TITLE:
Efficacy of whole PTH assay and 1-84/non-(1-84) PTH ratio in patients on hemodialysis or undergoing parathyroidectomy

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CITATION:
Kato, Yuji ...[et al]. Efficacy of whole PTH assay and 1-84/non-(1-84) PTH ratio in patients on hemodialysis or undergoing parathyroidectomy. 泌尿器科紀要 2004, 50(11): 755-762

ISSUE DATE:
2004-11

URL:
http://hdl.handle.net/2433/113496

RIGHT:
EFFICACY OF WHOLE PTH ASSAY AND 1-84/NON-(1-84) PTH RATIO IN PATIENTS ON HEMODIALYSIS OR UNDERGOING PARATHYROIDECTOMY

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For measurement of parathyroid hormone (PTH), the intact PTH (I-PTH) assay is generally used. However, I-PTH assay apparently detects PTH 7-84 fragments, in addition to PTH 1-84. The whole PTH (W-PTH) assay exhibits no cross-reactivity with PTH 7-84. Ratio of PTH 1-84 to non-(1-84) PTH has been proposed as a non-invasive indicator of bone turnover in patients with end-stage renal disease (ESRD). We evaluated the efficacy of W-PTH assay and 1-84/non-(1-84) PTH ratio in hemodialysis patients and patients who had undergone parathyroidectomy. PTH levels were measured using W-PTH and I-PTH assays in 138 hemodialysis patients, 27 healthy controls and 10 patients who were scheduled to undergo parathyroidectomy for secondary hyperparathyroidism. Alkaline phosphatase, bone alkaline phosphatase and intact osteocalcin were also measured for comparison with 1-84/non-(1-84) PTH ratio. W-PTH was strongly correlated with I-PTH in both groups, and with all three bone metabolic markers in hemodialysis patients. In hemodialysis patients, both PTH and bone metabolic markers were significantly lower in the subgroup with a PTH ratio < 1.0 than in the subgroup with a PTH ratio ≥ 1.0. However, PTH ratio exhibited no significant correlation with bone metabolism markers. PTH ratio was higher after parathyroidectomy than before. W-PTH and I-PTH assays offer identical indicators of bone metabolism in ESRD patients. However, 1-84/non-(1-84) PTH ratio may be of limited diagnostic use regarding bone turnover if a cut-off value of 1.0 is used. PTH 1-84 may be secreted relatively more than non-(1-84) PTH after parathyroidectomy, due to decreases in serum calcium. As the W-PTH assay allows easy calculation of non-(1-84) PTH by subtracting the I-PTH value, this assay contributes to identification of the function of non-(1-84) PTH fragments in various conditions.

Key words: Parathyroid hormone, Intact PTH, Whole PTH, Renal osteodystrophy, Bone turnover, Bone metabolic marker

INTRODUCTION

Measurement of parathyroid hormone (PTH) is indispensable for evaluation of parathyroid function. PTH represents one of the most important indicators for diagnosis and appropriate treatment of primary and secondary hyperparathyroidism and renal osteodystrophy (ROD) in patients with end-stage renal disease (ESRD). Almost all commercially intact PTH (I-PTH) assays are represented as detecting only human PTH 1-84. However, recent studies using high performance liquid chromatography suggest that most assays measure not only PTH 1-84, but also non-(1-84) PTH fragments such as PTH 7-84. Conventional “intact” PTH assays thus do not accurately indicate parathyroid function.

One new immunoradiometric assay for measurement of complete PTH molecules is known as the whole PTH (W-PTH) assay. This assay eliminates cross-reactivity from PTH 7-84 by using double antibodies to the 1-4 region of the amino-terminal and 39-84 region of the carboxyl-terminal in PTH 1-84. In recent studies comparing the W-PTH assay and several I-PTH assays, W-PTH concentration was 30-60% lower than that of I-PTH in ESRD patients and non-(1-84) PTH fragments might cause this difference between PTH values. Non-(1-84) PTH fragments accumulate under uremic conditions, but their biological effects are not well understood. Non-(1-84) PTH fragments do not bind to PTH/PTH-related protein receptors for PTH 1-84, and another receptor termed the C-PTH receptor has been identified on osteoblastic cells and
osteocytes\textsuperscript{12,13}. Moreover, PTH 1–84 antagonizes the calcemic actions of PTH 1–84, and these actions may be mediated through C-PTH receptors\textsuperscript{4,15}. These molecules are thus considered as factors contributing to skeletal resistance to PTH and adynamic bone disease (ABD) in ESRD patients\textsuperscript{16,17}. Monier-Faugere et al. proposed that the ratio of PTH 1–84 to non-(1–84) PTH (1–84/non-(1–84) PTH ratio) could offer a good indicator of bone turnover in ESRD patients\textsuperscript{18}. However, this proposal has been controversial because such findings have not yet been confirmed. Moreover, no reports are available on changes in W-PTH and 1–84/non-(1–84) PTH ratio after parathyroidectomy (PTx).

The purpose of this study was to evaluate the utility of W-PTH assay in hemodialysis patients compared with conventional I-PTH assay and serum bone metabolic markers. In addition, we examined the changes in these parameters after PTx.

**SUBJECTS AND METHODS**

Subjects were 138 patients (62 women, 76 men) undergoing hemodialysis (HD group), 27 volunteers (3 women, 24 men) with normal renal and parathyroid function (Control group), and 10 patients scheduled to undergo total PTx with forearm implantation\textsuperscript{19} due to severe secondary hyperparathyroidism (PTx group). In the HD group, the mean duration of hemodialysis treatment was 8.4±0.64 years (range, 3 months to 29 years). This group received hemodialysis for 3–5 h 2–3 times/week. Patients receiving therapy with vitamin D for secondary hyperparathyroidism were also included in this study. In the PTx group, all patients received postoperative calcium carbonate supplements and calcitriol (starting at 2 jg/day, increasing to 4 jg/day as necessary). Written informed consent was obtained from all participants.

Blood samples were collected before hemodialysis and meals in the HD group, and before meals in the Control group. In the PTx group, blood samples were obtained before PTx, and 1 day, 1 week and 1 month after PTx. Samples were centrifuged at 3,000 rpm for 15 min and supernatants were immediately frozen and stored at −80°C until required. Plasma PTH value was measured using both W-PTH assay (Scantibodies Laboratory, Santee, CA) and I-PTH assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). Alkaline phosphatase (ALP), bone alkaline phosphatase (BAP), intact osteocalcin (BGP-I), and serum calcium and phosphorus were also determined in each group. The 1–84/non-(1–84) PTH ratio was calculated as follows: PTH 1–84 value was obtained by W-PTH assay, and non-(1–84) PTH value was calculated by subtraction of W-PTH value from I-PTH value.

Values are presented as mean±standard error of the mean. Statistical analyses were performed using linear regression analysis, correlation analysis (Spearmann's r correlation coefficients), Mann-Whitney U-test and repeated ANOVA followed by Dunnett's test. All statistical analyses were performed using Stat View 5.0 for Windows software (SAS Institute Inc, Cary NC), and values of P<0.05 were considered significant.

**RESULTS**

Mean values for parameters obtained from the HD and Control groups are summarized in Table 1. W-PTH values were always lower than I-PTH values in each group. W-PTH/I-PTH ratios were 58.7% and 64.4% in the HD and Control groups, respectively. In both groups, direct linear correlations between W-PTH and I-PTH were noted (Fig. 1). In the HD group, significant correlations between W-PTH and the 3 bone metabolic markers were found (Fig. 2) and these correlations were basically identical to correlations between I-PTH and bone metabolic

<table>
<thead>
<tr>
<th>Subject</th>
<th>HD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.3±1.1</td>
<td>30.5±2.2</td>
</tr>
<tr>
<td>W-PTH (pg/ml)</td>
<td>199.8±23.1</td>
<td>22.5±2.4</td>
</tr>
<tr>
<td>I-PTH (pg/ml)</td>
<td>336.0±37.3</td>
<td>41.0±2.9</td>
</tr>
<tr>
<td>non-(1–84) PTH (pg/ml)</td>
<td>139.4±16.2</td>
<td>18.5±3.0</td>
</tr>
<tr>
<td>1–84/non-(1–84) PTH</td>
<td>2.0±0.2</td>
<td>2.5±0.4</td>
</tr>
<tr>
<td>W-PTH/I-PTH (%)</td>
<td>58.7±1.2</td>
<td>64.4±2.8</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.9±0.1</td>
<td>9.6±0.1</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>64.0±0.1</td>
<td>3.9±0.6</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>285.1±11.1</td>
<td>142.4±9.6</td>
</tr>
<tr>
<td>BAP (U/l)</td>
<td>29.7±1.6</td>
<td>2.3±2.2</td>
</tr>
<tr>
<td>BGP-I (ng/ml)</td>
<td>63.0±7.1</td>
<td>8.0±0.8</td>
</tr>
</tbody>
</table>

Mean±SEM; HD, hemodialysis; PTH, parathyroid hormone; W-PTH, whole-PTH; I-PTH, intact-PTH; ALP, alkaline phosphatase; BAP, bone alkaline phosphatase; BGP-I, intact osteocalcin.
Fig. 1. Correlations between values of whole PTH (W-PTH) and intact PTH (I-PTH) in a) hemodialysis patients (HD) or b) normal volunteers (Control). Positive correlations between W-PTH and I-PTH were noted in both groups.

Fig. 2. Correlations between W-PTH and a) alkaline phosphatase (ALP), b) bone alkaline phosphatase (BAP) or c) intact osteocalcin (BGP-I) levels in hemodialysis patients. Significant correlations were found between W-PTH and all three bone metabolic markers.
Table 2. Coefficients of correlation among parameters in 138 hemodialysis patients

<table>
<thead>
<tr>
<th></th>
<th>W-PTH (pg/ml)</th>
<th>I-PTH (pg/ml)</th>
<th>non-(1-84) PTH (pg/ml)</th>
<th>1-84/non-(1-84) PTH</th>
<th>non-(1-84) PTH/I-PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>0.081</td>
<td>0.070</td>
<td>0.044</td>
<td>-0.044</td>
<td>-0.055</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>0.068</td>
<td>0.105</td>
<td>0.135**</td>
<td>0.118</td>
<td>0.084</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>0.379*</td>
<td>0.336**</td>
<td>0.232***</td>
<td>0.252</td>
<td>0.216***</td>
</tr>
<tr>
<td>BAP (U/l)</td>
<td>0.675*</td>
<td>0.591*</td>
<td>0.395*</td>
<td>0.395</td>
<td>0.395**</td>
</tr>
<tr>
<td>BGP-I (ng/ml)</td>
<td>0.781*</td>
<td>0.724*</td>
<td>0.541*</td>
<td>-0.104</td>
<td>-0.213***</td>
</tr>
</tbody>
</table>

PTH, parathyroid hormone; W-PTH, whole-PTH; I-PTH, intact-PTH; ALP, alkaline phosphatase; BAP, bone alkaline phosphatase; BGP-I, intact osteocalcin. *p<0.01, **p<0.001, ***p<0.0001.

markers (Table 2). Non-(1-84) PTH was positively correlated with these bone markers, but correlations were weaker than those for each PTH value and bone markers. No correlation was found between 1-84/non-(1-84) PTH ratio and the 3 bone metabolic markers or between serum calcium and 1-84/non-(1-84) PTH ratio (Table 2).

The HD group was divided into two subgroups according to 1-84/non-(1-84) PTH ratio i.e., ≥1.0 (47 women, 66 men; mean age, 59.1±1.2 years) or <1.0 (15 women, 10 men; mean age, 59.7±3.6 years). Duration of hemodialysis treatment in the subgroup with ratio ≥1.0 was 8.5±0.74 years, compared to 8.2±0.56 years in the subgroup with ratio <1.0, with no significant difference found between the two groups. W-PTH and I-PTH values were significantly higher in the ≥1.0 subgroup than in the <1.0 subgroup (Fig. 3). ALP, BAP and BGP-I were also higher in the ≥1.0 subgroup (P values of 0.014, 0.006 and 0.039, respectively). Serum calcium and phosphorus levels were 10.7±0.9 mg/dl and 6.4±0.2 mg/dl in the ≥1.0 subgroup, and 10.2±0.2 mg/dl and 6.5±0.4 mg/dl in the <1.0 subgroup, respectively, with no significant differences observed between the two subgroups. Of the 113 HD patients with a PTH ratio ≥1.0, I-PTH values were <100 pg/ml in 51, and mean BAP and BGP-I concentrations were 20.5±1.8 U/l and 16.9±1.8 ng/ml, respectively. Mean values of each parameter in the 10 patients before and after PTx are summarized in Table 3. W-PTH and I-PTH levels declined immediately after PTx, with gradual increases at 1 week or 1 month after PTx. Postoperative values of W-PTH, I-PTH, calcium, and phosphorus declined markedly, while values of bone metabolic markers were elevated compared with the baseline. The 1-84/non-(1-84) PTH ratio tended to be increased at both 1 week and 1 month after PTx compared to before PTx, but no significant differences were noted.

![Fig. 3. Mean levels of a) W-PTH, b) I-PTH, c) ALP, d) BAP and e) BGP-I in two subgroup of patients on hemodialysis: 1-84/non-(1-84) PTH ratio ≥1.0; and <1.0. Levels of all five parameters were significantly higher in the ≥1.0 subgroup than in the <1.0 subgroup. *p<0.05 by Mann-Whitney U-test.](image-url)
A diagnostic definition of bone turnover is required for choosing between different therapeutic approaches to the wide variety of ROD types. 1-PTH assay has been used as a non-invasive marker of bone metabolism, but this assay overestimates parathyroid-mediated osseous abnormalities in uremia by a factor of 2-2.5. This overestimation is attributed to cross-reactivity of 1-PTH assay to non-(1-84) PTH, predominantly involving PTH-7-84. Several authors have already investigated the efficacy of W-PTH assay, but the clinical significance of W-PTH values and 1-84/non-(1-84) PTH ratio remains controversial. The present study revealed that W-PTH values are strongly correlated with I-PTH values, not only in patients undergoing hemodialysis, but also in volunteers with normal renal function. Correlations between W-PTH and bone metabolic markers in hemodialysis patients were found to be identical to those between I-PTH and the same markers, and these results are compatible with those noted in previous reports. Indeed, W-PTH assay determines only PTH-1-84 and can provide a better evaluation of parathyroid function than I-PTH assay. However, our results suggest that W-PTH and I-PTH provide similar information regarding bone metabolism. Consequently, the W-PTH assay should, as with the I-PTH assay, be used in combination with other bone metabolic markers such as BAP or BGP-I to evaluate bone turnover.

ROD represents one of the most common complications in hemodialysis patients, and ABD characterized by very low bone turnover is considered one of the most frequent bone histological patterns at present. Although bone biopsy offers the most reliable method for evaluating bone turnover, this gold standard procedure cannot be performed repeatedly because of the invasiveness of the procedure. As a non-invasive index of bone turnover, Monier-Faugere et al. proposed that 1-84/non-(1-84) PTH ratio <1.0 indicates low turnover bone in ESRD patients. However, data from more recent reports have been contradictory. Nakanishi et al. and Reichel et al. found no correlation between 1-84/non-(1-84) PTH ratio and serum bone markers. Coen et al. and Salusky et al. demonstrated that 1-84/non-(1-84) PTH ratio did not indicate any particular bone histological patterns in adult patients on hemodialysis or in pediatric patients with peritoneal dialysis. The present study found no correlation between the 3 bone metabolic markers and 1-84/non-(1-84) PTH ratio. Moreover, although ALP, BAP and BGP-I values were higher in the subgroup with PTH ratio ≥1.0 compared to the <1.0 subgroup, half of the patients in the ratio ≥1.0 subgroup exhibited very low concentrations of PTH, ALP, BAP and BGP-I. Since it is well recognized that patients with I-PTH levels ≥100 pg/ml display low bone turnover, 1-84/non-(1-84) PTH ratio may be not needed to diagnose low bone turnover in these cases. Nakanishi et al. and Reichel et al. suggested that cut-off value for this ratio was probably inappropriate because few patients (1.0-5.6%) display a ratio <1.0, while ABD and low bone turnover is present at a frequency of 30-40% in most studies. Further investigation is thus needed to elucidate whether 1-84/non-(1-84) PTH ratio is clinically useful as an indicator of bone turnover.

Coen et al. and Slatopolsky et al. have shown that non-(1-84) PTH/total PTH ratio (Scantibodies Laboratory, Santee, CA) correlates strongly with serum calcium level. D’Amour et al. reported that increased serum calcium level following calcium infusion results in decreased 1-84/non-(1-84) PTH ratio. Santamaria et al. demonstrated that W-PTH, I-PTH and non-(1-84) PTH levels change with a sigmoidal response by induction of hypocalcemia, and hypercalcemia may represent a major up-regulator of non-(1-84) PTH fragments. However, we found no significant correlation between serum calcium and non-(1-84) PTH, 1-84/non-(1-84) PTH ratio or non-(1-84) PTH/I-PTH ratio.

### Table 3. Parameters in 10 patients before and after parathyroidectomy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-op</th>
<th>1 day</th>
<th>1 week</th>
<th>1 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>W-PTH (pg/ml)</td>
<td>735.5 ± 91.4</td>
<td>12.4 ± 4.3*</td>
<td>23.8 ± 12.0*</td>
<td>42.8 ± 13.9*</td>
</tr>
<tr>
<td>I-PTH (pg/ml)</td>
<td>1,201.4 ± 155.8</td>
<td>23.5 ± 6.2*</td>
<td>42.4 ± 23.5*</td>
<td>73.2 ± 27.8*</td>
</tr>
<tr>
<td>non-(1-84) PTH (pg/ml)</td>
<td>465.9 ± 67.1</td>
<td>11.0 ± 2.5*</td>
<td>18.5 ± 12.6*</td>
<td>27.9 ± 12.6*</td>
</tr>
<tr>
<td>I-84/non-(1-84) PTH</td>
<td>1.64 ± 0.1</td>
<td>1.17 ± 0.29</td>
<td>2.35 ± 0.63</td>
<td>2.09 ± 0.25</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.9 ± 0.28</td>
<td>8.6 ± 0.46*</td>
<td>8.2 ± 0.37*</td>
<td>7.9 ± 0.29*</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>7.2 ± 0.56</td>
<td>5.8 ± 0.48*</td>
<td>4.4 ± 0.47*</td>
<td>4.1 ± 0.53*</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>393.9 ± 34.9</td>
<td>436.3 ± 63.2</td>
<td>767.4 ± 171.6*</td>
<td>628.6 ± 148.9*</td>
</tr>
<tr>
<td>BAP (IU/l)</td>
<td>55.3 ± 7.0</td>
<td>57.8 ± 16.4</td>
<td>107.6 ± 28.6*</td>
<td>92.5 ± 15.6*</td>
</tr>
<tr>
<td>BGP-I (mg/ml)</td>
<td>174.3 ± 35.3</td>
<td>162.3 ± 59.1</td>
<td>218.2 ± 50.0*</td>
<td>105.1 ± 18.9*</td>
</tr>
</tbody>
</table>

Mean ± SEM; PTH, parathyroid hormone; W-PTH, whole-PTH; I-PTH, intact-PTH; ALP, alkaline phosphatase; BAP, bone alkaline phosphatase; BGP-I, intact osteocalcin. * Significance of differences determined by repeated analysis of variance. p<0.05 compared to pre-operative values.
These results concur with those reported by Coen et al.\(^9\). Moreover, no significant difference was observed in serum calcium concentration between the two subgroups. This discrepancy is probably due to differences in calcium conditions resulting from dynamic changes following induction of calcium or static condition.

The reason for the changes in PTH 1–84 and non-(1–84) PTH fragments in subjects who underwent PTx remain unclear. 1-PTH value after PTx is well known to decrease transiently to the lower limit of detection, and then increase gradually to normal levels. In this study, both W-PTH and I-PTH values tended to increase gradually after both 1 week and 1 month in all patients. Although not a significant change, the 1-84/non-(1-84) PTH ratio markedly increased above the preoperative level higher after 1 week or 1 month. These results indicate that elevation of PTH value after PTx is due to a relative increase in PTH 1–84 secretion or decrease in non-(1–84) PTH secretion from parathyroid tissue. As serum calcium values decreased significantly after PTx, this finding supports the theory that regulation of PTH 1–84 and non-(1–84) PTH depends on dynamic changes in the serum calcium level. One limitation of our study is the small subject population, and further examination of a large cohort is required to test this hypothesis.

In conclusion, W-PTH assay provides similar information to I-PTH assay as an index of bone metabolism, while 1-84/non-(1–84) PTH ratio may be of limited diagnostic use regarding bone turnover under uremic conditions if a cut-off value of 1.0 is used. After PTx, PTH 1–84 may be secreted relatively more than non-(1–84) PTH, due to decreases in serum calcium. One advantage in using the W-PTH assay is that non-(1–84) PTH can be easily calculated by subtracting the I-PTH value. This advantage should allow identification of the function of non-(1–84) PTH fragments in various conditions.

**ACKNOWLEDGMENT**

We wish to thank Syusaku Nakazono, Jyun-ichi Hori, Noriko Takashita, Aiko Funaba and Seiko Ono for their technical assistance.

**REFERENCES**


(Received on March 15, 2004) (Accepted on July 4, 2004)
慢性透析患者および副甲状腺機能全摘出術施行患者における Whole PTH assay および 1-84/non 1-84 PTH ratio の有用性について
旭川医科大学医学部泌尿器科学講座（主任：八竹 直教授）
加藤 祐司，山口 聡，八竹 直
深川市立病院泌尿器科（主任：藤澤 真）
藤澤 真
道立紋別病院泌尿器科（主任：川上憲裕）
川 上 憲 裕

副甲状腺ホルモン（PTH）の測定は、副甲状腺機能や慢性腎不全における骨徐患の評価には不可欠である。PTH の新しい測定法である whole PTH（W-PTH）assay は、intact PTH（I-PTH）assay と異なり、生物活性を有する PTH 1-84 以外の PTH フラグメント（non 1-84 PTH）を測定することなく、副甲状腺機能の正確な評価が可能である。また PTH 1-84 と non 1-84 PTH の比（PTH ratio）は慢性透析患者における骨代謝回転の指標となると報告されているが、本邦における検討例は少ない。そこでわれわれは27名の慢性不全症と138名の慢性透析患者を対象として、W-PTH、I-PTH、PTH ratio を測定または算出し、骨型 ALP（BAP）やインテクトオステオカルシン（BGP-I）などの血中骨代謝マーカーと比較した。また 2 次性副甲状腺機能亢進症により副甲状腺機能全摘出術（PTx）を受けた10名の患者を対象として、手術前後のパラメーターの変化について検討し、W-PTH、PTH ratio の有用性を検討した。検査者と透析患者の間で W-PTH と I-PTH の間には非常に強い相関を認めた（p<0.001）。また透析患者群では、ALP、BAP、BGP-I と W-PTH または I-PTH の間にはそれぞれ有意な相関を認めたが、PTH ratio と各骨代謝マーカーとの間に相関を認めなかった。PTH ratio が1.0以上の患者群は1.0未満の群よりも、W-PTH、I-PTH、BAP、BGP-I は有意に高値であったが、ratio が1.0以上の患者113名のうち51名は I-PTH が 100 pg/ml 以下であった。PTH ratio は PTx 施行後に施行前の値よりも増加する傾向を認めた。今回の検討から W-PTH assay は I-PTH assay とは同様に慢性透析患者における骨代謝の指標となると考えられた。しかし PTH ratio はカットオフ値が1.0の場合、骨代謝回転の診断には限界があると思われた。PTx 後の PTH 分泌は血清カルシウムの低下により、non 1-84 PTH よりも PTH 1-84 が相対的に多く分泌される可能性が示唆された。W-PTH assay の利点は non 1-84 PTH を算出できることであり、また十分には解明されていない PTH フラグメントの生物学的役割について検討する際には非常に有用である。

(泌尿紀要 50：755-762，2004)