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A Prospective Evaluation of Novel Methods to Intraoperatively Distinguish Parathyroid Tissue Utilizing a Parathyroid Hormone Assay[1]

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Background. **Frozen section analysis has traditionally been used to confirm the identity of parathyroid tissue intraoperatively; however, it is time-consuming and costly and requires the excision of a significant portion of tissue. An intraoperative biopsy and analysis with a parathyroid hormone (PTH) assay is a possible alternative; however, this technique has not been perfected.**

Methods. **Two hundred twenty-three tissue specimens were collected prospectively from patients undergoing neck exploration. Each specimen was sampled intraoperatively using three different biopsy techniques: a fine-needle aspiration (FNA) with 10 passes of a needle (FNA10), a FNA with 20 passes of a needle (FNA20), and a tissue biopsy of approximately 1.0 mm^3 (BIOPSY). The PTH concentration of each sample was determined via the Elecsys® 1010 PTH immunoassay. The final tissue diagnosis was determined by histology or operative data.**

Results. **Parathyroid samples from all techniques had higher median PTH concentrations than nonparathyroid samples. However, the accuracies for the detection of parathyroid tissue varied markedly (PTH cutoff of 1000 pg/ml): the accuracies of the FNA10 and FNA20 were 71 and 80%, respectively, while the BIOPSY was 99% accurate.**

Conclusions. **This is the first prospective study evaluating multiple methods to diagnose parathyroid tissue intraoperatively using a rapid PTH assay. We conclusively show that the BIOPSY technique is 99% accurate for the diagnosis of parathyroid tissue, and therefore, should be the method of choice when the intraoperative confirmation of parathyroid tissue is needed.**

Key Words: **parathyroid; hyperparathyroidism; parathyroid hormone; fine needle aspiration; intraoperative.**

INTRODUCTION

Primary hyperparathyroidism (1HPT) is the third most common endocrine disorder in the United States with an incidence of 28 per 100,000 and approximately 100,000 new cases each year. Despite extensive effort to develop chemotherapeutic agents for the treatment of 1HPT, surgical resection remains the only treatment conferring complete disease resolution [1–5].

Undoubtedly over the last 15 years the management of parathyroid surgery has changed dramatically, which is in part due to advancements that allow surgeons to better gauge the adequacy of tissue resection. In years past, successful parathyroidectomy relied upon a pathologist's tissue diagnosis in an intraoperative frozen section [6]. While this method is relatively accurate for the intraoperative identification of parathyroid tissue [7], it is time-consuming and costly and requires the excision of a large portion of tissue, rendering it impractical in many settings. Additionally, errors in the frozen section contribute significantly to the operative failures of parathyroidectomy [8, 9]. More recently, intraoperative parathyroid hormone monitoring has superceded the need for a frozen section tissue diagnosis [10–13]. However, when the intraoperative parathyroid hormone level does not fall after the suspected parathyroid gland has been resected, a confirmation of the tissue diagnosis is necessary. A previous study has proposed the use of an intraoperative fine-needle aspiration (FNA) in conjunction with the rapid PTH assay as an alternative to the frozen section [14]. While this

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technique shows great promise, further investigation is necessary to determine its reliability and accuracy as well as the ideal manner in which it is performed. Thus, in this study we compared three separate biopsy techniques that can be used in conjunction with the rapid parathyroid hormone (PTH) assay to establish an intraoperative parathyroid tissue diagnosis.

METHODS

Between May and August 2004, a total of 68 patients was enrolled in this IRB-approved protocol who underwent neck exploration for parathyroid and thyroid disorders. All operations were performed by two endocrine surgeons at the University of Wisconsin Hospital and Clinics.

Procedure

All surgically resected tissues were subjected to three separate biopsy techniques: a FNA with 10 passes of a needle (FNA10), a FNA with 20 passes of a needle (FNA20), and a biopsy of approximately 1.0 mm^3 (BIOPSY). All biopsy techniques were performed in an *ex vivo* fashion immediately following surgical resection. Additionally, no negative pressure was used while performing either FNA, and a single needle pass was defined as moving the needle into and out of the resected tissue. First the FNA10 and FNA20 were performed using a 3.0-ml syringe and separate 1.5-inch 23-gauge needles. Their contents were immediately flushed into separate 5.0-ml Vacutainers® containing 2.0 ml of preservative-free saline. The needle was flushed by aspirating the saline with approximately five plunges of the syringe. Last, the BIOPSY was performed using a scalpel, a 3.0-ml syringe, and a 1.5-inch 18-gauge needle. The 1.0-mm^3 portion of tissue was placed into a separate Vacutainer®, where it was aspirated through the shaft of the needle approximately five times. The Vacutainers® were then brought to the University of Wisconsin Hospital and Clinic's core laboratory where the PTH concentration was determined by the Roche Elecsys® (Roche, Indianapolis, IN) 1010 immunology analyzer.

Upon arrival in the core laboratory, all Vacutainers® were centrifuged for 7.0 min at 25°C and 2880 revolutions per minute. The supernatants were then transferred to separate plastic vials used by the Elecsys® 1010 analyzer. Depending upon laboratory personnel availability, samples were either analyzed or frozen at −80°C. Furthermore, all samples that were initially frozen were analyzed within 30 days of collection.

Biochemical Assay

The Elecsys® 1010 analyzer utilizes a novel, single-step antibody sandwich electrochemiluminescence assay that determines the concentration of intact PTH [15]. A biotin-labeled monoclonal antibody binds to the N-terminal fragment (1–37) and a ruthenium-labeled monoclonal antibody binds to the C-terminal fragment (38–84). Although the epitopes recognized by the N-terminal and C-terminal antibodies are (26–32) and (55–64), respectively, binding is also dependent upon the presence of residues 1–8 [15], thus, reducing binding to truncated PTH metabolites. Additionally, paramagnetic microparticles coated with streptavidin, a biotin binding protein, are added to the mixture of antibody–PTH complexes and larger complexes are formed. These complexes are then transferred to a tripropylamine (TPA) buffered solution where they are then captured by a magnetic electrode and the unbound reagents are rinsed away. Voltage is then applied to the captured complexes, oxidizing both the TPA and the ruthenium, producing an excited state of ruthenium. The spontaneous decay of the excited ruthenium emits light at 620 nm, which is then quantified. The amount of light emitted is directly correlated to the amount of bound intact PTH.

The analyzer was calibrated according to the Roche guidelines, which generates a standard curve for a set amount of reagent used. The Elecsys® PTH assay has a analytical range of 1.20 to 5000 pg/ml with a sensitivity of less than 2.70 pg/ml [15]. Samples with PTH concentrations greater than the upper limit of the assay were reported as >5000 pg/ml. All nonparathyroid samples were expected to fall within the lower portion of the analytical range, while the parathyroid samples were expected to have significantly high PTH concentrations. Laboratory technicians were blinded to the tissue diagnosis of all samples.

The final tissue diagnosis of all specimens was made by permanent histological evaluation, with the exception of the adipose specimens, which were collected from the subcutaneous space. These tissue diagnoses were then compared with the PTH concentration of its corresponding biopsy samples and the data were recoded in an Excel spreadsheet. For each biopsy technique, the sensitivities, specificities, and accuracies for the detection of parathyroid tissue were calculated using a PTH cutoff of 1000 pg/ml.

RESULTS

Patient Data

The mean age of the patients was 56 ± 14 years and 77% were women. Forty-three (86%) operations were performed for primary hyperparathyroidism, one (2%) for secondary hyperparathyroidism, and six (12%) for tertiary hyperparathyroidism. Additionally, thyroid, lymphatic, and adipose specimens were collected from 18 operations for thyroid disorders.

Tissues

A total of 223 tissue specimens was collected from 66 of the 68 patients with a total of 81 (36%) parathyroid, 73 (33%) thyroid, 40 (18%) adipose, and 29 (13%) lymphatic specimens.

Tissue PTH Values

All except two samples were biopsied by all three techniques. Two parathyroid samples were not large enough to undergo either FNA. The median tissue PTH concentrations for the 81 parathyroid samples were 726, 1455, and 5000 pg/ml by FNA10, FNA20, and BIOPSY, respectively (Table 1). Additionally, when these samples were stratified by clinical diagnosis, gland weight, and preoperative serum PTH level, the

TABLE 1

Tissue-Specific PTH Values

	Parathyroid	Thyroid	Adipose/lymphatic
FNA10	726 (34–5000)	34 (15–37)	33 (25–242)
FNA20	1455 (31–5000)	33 (16–38)	34 (21–49)
BIOPSY	5000 (63–5000)	23 (9–92)	33 (12–340)
N	81	73	69

Note. Median (range) PTH concentration in pg/mL.
PTH = parathyroid hormone; FNA = fine needle aspiration.