

Usefulness of a rapid immunometric assay for intraoperative parathyroid hormone measurements

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Abstract

Intraoperative parathyroid hormone (IO-PTH) measurements have been proposed to improve operative success rates in primary, secondary and tertiary hyperparathyroidism (PHP, SHP and THP). Thirty-one patients requiring parathyroidectomy were evaluated retrospectively from June 2000 to January 2002. Sixteen had PHP, 7 SHP and 8 THP. Serum samples were taken at times 0 (before resection), 10, 20 and 30 min after resection of each abnormal parathyroid gland. Samples from 28 patients were frozen at -70°C for subsequent tests, whereas samples from three patients were tested while surgery was being performed. IO-PTH was measured using the Elecsys immunochemiluminometric assay (Roche, Mannheim, Germany). The time necessary to perform the assay was 9 min. All samples had a second measurement taken by a conventional immunofluorimetric method. We considered as cured patients who presented normocalcemia in PHP and THP, and normal levels of PTH in SHP one month after surgery and who remained in this condition throughout the follow-up of 1 to 20 months. When rapid PTH assay was compared with a routine immunofluorimetric assay, excellent correlation was observed ($r = 0.959$, $P < 0.0001$). IO-PTH measurement showed a rapid average decline of 78.8% in PTH 10 min after adenoma resection in PHP and all patients were cured. SHP patients had an average IO-PTH decrease of 89% 30 min after total parathyroidectomy and cure was observed in 85.7%. THP showed an average IO-PTH decrease of 91.9%, and cure was obtained in 87.5% of patients. IO-PTH can be a useful tool that might improve the rate of successful treatment of PHP, SHP and THP.

Key words

- Parathyroid hormone
- Intraoperative PTH measurement
- Primary hyperparathyroidism
- Secondary hyperparathyroidism
- Tertiary hyperparathyroidism

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Introduction

Primary hyperparathyroidism (PHP) is a hypercalcemic disease due to an abnormal increase in parathyroid hormone (PTH) secretion by one or more parathyroid glands. The hallmark of this condition is the pres-

ence of high levels of calcium and high or inappropriate levels of PTH. PHP is more common in women than men and increases with age in both sexes (1). Since PHP was first described, parathyroidectomy has remained the only definitive therapy when the disease is symptomatic (2). The goal of para-

thyroidectomy is the excision of the abnormal parathyroid gland(s) with preservation of the normal ones in order to achieve and maintain a postoperative normocalcemic state (3). Success rates for surgical treatment depend on the skill and experience of the surgeon in recognizing the pathologic changes and excising the correct amount of hyperfunctioning parathyroid tissue (4). Several methods have been proposed to aid the surgeon in deciding about the amount of parathyroid tissue to be resected. One of these approaches is intraoperative monitoring of PTH levels. Since the intact molecule of PTH containing residues 1 to 84 has a half-life of only 3 to 4 min (5-9), intraoperative assays are capable of quantitatively demonstrating the continued hypersecretion by remaining parathyroid tissue after excision of one or more enlarged glands (10).

Secondary hyperparathyroidism (SHP) is an acquired disorder most commonly seen in end-stage renal disease, since the uremic state presents a continuous stimulus to the parathyroid gland. About 5 to 10% of patients with chronic renal failure treated with long-term dialysis require surgery for SHP (11). The term tertiary hyperparathyroidism (THP) is used to describe the evolution of SHP to a state of apparent autonomous PTH secretion, resulting in elevated levels of serum calcium resembling PHP (12). In experienced hands, both subtotal parathyroidectomy and total parathyroidectomy with autotransplantation present good therapeutic results (13). Persistence/recurrence indicates an inadequate resection of hyperfunctioning tissue (14,15). Reoperation is necessary for recurrent SHP in 15% of cases and presence of supernumerary glands, inadequate initial parathyroidectomy, and continued hyperplasia of remnant tissue are all potential contributors to this rate of failure (16).

The usefulness of a rapid intraoperative PTH measurement relates to the need to avoid postoperative persistent hypercalcemia in patients with PHP. Since failure to

cure these patients is most often due to multiglandular disease not recognized by the surgeon or due to ectopic adenomas, measurement of intraoperative PTH can reduce the risk of failure by providing information about possible remnant tissue (10,17). Another potential utility of intraoperative PTH is in minimally invasive surgeries and the need to achieve high cure rates with this procedure (18).

As in PHP, the intraoperative determination of intact PTH may be useful in surgery of patients with chronic renal failure and hyperparathyroidism, suggesting missed or hyperfunctioning supernumerary glands, when levels fail to decrease after parathyroid tissue removal (19). The aim of the present study was to observe retrospectively the decay profile of intact PTH in patients undergoing parathyroidectomy for PHP, refractory SHP or THP and to compare it to the cure observed in postoperative follow-up, in order to assess the utility of this procedure in predicting cure.

Patients and Methods

Patients

Thirty-one patients requiring parathyroidectomy were evaluated retrospectively during a period of 20 months (June 2000 to January 2002) at Escola Paulista de Medicina, Federal University of São Paulo, São Paulo, SP, Brazil. Sixteen had PHP, and fifteen had hyperparathyroidism related to chronic renal failure. Diagnosis of PHP was based on clinical features and confirmed by the findings of high total and/or ionized calcium levels, and high or inappropriate levels of intact PTH associated with normal renal function. Criteria for surgery in PHP were based on rules established by the National Institutes of Health (NIH, Bethesda, MD, USA) Consensus Conference of 1990 (20).

Among the patients with hyperparathyroidism secondary to chronic renal failure,

7 had refractory SHP in dialysis treatment and 8 had THP (6 with renal transplantation and functioning grafts and 2 in dialysis treatment). Refractory SHP patients had severe SHP without hypercalcemia, and the term THP was used to describe the evolution of SHP to a state of apparent autonomous PTH secretion, resulting in elevated levels of serum calcium resembling PHP (12).

Specific indications for parathyroidectomy in hyperparathyroidism related to renal disease include a) persistent hypercalcemia, b) intractable pruritus not responding to intensive dialysis or to other medical interventions, c) progressive extraskeletal calcifications and/or persistent hyperphosphatemia despite the continued use of dietary phosphorus restriction and phosphate-binding agents, d) severe bone pain or fractures, and e) the development of calciphylaxis (21).

Patients were divided into three groups: the first included those with PHP, the second patients with refractory SHP and the third group patients with THP. Data analysis was carried out on these groups because of the particular characteristics and evolution of the patients. Characteristics of patients and preoperative laboratory findings are shown in Table 1. This study was approved by the University Ethics Committee (approval No.

886/00) and informed consent was obtained from all patients.

Methods

Baseline peripheral venous blood samples were obtained immediately after induction of anesthesia. After the parathyroid adenoma was removed, additional samples were collected at 10, 20 and 30 min after excision. When patient presented with multiglandular disease, blood samples were obtained 10, 20 and 30 min after each glandular excision. Samples from 28 patients were frozen at -70°C for subsequent tests, whereas samples from 3 patients were tested while surgery was being performed.

PTH was measured by the Elecsys PTH Immunoassay (Elecsys 1010 System, Roche, Mannheim, Germany). The test is an immunometric assay based on monoclonal antibodies, magnetic particles as the solid phase and ruthenium complex as the chemiluminescent label. The time needed to perform the assay was 9 min. Analytical sensitivity (lower detection limit) was 1.20 pg/ml. To validate the rapid PTH assay, 170 samples were also submitted to a standard immunofluorimetric assay (22). During the postoperative follow-up (1 to 20 months after sur-

Table 1. Characteristics of patients and preoperative laboratory findings.

	Primary HP	Secondary HP	Tertiary HP
No. of patients	16	7	8
Sex (female:male)	10:6	4:3	5:3
Mean age (years)	52 (12-80)	31.7 (22-58)	40 (23-54)
Mean preoperative iPTH (RV: 10-70 pg/ml)	248.6 (86-1019)	1312.5 (248-2500)	503.2 (140-1774)
Mean preoperative ionized calcium (RV: 1.12-1.32 mmol/l)	1.45 (1.32-1.89)	1.20 (1.13-1.27)	1.56 (1.37-2.06)
Years on dialysis		6 (3-14)	7.8 (4-14)
Years of renal graft (N = 6)			1.5 (0.16-4)

HP, hyperparathyroidism; iPTH, intact parathyroid hormone; RV, reference value. Range is shown in parentheses.

gery), we considered as cured patients who presented normocalcemia in the case of PHP and THP, and normal PTH levels in SHP.

Serum total calcium, phosphorus, total alkaline phosphatase and urinary calcium were measured by standard automatic assays. Serum ionized calcium was measured using an ion-specific electrode (AVL 9180 Electrolyte Analyzer, Roswell, GA, USA), with a reference value of 1.12 to 1.32 mmol/l.

Statistical analysis

Data were analyzed by the paired *t*-test and Spearman correlation test. A *P* value of <0.05 was considered to be significant.

Results

The correlation between the rapid PTH assay and the standard immunofluorimetric assay was $r = 0.959$ ($P < 0.0001$) (Figure 1).

Among the 16 patients with PHP, 14 had uniglandular disease and 2 multiglandular disease. In the 14 patients with PHP and uniglandular disease there was an average decrease of 78.8% in intact PTH concentrations 10 min after abnormal parathyroid resection. Intact PTH levels continued to fall 20 and 30 min after abnormal parathyroid removal by about 83.7 and 87.1% from the baseline values, respectively. There was a significant difference in PTH values between

times 10 and 20 min ($P = 0.0170$) and between times 20 and 30 min ($P = 0.0027$) after resection.

It is interesting to note that in PHP with multiglandular disease ($N = 2$), as expected, the decay profile was different from that for uniglandular disease, with a stepwise decrease in intraoperative PTH concentrations following each gland removal. The percent changes in PTH from baseline for the 14 patients with PHP with uniglandular disease are presented in Figure 2A and the decay profile for PHP patients with multiglandular disease is presented in Figure 2B.

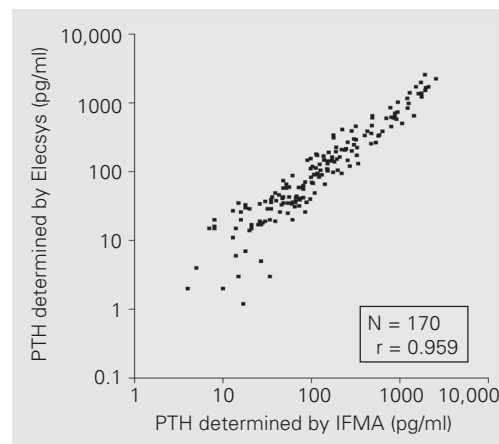
All patients with PHP were considered cured during the postoperative follow-up, with a mean 78.8% reduction in PTH levels, suggesting that the assay could predict cure in this group of patients.

As expected when multiglandular disease is involved, measurements of intraoperative rapid intact PTH in refractory SHP patients followed a sequential decrease pattern after each gland removal. Patients with refractory SHP showed a mean intact PTH decrease of 89% 30 min after total parathyroidectomy, indicating high cure rates. In this group, cure was observed in 85.7% (6/7) of patients. In 5 of these 7 refractory SHP patients, intact PTH absolute values in the final intraoperative samples were still high (mean 128.5 pg/ml, range: 83-311 pg/ml), but during the postoperative follow-up 85.7% of SHP patients were considered cured. The PTH decrease observed in THP patients resembled that observed in PHP, with a faster PTH reduction than observed in SHP (Figure 2C). Patients with THP had a mean rapid intact PTH decay of 91.9% from baseline 30 min after removal of all recognized parathyroid tissue, with a cure rate of 87.5% (7/8).

Discussion

Failure to surgically cure hyperparathyroidism is usually related to multiglandular disease, ectopic adenomas, misinterpretation

Figure 1. Correlation between the results of rapid intraoperative intact parathyroid hormone (PTH) determined by the Elecsys immunochemiluminometric assay and by a standard immunofluorimetric assay (IFMA). *N* = number of patients; *r* = correlation coefficient.



of frozen-section pathologic findings, and incorrect diagnosis of PHP (23,24). Attempting to decrease failure rates, experienced surgeons have reported large series with excellent results using different surgical approaches to parathyroidectomy (25). Surgical treatment of PHP has undergone some changes in recent years. The standard technique of bilateral neck exploration with identification of parathyroid adenoma together with the three other normal glands is being replaced in selected cases at many medical centers with a less time-consuming procedure of unilateral neck exploration based on preoperative localization by ^{99m}Tc -sestamibi scans (26). The utility of a test that can assure the surgeon that multiglandular disease is not present after the excision of a large parathyroid gland, or that can help localize intraoperatively an eluding adenoma, and quantitatively confirm the adequate excision, is clear.

Secretion of PTH is suppressed in the remaining normal parathyroid glands after removal of all hyperfunctioning tissue (9) and this, coupled with the rapid clearance of intact PTH, allows the measurement of intraoperative PTH to determine its disappearance rates. The clinical utility of rapid intraoperative PTH measurements in parathyroidectomy was first reported in 1988 using a modified intact PTH immunoradiometric assay (27). The authors observed that PTH concentrations declined to a mean of 40% of baseline values 15 min after successful parathyroid adenomectomy in 12 patients, and they suggested that the surgical judgment and skill of the surgeon to determine surgical cure could be complemented with the intraoperative PTH assay.

Thereafter, rapid assays were developed using radioactive (4,28,29) as well as non-radioactive formats (10,30). Irvin III and Deriso (10) documented an immunochemiluminometric assay for intact PTH (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) for use as a rapid intraoperative PTH

assay in surgery for PHP, and reported a sensitivity of 97%, specificity of 100%, and an overall accuracy of 97% (31). Gordon and co-workers (32) suggested that a 50% decrease in the 10-min post-excision sample accurately predicts the disease state in 89% of patients with PHP. Findings of 88% accuracy in predicting cure using the criterion of

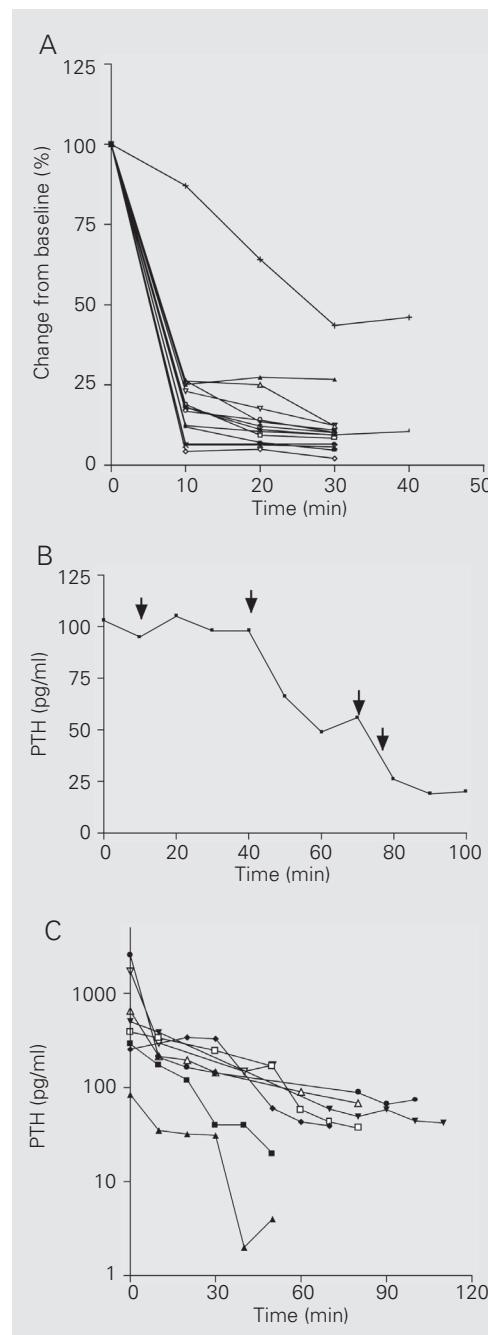


Figure 2. Intraoperative circulating concentrations of intact parathyroid hormone (PTH) during parathyroid gland resection. Intact PTH was determined by the 9-min Elecsys assay system. *A*, Reduction of intact PTH in 14 primary hyperparathyroid patients with a single adenoma. Data are reported as percent. *B*, Reduction of intact PTH (pg/ml) in primary hyperparathyroid patients with multiglandular disease. Arrows indicate parathyroid gland resection. *C*, Intact PTH concentration (pg/ml) of 8 tertiary hyperparathyroid patients.

a 50% decrease at 5-10 min have been reported, and of 97% if patients with delayed decreases of intraoperative PTH are included (33).

The assay used in the present study showed a good correlation with a standard PTH immunofluorimetric assay used in our routine ($r = 0.959$, $P < 0.0001$) and documented the rapid decline in PTH levels after excision of significant amounts of parathyroid tissue, as previously described by other authors (4,10,27,29,30,33) with similar assays.

All patients with PHP were considered to be cured after parathyroidectomy and this correlated with an average 78.8% reduction of PTH values from baseline at 10 min after adenoma removal, confirming the ability of the assay to predict cure. Patients with SHP showed an average decrease of 89% 30 min after parathyroidectomy with the assay showing the ability to predict cure in 85.7% of patients. Even if final intraoperative PTH levels were still high in SHP patients, the postoperative follow-up showed normal PTH

levels in 85.7%. This pattern of delayed PTH clearance has been described in SHP patients receiving dialysis compared to patients with normal renal function (19). The decreased renal function might explain the final abnormal intraoperative PTH levels in patients that eventually were considered to be cured. In these cases it may be of interest to consider the percent decay in intraoperative PTH values, instead of absolute values after total parathyroidectomy. Among patients with THP, the rapid PTH decay profile showed a pattern resembling PHP, probably due to the presence of a functioning renal graft in these patients ($N = 6$). In THP patients, intraoperative PTH was able to predict cure in 87.5% of cases, with a mean 91.9% decrease in PTH values from baseline to 30 min after parathyroidectomy. In conclusion, the rapid PTH assay used in this study might be useful to improve success rates in parathyroidectomy surgeries in primary, secondary and tertiary hyperparathyroidism.

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