

Intraoperative Parathyroid Aspiration and Parathyroid Hormone Assay During Parathyroidectomy for Primary Hyperparathyroidism

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ABSTRACT: **Background:** Direct aspiration from suspected pathological tissue and rapid parathyroid hormone analysis may offer a reliable, cost effective alternative to currently used “gold standard” tests.

Objectives: To validate the accuracy of intraoperative measurements of parathyroid hormone levels in parathyroid adenomas.

Methods: A prospective study included 22 patients diagnosed with primary hyperparathyroidism who underwent parathyroidectomy due to an adenoma or hyperplasia. Aspirations of tissues extracted from three adjacent areas (the pathological parathyroid, thyroid, and muscle tissues) were sent for rapid parathyroid hormone analysis. The assay values of these tissue aspirates were compared to the results of the pathology report based on frozen section analysis and the final pathology report.

Results: All assay results were significantly higher for parathyroid tissue 16,800 to 1,097,986 pmol/L (median 26,600), than for either thyroid 1.7 to 415 pmol/L (median 6.5), $P < 0.001$, or muscle tissue 1.1 to 1230 pmol/L, (median 11.3), $P < 0.001$. All tissues showing high parathyroid assay values were also verified by pathology examinations: 7 had adenomas and 15 had a differential diagnosis of adenoma or hyperplasia. The frozen section identified all but one (false negative). Rapid intraoperative parathyroid levels > 1500 predicted parathyroid tissue with a 99% level of confidence, while levels between 1000 and 1500 predicted it with 95% confidence. The intraoperative parathyroid hormone assay showed $> 70\%$ decrease in 15/21 cases.

Conclusions: Rapid intraoperative parathyroid hormone analysis is a reliable and precise technique, equally accurate for frozen section analysis in predicting with high certainty intraoperative parathyroid tissue.

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KEY WORDS: parathyroid adenoma, parathyroid hormone, parathyroidectomy, frozen section, quick parathyroid hormone (PTH)

For six decades, until 1990, the accepted technique for performing a parathyroidectomy was bilateral neck exploration (BNE), an approach that yielded a high cure rate of over 95% in patients with primary hyperparathyroidism but resulted in a large scar and potential complications. Transient hypocalcemia was reported in 15–25% of cases and there was risk of damage to the recurrent nerve. The modern technique currently used is the minimally invasive approach that requires early identification and localization of the pathological gland prior to the surgery. Compared to BNE, this approach entails less tissue trauma and leads to a significantly reduced complication rate (approximately 5%) for developing postoperative transient hypocalcemia [1].

In the minimally invasive approach, it is common to use an intraoperative technique to confirm that the removed tissue is parathyroid tissue (and not a lymph node or thyroid tissue). The two most common techniques used in our department are the frozen section method and quick parathyroid hormone (PTH) assay.

The frozen section biopsy method requires sending the preparation identified during surgery as a pathological parathyroid gland for pathology confirmation. The disadvantages of the method are:

- It is time consuming. In our hospital, the process of transferring the tissue to the pathology department, specimen preparation and, analysis take about 30 minutes, during which the surgery is stopped until the pathology results arrive.
- It is expensive: \$300 according to a university hospital cost estimate [2].
- Possible inaccurate pathology results (false negative) in cases of intrathyroidal parathyroid glands, parathyroid glands with a microfollicular pattern, or when the parathyroid gland contains considerable fatty tissue [3].

The quick intraoperative PTH (IOPTH) analysis allows detection of a rapid decrease in blood levels of PTH during parathyroidectomy. A decrease of more than 50% to 70% from the baseline IOPTH level pre-incision, as measured at the

beginning of the surgery, compared to pre-excision IOPTH, measured 10 minutes after removal of the suspected gland, accurately predicts the success of the surgery [4]. This method has been criticized in the literature claiming that it might be inaccurate due to manipulation of the gland during the surgery before drawing blood for measuring baseline PTH level or, alternatively, parallel thyroid gland surgery might lead to false positive results, while manipulation of the gland before the second blood analysis (after removal of the gland) might lead to false negative results [5].

In this study, we assessed an additional technique that may provide an alternative to frozen section analysis and IOPTH assay. The fine-needle aspirate (FNA) PTH technique was first described in 1983 by Doppman [6] but came into use in the late 1990s [5]. This procedure entails extracting a tissue sample with a needle and sending it for analysis to determine PTH concentration. The sensitivity and specificity of the test showed 91–100% accuracy in identification of parathyroid tissue [3]. A number of studies have shown that this test may help in both pre- and intraoperative localization of the parathyroid gland [2,3,6], thus demonstrating the FNA–PTH technique is faster and equal in accuracy, offering a reliable alternative to the currently used tests, which are considered as the gold standard.

The aim of the present study was to assess the accuracy of the FNA–PTH technique to demonstrate the higher PTH levels in the pathological parathyroid gland, compared to those in adjacent tissues (muscle, thyroid).

PATIENTS AND METHODS

The study was conducted at a single medical center between May 2014 and September 2015. The study included patients over 18 years of age who had been diagnosed with primary hyperparathyroidism and who underwent first parathyroidectomy to remove the suspected gland/s. We used technetium (^{99m}Tc) sestamibi (MIBI) scan and neck ultrasound for localization of the tumor. Magnetic resonance imaging (MRI) and four-dimensional computerized tomography (4DCT) imaging were performed when the previous imaging methods failed in detection and localization of the tumor.

Patients with non-primary hyperparathyroidism or multiple endocrine neoplasia (MEN) syndromes, patients who underwent previous parathyroid surgery, or patients younger than 18 years of age were excluded from the study.

SURGICAL TECHNIQUE

The surgery began with a neck incision common in parathyroidectomy, including dissection and search for the pathological gland. All the surgeries were performed by the same experienced surgeon. Once the pathological gland was identified, the anesthesiologist performed a blood test to determine the patient's rapid PTH level. The suspected glands were excised,

removed, and placed on a sterile surface. Aspirations from muscle tissue identified in the surgical field (strap muscle), thyroid tissue, and suspected parathyroid gland were performed using a 23-gauge needle connected to a 3 ml syringe containing 2 ml of saline that was inserted into the tissue. Back and forth movements of the piston were performed to optimize the extraction of the material. Contents of the syringe were injected into ethylenediaminetetraacetic acid (EDTA) tubes and transferred in an ice bag to the endocrine laboratory for analysis of the PTH levels. The assays were performed after dilution for reference range, centrifugation for 5 minutes, and chemiluminescence. The results are expressed in pg/ml. The same laboratory performed the measurements of serum PTH preoperatively.

The parathyroid gland was sent for a frozen section test. A blood test for PTH was performed at the beginning of the surgery and about 15 minutes after excision of the gland. When the results of the frozen section test identified pathologic parathyroid tissue and, in addition, quick PTH level in blood showed a decrease of more than 50%, the surgery ended. In cases in which one or both of the above conditions were not met, the surgery continued in search of another suspected parathyroid gland. We compared the tissue aspirates from the three types of examined tissues. In addition, we verified through the pathologist's final confirmation that parathyroid tissue was indeed pathological.

STATISTICAL ANALYSIS

Paired *t*-tests and Wilcoxon signed-rank tests were performed to analyze differences between the parameters. Data were analyzed using SAS[®] version 9.3 (SAS Institute, Cary, North Carolina, USA). *P* < 5% was considered statistically significant.

RESULTS

A parathyroidectomy was performed on 22 patients (17 female), age range 22–76 years, mean age 56.1 years. Patients' calcium levels before the surgery ranged from 10.8 to 13.25 mg/dl, mean 11.8 mg/dl. Calcium levels at least 6 months post-surgery were in the normal range (8.6–10.18 mg/dl, mean 9.45 mg/dl) for all patients.

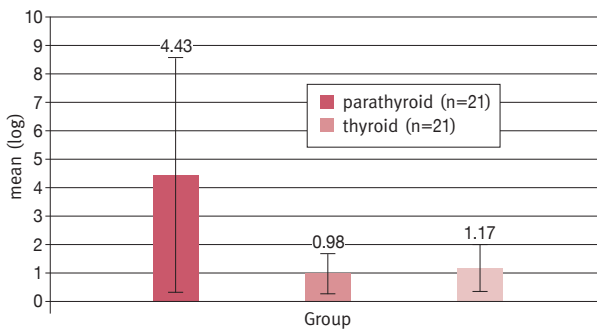
According to the final pathology reports, all patients underwent removal of a pathological parathyroid gland. In 7 patients, adenoma was confirmed (normal parathyroid tissue in circumference), while in 15 patients such tissue in circumference was not identified with certainty, and the differential diagnosis included adenoma and hyperplasia. One case included adenoma and papillary thyroid cancer. There were no cases of carcinoma.

The frozen section results confirmed pathological parathyroid tissue in 21 of the 22 patients. In one case, a frozen section provided negative result for parathyroid tissue, whereas the final report for the same patient confirmed that the preparation submitted to pathologists during the surgery was pathological parathyroid (false negative of frozen section).

Table 1. Patient details

Patients	N=22 17 females, 5 males
Age (years)	22–76 Mean 56.1
Calcium level before surgery (mg/dl)	13.25–10.8 Mean 11.8
Calcium levels 6 months post-surgery (mg/dl)	8.6–10.18 Mean 9.45

Figure 1. Mean log transformed results per group, parathyroid vs. thyroid: $P < 0.05$, parathyroid vs. muscle: $P < 0.05$, muscle vs. thyroid: NS. Standard error reflects a confidence interval of 95%



Quick PTH tests were performed in 21 patients (in one case the test was not performed due to technical reasons). Nineteen patients showed a decrease greater than 50% from the level before removal of the pathological gland, and 15 of 21 cases showed a decrease of more than 70%. Of six cases with decrease less than 70%, the final histologic diagnosis for four patients was not certain (adenoma vs. hyperplasia), and two had a final diagnosis of adenoma.

The values of rapid intraoperative PTH from the parathyroid gland ranged from 1680 to 1,097,986 pmol/L, median 26,600 pmol/L.

The values of rapid intraoperative PTH from the thyroid gland ranged from 1.7 to 415 pmol/L, median 6.5.

The values of rapid intraoperative PTH from the strap muscle ranged from 1.1 to 1230 pmol/L, median 11.3.

According to the Wilcoxon signed-rank test the difference in PTH values between parathyroid and thyroid tissue was statistically significant, ($t = 15.86, P < 0.001$).

There was also a statistically significant difference in PTH values between parathyroid and muscle tissue ($t = 14.52, P < 0.001$).

However, the difference in PTH values between the muscle and thyroid tissue was not statistically significant.

We concluded at a confidence level of 99% that rapid intraoperative PTH levels > 1500 pmol/L predict parathyroid tissue with 99% confidence, while levels between 1000 to 1500 pmol/L predict it with 95% confidence.

DISCUSSION

Our findings suggest that measurement of PTH levels during parathyroidectomy is an effective and accurate technique to aid surgeons in predicting and confirming that the removed tissue is indeed parathyroid tissue. Our study and previous reports have shown that the lowest IOPTH level measured from parathyroid tissue are significantly higher than the IOPTH level sampled from adjacent tissues [7,8].

Pelizzo et al. [9] demonstrated that this technique could be helpful in metastatic thyroid cancer that requires neck dissection of lymph nodes, which often involves a difficulty to visually distinguish between the pathological lymph node to be removed and the parathyroid tissue. During surgery the gland is removed and tested by frozen section. In cases in which the pathologist confirms that it is a lymph node, the surgery continues; however, when the test identifies parathyroid tissue, the surgeon must perform parathyroid autotransplantation, which may cause transient hypocalcemia in up to 43% of cases [10].

Rapid intraoperative measurement of PTH performed by fine needle aspiration has been previously demonstrated as a good parameter for the differential diagnosis of parathyroid vs. lymph node tissue, showing the lowest level of PTH from parathyroid tissue was 85 pmol/L, whereas the highest level measured in lymph nodes was 20.4 pmol/L without overlap [9].

Another study compared 223 intraoperative measurements of FNA-PTH levels in parathyroid tissue to thyroid and lymph tissues demonstrated that values greater than 84 pmol/L are 100% sensitive and specific to predict parathyroid gland [5].

A validation study using rapid intraoperative assay compared measurement of PTH levels in parathyroid tissue to thyroid and muscle tissues, and demonstrated that the lowest level measured in parathyroid tissue was 675 pmol/L, whereas the highest level in non-parathyroid tissue was 248 pmol/L [2].

A study of 65 parathyroid surgeries performed by the same surgeon showed that while PTH values were always higher than 1500 pmol/L, the highest values measured in thyroid, thymus, and fat tissue were 562 pmol/L, 71 pmol/L, and 47 pmol/L, respectively [3].

This technique of measuring PTH is also instrumental in localization tests performed preoperatively. The common methods for pathological parathyroid gland localization are MIBI scans and ultrasound. When these methods fail to identify the patient's pathological gland(s), additional imaging modalities are used, such as MRI, 4DCT, venous sampling, and intraoperative scintigraphy.

Numerous studies in recent years have demonstrated that measurement of the PTH levels sampled preoperatively with ultrasound from the suspected gland is highly sensitive and specific, and sometimes its accuracy exceeds those of standard localization tests [7,8].

Table 2. Descriptive statistics of the parathyroid hormone measurements

	N	Mean	Standard deviation	CV	Minimum	Q1	Median	Q3	Maximum	95_LCLM	95_UCLM
Parathyroid	21	94914.10	242389.20	255.38	1680.00	12198.00	26600.00	43360.00	1097986.00	-15420.15	205248.34
Thyroid	21	34.87	91.47	262.35	1.70	4.00	6.50	10.50	415.00	-6.77	76.50
Muscle	21	113.20	315.13	278.38	1.10	5.64	11.30	31.10	1230.00	-30.24	256.65

CV = confidence value, LCLM = lower confidence limit mean, UCLM = upper confidence limit mean

From a practical perspective, this technique may be useful as a preoperative tool when imaging tests are inconclusive in localization of the pathological gland, as in cases of thyroid nodules, small adenoma, or ectopic location of the adenoma. These conditions increase the risk for MIBI scan failure to accurately identify the suspected gland (false negative) [5,8]. Therefore, this technique may be added to the intraoperative techniques when the initial localization tests fail to identify the suspected gland.

Moreover, currently two preoperative imaging tests are needed, an anatomical ultrasound examination and an imaging physiological examination. Results of the aforementioned studies point to an option to omit the imaging examination (which is expensive, invasive, and time consuming) and instead conduct the anatomical ultrasound examination and the physiological examination using ultrasound-directed needle aspiration of PTH together [7,8].

Nonetheless, doubts have been expressed concerning safety of fine-needle aspiration from parathyroid gland and its effect on the future surgical procedure. Norman and co-authors [11] noted the disadvantages of this test by comparing histology results demonstrating that 77% of the surgically removed parathyroid glands that had previously undergone preoperative fine-needle aspiration demonstrated fibrosis vs. 4.3% of the glands that had not undergone aspiration. Furthermore, the fibrosis could be mistaken as a malignancy on histology [11]. In addition, the operative time was doubled in cases of glands that had undergone aspiration.

Frozen section examination, in contrast to other computerized laboratory tests, is subjective and thus the results are dependent on the pathologist's level of knowledge and experience.

Hatami et al. [12] examined 306 frozen section specimens from various tissues (e.g., thyroid, ovary, parathyroid, and lymph nodes). In six cases (2.03%) they found a discrepancy between the results of the frozen section and the final tissue diagnosis. The overall reported sensitivity and specificity were 92.95% and 99.55%, respectively [12].

A response article stated that these disadvantages may be overcome through the use of small needles (27 Gy) and by limiting the number of aspiration passes to one or two per gland [13].

It is important to note that in previous studies it was not possible to distinguish between normal and abnormal parathyroid tissue based on the PTH levels. In addition, it was not possible to distinguish between different pathological conditions (hyperplasia vs. adenoma) based on the PTH levels.

Concluding this test is limited in its ability, and can only confirm identification of the parathyroid tissue [2,8,9,14].

Interestingly, IOPTH levels sampled from normal parathyroid glands in patients with primary hyperparathyroidism were significantly lower than IOPTH levels from the pathological gland in the same patient [3,15], which is associated with the negative feedback from the pathological gland to the normal glands in cases of primary hyperparathyroidism.

Concurrent with previous reports, PTH values in our study were high and varied within a large range [2,9,14,15]. The reasons the test results of different patients vary within a large range in all these studies is unclear; however, we can suggest that lack of standardization in aspiration of the material from the parathyroid gland and in quantity of dilution with liquid in the syringe after aspiration are contributing factors. Therefore, this technique is considered operator dependent. Moreover, we cannot conclude that an extremely high level of PTH (in our study, one patient with adenoma showed PTH level over one million) is indicative of a more aggressive disease than in patients who have lower measured levels of PTH.

Although termed "quick PTH assay" the time measures are usually not reported in the literature. From our experience, the time required to attain results from each PTH assay is measured in minutes while waiting for pathology results of each frozen section examination is estimated to take approximately 40 minutes.

The FNA-PTH testing kit costs less than US\$100 per patient. This is relatively inexpensive since the reagent can be used for multiple patients on multiple days (within the expiration date). Frozen sections cost an estimated US\$310 (pathologist's work and cost of preparing the frozen section). In addition, FNA-PTH results may reduce the number of reoperations needed. Thus, FNA-PTH is less expensive and more cost effective to the system than the frozen section [16].

Finally, FNA-PTH results show higher accuracy than the frozen section test. IOPTH levels greater than 1500 pmol/L predicted parathyroid tissue in 99% accuracy; whereas, levels 1000–1500 Pmol/L had 95–99% accuracy.

As previously demonstrated, and in accordance with our results, the PTH values in the parathyroid tissue were significantly higher compared to those measured in adjacent tissues (thyroid, muscle), thus differential diagnosis for high PTH does not exist. Therefore, we perceive no usefulness for additional PTH assays of other tissue samples after attaining samples with high PTH values.

In our study, the quick PTH assay test had 100% sensitivity and 100% positive predictive value (PPV) (no false negative results). The frozen section test had sensitivity of 95.5% (one false negative). The quick PTH assay using strict standard cutoff value of 70% had sensitivity of 71.4% with 6 false negative cases.

IOPH levels measured during parathyroidectomy in the parathyroid tissue are higher than the PTH levels measured in adjacent tissues (thyroid, muscle). High levels of PTH in the suspected tissue during parathyroidectomy accurately predict parathyroid tissue.

Direct aspiration of the suspected adenoma is quick, easy, cost effective, and reliable for confirming it is tissue of parathyroid origin and thus may offer an alternative to the intraoperative tests currently used worldwide for identification of parathyroid tissue.

CONCLUSIONS

In conclusion, we can suggest that using the FNA-PTH technique may offer a good alternative to the frozen section examination by providing a pronounced advantage in saving time during surgery, reducing overall cost, and ensuring higher accuracy. A shorter operative time is achieved since the FNA-PTH results can be obtained faster than both the frozen section method and the quick PTH test, and can be used as a complementary modality when the surgeon is already planning to use the assay. Taken together, these options may lead to changes in the order of procedures during parathyroidectomy.

Additional studies are needed, especially in non-standard situations in which abnormal parathyroid was not preoperatively identified with certainty, during reoperations, and determining less common pathologies, such as hyperplasia and carcinoma, to show that this technique is effective and can replace existing techniques used widely today.

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Capsule

For diabetes screening, race matters

Type 2 diabetes is diagnosed and monitored by a blood test for HbA1c, a modified form of hemoglobin produced when blood glucose is high. HbA1c levels can be influenced by genetic variants unrelated to glucose homeostasis. To examine whether such variants affect the reliability of the HbA1c test, **Wheeler** et al. studied 60 genetic variants in nearly 160,000 people of different ancestries. They identified a specific variant that, by shortening the lifespan of red blood

cells, reduced HbA1c levels irrespective of blood glucose. This variant occurs almost exclusively in individuals of African ancestry. The results suggest that about 650,000 African Americans with type 2 diabetes may be misdiagnosed as healthy if they are screened solely by the HbA1c test.

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