment of newly diagnosed patients with PHP1 is necessary for the better evaluation of basic PTH levels among them.

Conclusion

In the present study, the conformity test showed that the PTH(1–84) immunoassay presented a similar ability in examining PTH levels among patients with PHP1 and normal control groups compared with the iPTH assay which was reflected in the Bland–Altman plot. According to the Kappa value, the conformity of PTH(1–84) and iPTH in detecting elevated PTH levels was better in the PHP1 group than in the PHPT group. It was suggested that the relationship between the values of iPTH and PTH(1–84) was different in PHP1 patients comparing with PHPT patients. Further studies are needed for a better understanding of the mechanisms of the differences between these assays.

Compliance with ethical standards

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