

PTH secretion of “manipulated” parathyroid adenomas

Philipp Riss · Reza Asari · Christian Scheuba ·
Christian Bieglmayer · Bruno Niederle

Received: 20 February 2009 / Accepted: 8 April 2009 / Published online: 25 April 2009
© Springer-Verlag 2009

Abstract

Purpose Increased secretion of parathyroid hormone (PTH) and its fragments intraoperatively may influence PTH monitoring. The purpose of this study was to investigate whether “intended intraoperative manipulation” of parathyroid adenomas through mechanical stimulation (through squeezing or manual rubbing) would lead to increased PTH excretion. The different PTH fragments that result from this kind of manipulation were correlated and analyzed.

Methods The enlarged glands of six consecutive patients who underwent open minimally invasive parathyroid exploration were “manipulated” for 30 s as soon as they had been identified. Blood samples were drawn before skin incision, at the beginning of the manipulation, 30 s, and at 2-, 5-, 10-, and 15-min intervals. Serum levels of (1-84) PTH were measured and (7-84)PTH was calculated.

Results An increased PTH secretion was documented in four of six “manipulated” single adenomas (mean PTH±SD 312±497 pg/ml). The PTH of one patient rose from 343 to 1,747 pg/ml. The ratio of (1-84)PTH to (7-84)PTH was 1.3±0.6 (median±SD):1 at “baseline” and 1.4±0.2:1 after manipulation. The coefficient of determination (R^2) for the “baseline values” and for the values after manipulation is $R^2=0.9816$ and $R^2=0.9985$, respectively.

Conclusions First, secretion of PTH varies widely after manual manipulation of adenomas. Second, PTH fragments circulate in the same ratio before and after “manipulation.”

Keywords Primary hyperparathyroidism · Intraoperative PTH monitoring · Manipulation · PTH fragments

Introduction

Intraoperative intact parathyroid hormone (iPTH) monitoring using a quick PTH assay (QPTH) is an important prerequisite for minimally invasive surgical procedures for primary hyperparathyroidism (PHPT). QPTH measurements are used to confirm intraoperatively the successful excision of a hyperfunctioning enlarged gland in patients with single-gland disease with a high sensitivity and indicate the presence of further hypersecreting glands in patients with multiple-gland disease with a high specificity depending on the interpretation criterion of the iPTH decline [1].

However, there are several pitfalls in the monitoring of QPTH: technical assay problems or blood sampling errors, difficulties in defining the “baseline values” from which the PTH drop is calculated, and very high or very low baseline values [1]. Circulating non-(1-84)PTH fragments cumulate in patients with renal insufficiency [2]. These fragments cross-react with commercially available QPTH assays resulting in artificially high PTH values followed by a prolonged intraoperative iPTH decline [2–4]. Patients with borderline elevated baseline iPTH values show differences in PTH kinetics and a higher number of missed multiple-gland diseases [5].

It has been previously shown that “PTH spikes” during mobilization of the enlarged parathyroid gland (“uninten-

P. Riss (✉) · R. Asari · C. Scheuba · B. Niederle
Section “Endocrine Surgery,” Division of General Surgery,
Department of Surgery, Medical University of Vienna,
Währinger Gürtel 18-20,
Vienna 1090, Austria
e-mail: philipp.riss@meduniwien.ac.at

C. Bieglmayer
Clinical Institute for Medical and Chemical Laboratory
Diagnostics, Medical University of Vienna,
Vienna, Austria

tional manipulation”) lead to a prolonged PTH decline in at least 15% of the patients [6]. These “PTH spikes” may occur irrespective of the surgical procedure performed [6]. Up to now, it has not been evaluated if “manipulated” parathyroid adenomas secrete “whole” (1-84)PTH or different PTH fragments in a higher amount which may complicate the interpretation of the intraoperative PTH curve.

The purpose of this experimental “in vivo study” was to evaluate if “intended” manipulation by squeezing and rubbing of an enlarged hypersecreting parathyroid gland leads to “PTH spikes” followed by secretion of PTH fragments. Furthermore, circulating PTH fragments were measured and calculated before and after “intended” manipulation, respectively, to evaluate the ratio of their secretion.

Materials and methods

Demography and surgery

Six consecutive patients (five females, one male) with biochemically proven PHPT and normal renal function (creatinine ≤ 1.2 mg/dl) were included in this study. Mean preoperative serum calcium levels were 2.9 mmol/l (range 2.70–3.00 mmol/l; normal range 2.0–2.6 mmol/l), mean preoperative iPTH values were 130.3 pg/ml (range 89 – 250 pg/ml; normal range 10–65 pg/ml).

^{99m}Tc sestamibi scan and high-resolution ultrasound localized preoperatively one enlarged gland in all six patients. Additionally, high-resolution ultrasound excluded concomitant thyroid disease [7]. Therefore, all patients were candidates for open minimally invasive (targeted, focused) parathyroid exploration.

All patients gave informed consent for their participation in this “in vivo” study.

Intended manipulation— intraoperative/postoperative assays

After exposing the preoperatively localized enlarged parathyroid gland, it was intentionally “manipulated” for 30 s before cutting off any venous blood vessel. Manipulation was carried out through manual squeezing or rubbing of the enlarged gland. The aim was to stimulate secretion of PTH. Blood samples were drawn after the start of anesthesia before skin incision (baseline value), after visual identification of the gland, at the beginning of the “manipulation,” after 30 s, and then after 2, 5, 10, and 15 min, respectively.

One single enlarged gland was excised in all six patients. To confirm excision of all hyperfunctioning parathyroid tissue, blood samples were drawn at 5, 10, and 15 min after excision of the gland.

A commercially available QPTH assay (Elecsys 1010, Roche, Mannheim, Germany) was used to monitor iPTH during surgery [7].

By definition, cure was predicted in all six patients documenting a more than 50% decline of the PTH level from the “baseline value” [8] 10 min after excision of the enlarged gland.

Because of the lack of a quick whole (1-84)PTH assay [3], blood samples were reanalyzed after surgery using an iPTH and a bio-intact (1-84)PTH assay (both Nichols Institute Diagnostic, San Juan Capistrano, CA, USA). The bio-intact (1-84)PTH assay only measures the circulating “whole” PTH molecule ((1-84)PTH) and uses two antibodies: the first polyclonal antibody binds to the first six amino acids of the N-terminal part of the PTH molecule. The second antibody is directed towards the mid- and C-terminal part of the molecule (amino acid sequence 39–84; [8]). Non-(1-84)PTH fragments were calculated subtracting (1-84)PTH values from iPTH values.

All values (PTH levels, ratio, minutes) are provided in median \pm SD.

Parathyroid gland volume

Volumes (in milliliter) of removed glands were calculated on the basis of a prolate ellipsoid [$v = (\pi/6) \times (D_1 \times D_2 \times D_3)$].

Results

An increased iPTH secretion was documented intraoperatively in four of six (67%) single adenomas (312 ± 497 pg/ml). In two patients, manipulation of the enlarged glands did not lead to a change in PTH secretion (Fig. 1).

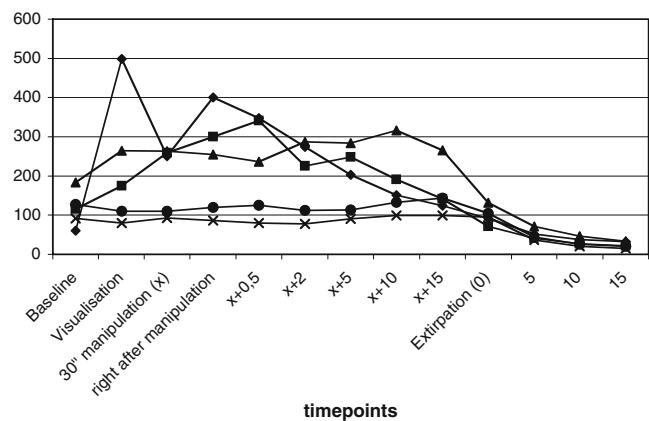


Fig. 1 Intraoperative intact PTH curves using a quick PTH assay of five patients with PHPT—PTH levels before and after 30 s (x) of squeezing and rubbing (=intended manipulation) of the parathyroid adenoma (excluded patient no. 2 with extensive PTH excretion after “manipulation”—see Fig. 2)

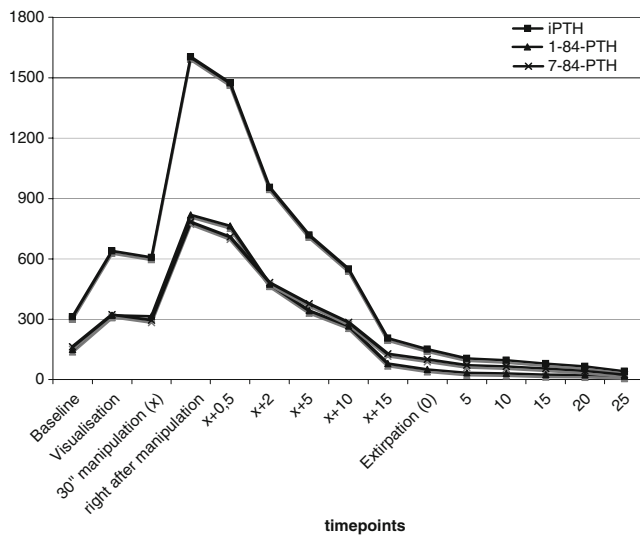


Fig. 2 Patient no. 2 with extensive PTH secretion after “manipulation” of a single adenoma (time points in minutes)

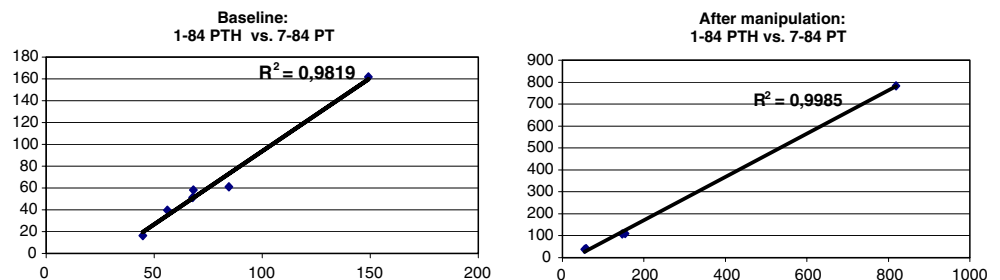
The median volume of all six glands was 1.58 ml (range 0.65 to 10.8 ml).

iPTH rose from 343 to 1,747 pg/ml in one patient after 30 s of squeezing (Fig. 2). Although this patient had the largest gland (10.8 ml), there was no correlation between gland sizes and the extent of spikes in the analysis of the data for all patients ($R^2=0.44351$ without this patient, Fig. 4). Median gland volume was 0.83 ml (ranging from 0.34 to 5.65 ml). Furthermore, there was no correlation between increase of iPTH after manipulation and preoperative calcium or PTH, respectively ($R^2=0.2727$ and $R^2=0.64657$).

PTH fragments

The ratio of (1-84)PTH to (7-84)PTH was $1.3\pm 0.6:1$ at “baseline value” and $1.4\pm 0.2:1$ after manipulation. The coefficient of determination (R^2), comparing (1-84)PTH values with (7-84)PTH values of all patients, was $R^2=0.9816$ for the “baseline values” and $R^2=0.9985$ for the values after manipulation, respectively (Fig. 3).

Fig. 3 Ratio of (1-84)PTH versus (7-84)PTH at baseline and 30 s after manipulation comparing all patients ($n=6$). R^2 , coefficient of determination



PTH half-life

Due to a lack of PTH decay right after manipulation, the PTH half-life could not be calculated. After excision of the hyperfunctioning gland, the half-life of PTH (QPTH assay) was 4.31 ± 1.01 min, of iPTH 4.19 ± 13.76 min, of (1-84)PTH 5.42 ± 4.08 min, and of (7-84)PTH 5.5 ± 9.43 min.

iPTH values after removal of the single gland followed an exponential decay in all six patients according to the well-known criteria for the intraoperative interpretation of the iPTH decay using QPTH assay.

As defined by normal iPTH and calcium levels, long-time follow-up confirmed cure from PHPT in all six patients more than 1 year after surgery.

Discussion

QPTH monitoring is used during surgery for PHPT to predict and, in most instances, to confirm that the hyperactive parathyroid tissue has been successfully excised especially in minimally invasive (targeted, limited) surgery [9]. Nevertheless, due to several possible pitfalls, a careful interpretation of the intraoperative iPTH decay by an experienced team is necessary [1, 9].

Although QPTH monitoring is used in all patients undergoing surgery for PHPT in our department [1, 6, 8], only six patients were randomly selected for participation in this “in vivo” study due to the complex study design.

The aims were to examine first if the “manipulation” of enlarged parathyroid glands in patients with PHPT leads to “PTH spikes” and second which types of PTH fragments are excreted.

In this study, “PTH spikes” were documented in four of six patients. In one patient with a very large adenoma (5.65 ml), iPTH rose from 343 to 1,747 pg/ml after “manipulation.” However, no correlation between gland sizes and PTH spikes could be documented when analyzing five of six patients (Fig. 4).

Two of six patients showed no “PTH spikes” in spite of intensive manipulation. Reasons for this remain

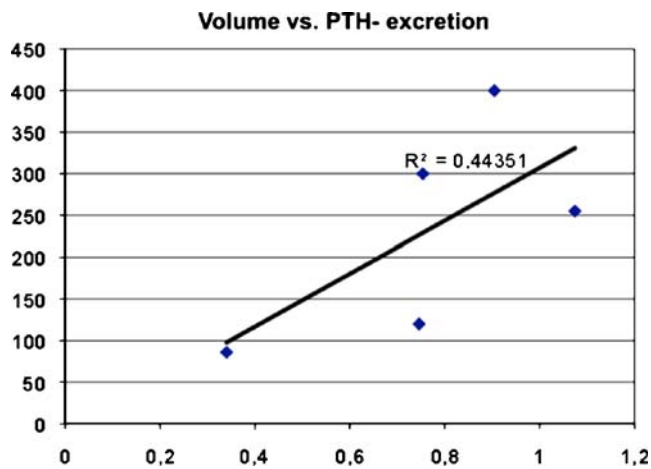


Fig. 4 No correlation between volumes and PTH excretion after manipulation, $n=5$; (excluded patient no. 2 with extensive PTH excretion after “manipulation”—see Fig. 2). x -axis, parathyroid gland volume (ml); y -axis, PTH after manipulation (pg/ml); R^2 , coefficient of determination

unclear. One of these patients had the second largest adenoma (1.07 ml). Although a standardized study protocol was used, a comparison of PTH secretion between various glands was difficult because neither the extent of secretion nor the type of secreted PTH fragments can be predicted.

Yang et al.[10] described the occurrence of “PTH spikes” before excision of the hyperfunctioning parathyroid glands which may lead to a false interpretation if not documented. Such “PTH spikes” may occur intraoperatively due to “unintended” manipulations during mobilization of the adenoma. As published recently[6], “PTH spikes” occur in 15% of the patients with PHPT undergoing parathyroid exploration, independent of the surgical approach applied (bilateral exploration or open minimally invasive surgery). Furthermore, this study[6] shows that criteria without a strict definition of the “baseline level” (such as referring to the “highest value before extirpation of the enlarged gland”) may miss “PTH spikes” and therefore may incorrectly confirm cure before any gland (enlarged or normal) is excised[1]. If “PTH spikes” are detected before excision of the enlarged glands, further blood samples (at 15-, 20-, and 25-min intervals) have to be drawn [6]. A prolonged PTH decline into the required range (exponential decay from “spike value”) may document cure. Emmolo et al. [11] found a spike in only three of 125 patients but without drawing blood right before excision of the gland. One must suspect that the described false-negative results (no adequate decline within 10 min after extirpation despite cure) also result from unrecognized spikes.

It cannot be predicted in which patient “PTH spikes” will occur intraoperatively. Furthermore, “PTH spikes” always occur during the mobilization of the gland. Even

gentle handling of the hyperfunctioning parathyroid tissue during limited minimally invasive exploration using short incisions cannot prevent an increased excretion of PTH and its fragments. It was reported recently [11] and also confirmed in this study that manipulation of normal parathyroid glands does not lead to measurable PTH spikes.

In this study, the enlarged hyperfunctioning parathyroid gland was “intentionally manipulated” in six consecutive patients. All patients had evidence of single-gland disease in preoperatively performed localization studies following a standard protocol [8, 12].

It was primarily investigated if a “manipulation” of the enlarged parathyroid glands in patients with PHPT leads to “PTH spikes” and secondly which types of PTH fragments are excreted.

Different PTH fragments are—in small amounts—secreted by the parathyroid glands themselves. In the Kupffer cells of the liver, the whole PTH molecule undergoes a fast metabolism. The whole (1-84)PTH molecule is split up into various fragments [13–16]. These fragments are afterwards eliminated by the kidney. In patients with reduced renal function, it is known that (7-84)PTH fragments (C-terminal fragments) accumulate [4]. Cross-reacting PTH assays [7] lead then to falsely high PTH values (longer half-life of PTH fragments), prolonging the intraoperative PTH decay although all hyperfunctioning tissue has been excised [4]. This phenomenon was described previously [6] but could be documented only in a small number of patients with PHPT [6]. In patients with secondary (renal) hyperparathyroidism, a (1-84)PTH assay seems to be more helpful in confirming “sufficient” parathyroidectomy intraoperatively [3].

The present study also evaluates to what extent PTH fragments are secreted by “manipulated” glands and if there is a change in the ratio of secreted PTH fragments before and after “manipulation.” The data indicate no change in the ratio of (1-84)PTH to (7-84)PTH before and after manipulation (1.3:1 vs. 1.4:1) with high coefficients of determination in patients with normal renal function.

Median half-life of the different PTH fragments was also comparable in spite of the small number of patients and the wide range of values.

Conclusion

After “intended manipulation,” not all parathyroid adenomas showed increased PTH secretions [6]. There seems to be no correlation between gland size and maximal secretion of PTH after “manipulation.” Furthermore, the ratio of the different PTH fragments does not change after “intended manipulation.” Therefore, no interpretation problems of the

intraoperative PTH curve due to a higher (or lower) amount of PTH fragments can be expected. If “PTH spikes” occur due to “unintended manipulation” [6], the increase of PTH seems to be no problem during PTH monitoring applying the “Vienna Criterion” [1] because “PTH spikes” before extirpation of the enlarged parathyroid gland can be recognized.

Acknowledgement The study was supported by “Jubiläumsfonds der Österreichischen Nationalbank” grant 9307.

Special thanks to Peter Pollack, MD, for his critical revision of the manuscript.

References

- Riss P, Kaczirek K, Heinz G, Bieglmayer C, Niederle B (2007) A “defined baseline” in PTH monitoring increases surgical success in patients with multiple gland disease. *Surgery* 142:398–404. doi:10.1016/j.surg.2007.05.004
- Bieglmayer C, Kaczirek K, Prager G, Niederle B (2006) Parathyroid hormone monitoring during total parathyroidectomy for renal hyperparathyroidism: pilot study of the impact of renal function and assay specificity. *Clin Chem* 52:1112–1119. doi:10.1373/clinchem.2005.065490
- Kaczirek K, Prager G, Riss P, Wunderer G, Asari R, Scheuba C, Bieglmayer C, Niederle B (2006) Novel parathyroid hormone (1-84) assay as basis for parathyroid hormone monitoring in renal hyperparathyroidism. *Arch Surg* 141:129–134. doi:10.1001/archsurg.141.2.129 discussion 34
- Kaczirek K, Riss P, Wunderer G, Prager G, Asari R, Scheuba C, Bieglmayer C, Niederle B (2005) Quick PTH assay cannot predict incomplete parathyroidectomy in patients with renal hyperparathyroidism. *Surgery* 137:431–435. doi:10.1016/j.surg.2004.12.017
- Miller BS, England BG, Nehs M, Burney RE, Doherty GM, Gauger PG (2006) Interpretation of intraoperative parathyroid hormone monitoring in patients with baseline parathyroid hormone levels of <100 pg/ml. *Surgery* 140:883–889. doi:10.1016/j.surg.2006.07.016 discussion 9–90
- Riss P, Kaczirek K, Bieglmayer C, Niederle B (2007) PTH spikes during parathyroid exploration—a possible pitfall during PTH monitoring? *Langenbecks Arch Surg* 392:427–430. doi:10.1007/s00423-006-0125-6
- Bieglmayer C, Prager G, Niederle B (2002) Kinetic analyses of parathyroid hormone clearance as measured by three rapid immunoassays during parathyroidectomy. *Clin Chem* 48:1731–1738
- Prager G, Czerny C, Kurtaran A, Passler C, Scheuba C, Bieglmayer C, Niederle B (2001) Minimally invasive open parathyroidectomy in an endemic goiter area: a prospective study. *Arch Surg* 136:810–816. doi:10.1001/archsurg.136.7.810
- Ruda JM, Hollenbeak CS, Stack BC Jr (2005) A systematic review of the diagnosis and treatment of primary hyperparathyroidism from 1995 to 2003. *Otolaryngol Head Neck Surg* 132:359–372. doi:10.1016/j.otohns.2004.10.005
- Yang GP, Levine S, Weigel RJ (2001) A spike in parathyroid hormone during neck exploration may cause a false-negative intraoperative assay result. *Arch Surg* 136:945–949. doi:10.1001/archsurg.136.8.945
- Emmolo I, Corso HD, Borretta G, Visconti G, Piovesan A, Cesario F, Borghi F (2005) Unexpected results using rapid intraoperative parathyroid hormone monitoring during parathyroidectomy for primary hyperparathyroidism. *World J Surg* 29:785–788. doi:10.1007/s00268-005-7751-y
- Prager G, Czerny C, Ofluoglu S, Kurtaran A, Passler C, Kaczirek K, Scheuba C, Niederle B-A (2003) Impact of localization studies on feasibility of minimally invasive parathyroidectomy in an endemic goiter region. *J Am Coll Surg* 196:541–548. doi:10.1016/S1072-7515(02)01897-5
- Pillai S, Zull JE (1986) Production of biologically active fragments of parathyroid hormone by isolated Kupffer cells. *J Biol Chem* 261:14919–14923
- Bringhurst FR, Segre GV, Lampman GW, Potts JT Jr (1982) Metabolism of parathyroid hormone by Kupffer cells: analysis by reverse-phase high-performance liquid chromatography. *Biochem* 21:4252–4258. doi:10.1021/bi00261a011
- Habener JF, Rosenblatt M, Potts JT Jr (1984) Parathyroid hormone: biochemical aspects of biosynthesis, secretion, action, and metabolism. *Physiol Rev* 64:985–1053
- Potts JT Jr, Kronenberg HM, Rosenblatt M (1982) Parathyroid hormone: chemistry, biosynthesis, and mode of action. *Adv Protein Chem* 35:323–396. doi:10.1016/S0065-3233(08)60471-4