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Smaller parathyroids have higher near-infrared autofluorescence intensity in hyperparathyroidism

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ABSTRACT

Background: Intraoperative parathyroid gland identification can be challenging. Parathyroid glands have an intrinsic autofluorescence when excited by wavelengths in the near-infrared region. Studies using near-infrared cameras to detect parathyroid gland near-infrared autofluorescence have suggested improved identification. The pathologic parathyroid glands in primary hyperparathyroidism have variable near-infrared autofluorescence intensity, but how this correlates with different characteristics of hyperparathyroidism is unknown. Our objective was to correlate the fluorescent intensity of excited glands with clinical variables to enhance a surgeon's ability to identify parathyroid glands.

Methods: The data on patients undergoing surgery for primary hyperparathyroidism were collected. The images were collected intraoperatively with a handheld near-infrared device and analyzed. The data consisted of the ratio of mean parathyroid gland near-infrared autofluorescence over background (white gauze) near-infrared autofluorescence. The variables assessed for correlation with autofluorescence intensity were gland volume and weight, preoperative serum calcium and parathyroid hormone, age, body mass index, and sex. The images were quantified by Image J software (National Institutes of Health, Bethesda, MD). The lasso regression was analyzed by R version 4.1.3 to calculate adjusted *P* values (R Foundation for Statistical Computing, Vienna, Austria).

Results: From 2017 to 2021, 131 patients with primary hyperparathyroidism underwent parathyroidectomies of 151 parathyroid glands. The mean near-infrared autofluorescence intensity of parathyroid glands had a negative correlation with weight with lighter glands fluorescing more ($P = .019$) and a positive correlation with age with glands from older patients fluorescing more ($P = .013$). There were no significant correlations with preoperative serum calcium and parathyroid hormone, body mass index, and sex ($P > .05$).

Conclusion: In patients with primary hyperparathyroidism, we found that autofluorescence intensity correlated with parathyroid gland weight and patient age. This suggested that near-infrared camera use may be particularly helpful in identifying smaller adenomas and in older patients.

Introduction

Identifying and localizing parathyroid glands (PGs)—both normal and pathologic—can be challenging even for experienced surgeons due to their small size, similar appearance to fat, and frequent ectopic locations. Not surprisingly, due to their appearance and location, unintended damage to healthy PGs is common after a total thyroidectomy.[1] These poor surgical outcomes can result in transient hypocalcemia in 5% to 35% of patients and permanent hypocalcemia in up to 7% of patients.[2] Moreover, 5% to 10% of parathyroidectomies were unsuccessful due to the inability to correctly localize pathologic PGs.[3] Therefore, accurate identification and proper removal of pathologic PGs is imperative for surgical success.

The current standard for identifying parathyroid pathology intraoperatively is a gross inspection of the surgical specimen, followed by a frozen section analysis.[4] Although frozen section analysis has been consistently used until now, it is time consuming and limited in scope as it examines a small fraction of the specimen.[5] Parathyroid hormone measurement from tissue is another

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commonly used intraoperative technique. However, it can be invasive, labor-intensive, costly, and time-consuming.[6] Lastly, indocyanine green infusion has been used in some institutions, but lacks evidence supporting applicability and sensitivity.[7] Additionally, many preoperative efforts such as ultrasound, sestamibi scintigraphy, computed tomography (CT), and magnetic resonance imaging have been used to help localize PGs. Although these modalities can be helpful, they are not always accurate and frequently are not localizing for mild disease, small adenomas, or multigland disase.[8–11]

The use of near-infrared autofluorescence (NIRAF) has been emerging in endocrine surgery to aid in the localization and evaluation of PGs. Exisiting literature has shown that PGs have an intrisic AF due to an uncharacterized fluorophore.[12] After an excitation wavelength in the near-infrared region (~785 nm) hits parathyroid tissue, an AF is emitted with a peak fluorescence around 820 nm. Since then, there have been many efforts to demonstrate an optical modality that uses NIRAF for a noninvasive identification of PGs. Imaging systems were developed to illuminate tissues with NIR light and collect the fluorescence emitted with a handheld camera. Mutliple studies have shown that PGs emit stronger NIRAF signals than the surrounding tissues, allowing for the identification of PGs with a 97% to 99% accuracy even in multicenter trials.[13] Many studies have been conducted evaluating the possiblility of intraoperative identification of parathyroid glands during thyroidectomies to avoid unintentinonal resection of parathyroid glands, including a randomized control trial.[13–17]

For patients with primary hyperparathyroidism (pHPT), less is known regarding the utility of parathyroid AF and what factors affect their fluorescence. One study of 96 patients undergoing parathyroidectomy for both primary and secondary hyperparathyroidism found a correlation of intensity with preoperative calcium and preoperative serum parathyroid hormone (PTH) levels based on the surgeon's subjective rank of fluorescence based on a scale of low-medium-high.[18] Another study compared the fluorescence from normal and excised parathyroid glands in 50 patients having surgery for hyperparathyroidism capturing images from both in situ and excised glands.[19] They found a higher intensity with normofunctioning glands compared with hyperfunctioning glands. We have noted an appreciable variability of NIRAF intensity in pathologic PG in pHPT and hypothesize that this may be related to their clinical or pathologic state. This study's objective was to correlate autofluorescent intensity with different clinical variabiles in pHPT patients in only glands pathologically proven to be parathyroid tissue (excised) in a large patient cohort.

Methods

Study design

This was a clinical study of prospectively collected data from patients undergoing neck exploration and parathyroidectomy for a clinical diagnosis of primary hyperparathyroidism between June 2017 and July 2021 by 2 experienced endocrine surgeons at a single institution. This study was approved by the Institutional Review Board of The Ohio State University. A total of 131 patients who underwent parathyroidectomies of a total of 151 glands were included in this study. The patients with a diagnosis of multiple endocrine neoplasia (1 or 2), secondary, tertiary hyperparathyroidism, or patients not demonstrating biochemical cure postoperatively were excluded from the study. Pertinent medical information such as PG weight, PG volume, preoperative serum calcium and parathyroid hormone, age, body mass index (BMI), and sex were collected.

Camera

Resected PGs were imaged with an intraoperative handheld NIR camera (PDE-Neo II; Hamamatsu, Mitaka USA, Inc, Denver, CO). It emits infrared light at 760 nm and captures NIR wavelengths of 790 to 830 nm. The camera was sterilely draped and positioned 5 cm above the surgical field for all of the imaging because fluorescence is affected by distance. With all of the operating room lights off, images were taken ex vivo with ambient light from the camera and then at NIR wavelengths as mentioned above.

Surgery

The PTH and calcium levels were measured for each patient to confirm the diagnosis of primary hyperparathyroidism. Most of the patients had preoperative localization studies including surgeon performed ultrasound, technetium-sestamibi scan, and 4-dimensional 4D CT scans. The intraoperative PTH levels were obtained to determine the extent of surgery. If the intraoperative PTH decreased >50% and into the normal physiological range after 10 minutes, further neck exploration was not performed. If the postoperative calcium levels did not fall within physiological range, the patient was not considered cured and was removed from the analysis.

Data analysis

Images from all of the patients were collected and then analyzed using Image J software (National Institutes of Health, Bethesda, MD). Using the region of interest tool, the borders of the PGs were selected and the mean autofluorescence (AF) intensity (0–255-pixel intensity) was measured. Then a similar-sized background region was selected and measured in the same manner. The background consisted of white gauze. Each mean parathyroid AF was normalized using its respective background AF. This ratio determined the overall average intensity of the gland and was used to compare between groups.

Statistics

The variables sex, age, BMI, preoperative serum calcium, and parathyroid hormone, gland volume and weight were normalized as mean = 0 and SD = 1. Lasso regression was analyzed by R version 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria) with the packages glmnet and selectiveInference to calculate adjusted P values. All of the research was undertaken with the approval of the Institutional Review Board.

Results

Out of 168 patients who underwent parathyroidectomy and consented to this study, 131 met inclusion criteria.

The cohort included 102 women (78%) and 29 men (22%), and the median patient age was 62 years old (Figure 1). The median BMI was 30.7 kg/m^2 (IQR, 26.5–36.8 kg/m^2). The median preoperative PTH level was 136.9 pg/dL (IQR, 103.4–197.1 mg/dL). At pathologic evaluation, the median parathyroid gland volume was 0.82 cm^3 (IQR, 0.39–1.82 cm^3), and the median gland weight was 0.50 g (IQR, 0.2–1.20 g; Figure 1).

The tuning parameter (λ) for the lasso regression was 0.0343, chosen by 10-fold cross-validation. The coefficient estimates of 5 variables (sex, BMI, pre-PTH, pre-calcium, and volume) were zeros. Age and weight were in the final model. In other words, the mean NIRAF intensity of PGs had a negative correlation with weight with lighter glands fluorescing more ($P = .019$; Figs 2, *A* and 3) and a

Variable	
Female gender: n (%)	102 (78)
Median age: years (IQR)	62 (54-70)
Median BMI: kg/m^2 (IQR)	30.7 (26.5-36.8)
Median pre-operative serum PTH: pg/dL (IQR)	136.9 (103.4-197.1)
Median pre-operative serum calcium: mg/dL (IQR)	10.9 (10.6-11.3)
Parathyroid gland volume: cm^3 (IQR)	0.82 (0.39-1.82)
Parathyroid gland weight: gm (IQR)	0.50 (0.2-1.0)

Figure 1. Demographic and clinical features of 131 patients who underwent parathyroidectomies for diagnosis of primary hyperparathyroidism. *BMI*, body mass index.

positive correlation with age with glands from older patients fluorescing more ($P = .013$; Figure 2, *B*). There were no significant correlations with preoperative serum calcium and parathyroid hormone, BMI, and sex ($P > .05$; Figure 2, *C*–*F*).

Discussion

This was one of the first and largest studies to objectively quantify images of intraoperative NIRAF intensity of excised parathyroid glands in patients undergoing surgery for primary hyperparathyroidism. Comparing the AF intensity with various clinical correlates, we found that intensity negatively correlates with PG weight, with lighter glands having more intense fluorescence and positively correlates with patient age, with glands from older patients having brighter fluorescence. We did not find that the intensity correlated with preoperative calcium or PTH level, BMI, sex, or volume. Our study focused only on excised glands that were all proven to be parathyroid tissue, and whose removal resulted in cure of the patient's hyperparathyroidism.

Our findings differed from the findings of DiMarco et al in that intensity did not correlate with PTH and calcium levels.[18] Possible explanations include differences in fluorescence quantification as

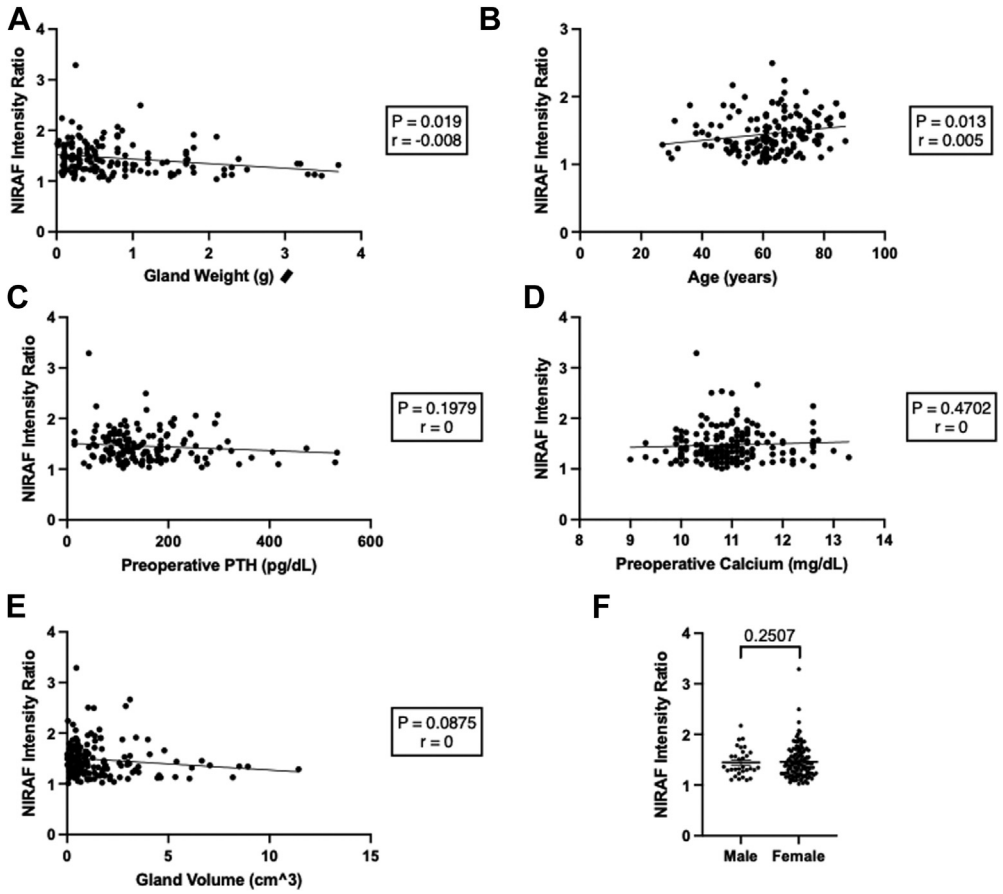

Figure 2. Scatter plots comparing near-infrared autofluorescence intensity ratio with the following respective clinical variables: *(A)* gland weight, *(B)* age, *(C)* preoperative serum parathyroid hormone, *(D)* preoperative serum calcium, *(E)* gland volume, and *(F)* the sex determined at birth of 131 patients who underwent parathyroidectomies for diagnosis of primary hyperparathyroidism with a total of 151 parathyroid gland resected. *NIRAF*, near-infrared autofluorescence; *PTH*,preoperative serum parathyroid hormone.

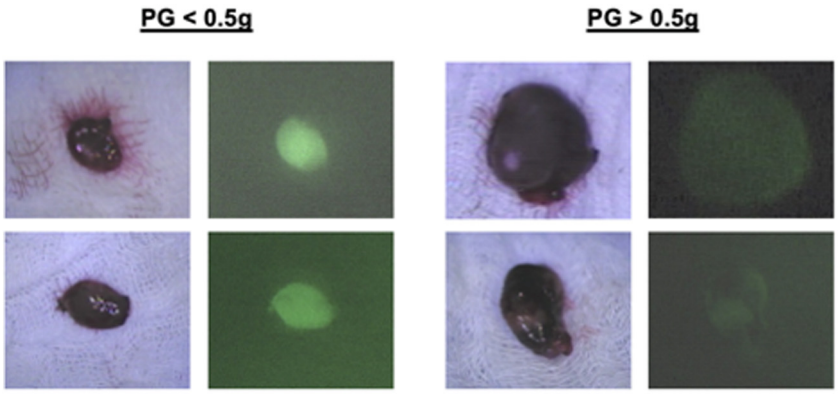

Figure 3. Representative ambient and near-infrared autofluorescence images of parathyroid glands weighing <0.5 grams (left) and >0.5 grams (right). *PG*, parathyroid glands.

subjectively determined by the surgeon, and including secondary hyperparathyroidism, as well as including the glands left in situ. In the study by Kose et al, the AF intensity correlated with more normo-functioning glands compared with hyperfunctioning glands and included both normal glands left in situ (as determined by the surgeon) and excised glands.[19] Generally, smaller adenomas are found in less severe disease; therefore, it is not surprising that in our study smaller glands had more fluorescence.

Intraoperative parathyroid gland identification can be difficult, especially for small glands, which are typically not localized as well on ultrasound, technetium sestamibi, or 4D CT scans.[8–11] Moreover, the current standards for identifying parathyroid pathology, such as frozen section analysis or parathyroid hormone measurement, can be invasive, labor-intensive, costly, and time-consuming.[7] For such reasons, there has been a rising interest in using noninvasive, light-based techniques as an alternative for intraoperatively to identifying PGs.

The discovery of the intrinsic AF of parathyroid glands in the NIR range led groups to evaluate the efficacy of handheld, real-time intraoperative NIRAF imaging technology as well as probe-based methods for noninvasive identification of parathyroid glands.[20,21] Moreover, further studies have shown promising preliminary data with using NIRAF technology in patients with various thyroid and parathyroid pathologies.[13,22,23] The ability to use AF to detect both normal and pathologic parathyroid glands in situ and confirm ex vivo shows potential to reduce time spent on neck exploration and minimize risk of injury to the recurrent laryngeal nerve, which enhances the safety of parathyroid and thyroidectomies. Despite there being many studies that confirm the feasibility and efficacy of NIRAF technologies in thyroid surgery, less is known regarding the relationship between parathyroid AF intensity and clinical correlates in primary hyperparathyroidism.

Our results showing that smaller parathyroid glands having brighter AF provide insight into how NIR cameras may be particularly helpful in patients who are suspected to have smaller adenomas. Studies have shown that technetium sestamibi and 4D CT scans, both popular preoperative localization procedures, are not as effective in detecting smaller glands.[8–11] Therefore, in clinical settings where the gland is suspected to be smaller by preoperative imaging or is not localized, our results suggested NIR imaging may be more beneficial in these scenarios.

Despite the increased use and advancement of NIRAF imaging technologies in the past decade, there were limitations to be considered. The first limitation was how this was a single institution study. Additionally, although our study demonstrated encouraging results for NIRAF technology to become a beneficial adjunct to parathyroid surgery, the true implications of this study will be seen when the patient outcome data are evaluated. Other limitations encountered during this study can give insight into the complexity of postresection procedures performed at the pathology lab. Our results indicated a significant fluorescent intensity difference when comparing gland weight, but not when comparing gland volume. It is interesting that we did not find volume of the gland to correlate with intensity, but only weight. We speculated that there was added uncertainty as multiple pathologists have been involved measuring the parathyroid glands. Moreover, each dimension (length, width, height) was measured by a pathologist with a small ruler. Because parathyroid glands are so small and can have irregular shapes, these measurements may not be as accurate as weight from a scale. Volume also requires 3 multiplied measurements as opposed to only 1 for weight, adding variability. Alternatively, weight may better represent the density of the gland. We believed that measuring the weight of the gland was a more accurate description of size of the gland than the volume, as the method to measure weight is more objective and standardized, and therefore better correlates with size of the gland.

In our current culture of health care, where there is a heavy emphasis on better patient outcome and efficient delivery of care, intraoperative parathyroid identification by AF detection for patients with primary hyperparathyroidism may be of substantial value as it is extremely safe, accurate, and fast when identifying parathyroid glands with apparent high accuracy. Moreover, the use of NIRAF technology to identify parathyroid glands has the potential to decrease overall costs by eliminating time required to obtain frozen section pathologic confirmation or intraoperative lab values, as it takes <3 minutes to use the handheld NIRAF camera.

In conclusion, parathyroid NIRAF intensity negatively correlates with weight with smaller glands having brighter fluorescence, and positively correlates with age, with older patients having brighter glands, suggesting intraoperative characterization of PGs by real-time NIR imaging may be a more useful adjunct during parathyroid surgery if preoperative parathyroid localization is unsuccessful, suggesting that the gland is small or the patient is older.

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Conflict of interest/Disclosure

John Phay is one of the inventors of a patent related to parathyroid autofluorescence imaging which was previously licensed to a company, AiBioMed, which is not a part of this study. None of the other authors have any conflicts of interest to declare related to this work.

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