Near-infrared Autofluorescence Features of Parathyroid Carcinoma

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Abstract

Introduction: Parathyroid carcinoma is very rare, and intraoperative definitive diagnosis can be elusive with currently available diagnostics. Near-infrared (NIR) autofluorescence is an emerging tool that identifies parathyroid glands in real time. It is not known whether NIR autofluorescence can detect parathyroid carcinoma intraoperatively.

Methods: Patients with preoperative suspicion for parathyroid carcinoma were identified from ongoing studies examining parathyroid autofluorescence with a NIR camera and probe. Specimens from these patients were examined intraoperatively to determine their autofluorescence patterns.

Results: Three patients with suspected parathyroid carcinoma were identified preoperatively. Intraoperative NIR autofluorescence imaging showed a relative lack of autofluorescence for all cases, in contrast to parathyroid adenomas and normal parathyroid glands, which typically exhibit significant autofluorescence. Final pathology confirmed parathyroid carcinoma in all cases.

Conclusion: Parathyroid carcinoma can be difficult to confirm prior to final pathology review. Our 3 cases suggest that absence of NIR autofluorescence may suggest the likelihood of parathyroid carcinoma, but more studies are needed to investigate this experience.

Key Words: parathyroid carcinoma, parathyroid autofluorescence, near-infrared autofluorescence

Parathyroid carcinoma is very rare, with a prevalence of approximately 0.005% [1-7]. It is typically hormonally active and accounts for 0.5% to 5% of cases of primary hyperparathyroidism [2, 3, 5, 6]. These patients often present with significantly elevated serum calcium levels and pronounced symptoms of hypercalcemia (eg, renal stones, bone pain, abdominal pain, nausea, vomiting, and fatigue) due to elevations in parathyroid hormone (PTH) 3 to 10 times the normal range [2, 4, 6, 7]. Between 30% and 76% of parathyroid carcinoma patients present with a palpable neck mass and may have associated symptoms of dysphagia or hoarseness [2, 6].

Operations for parathyroid carcinoma are more aggressive than those for benign parathyroid adenomas, typically involving an ipsilateral thyroid lobectomy and en bloc resection of any involved structures [7]. However, the diagnosis of parathyroid carcinoma can be challenging and, in absence of obvious invasion of nearby structures, may be difficult to differentiate from an atypical parathyroid tumor [8] until review of the final specimen by pathology. To date, there are no reliable preoperative or intraoperative tests to diagnose parathyroid carcinoma beyond a high degree of clinical suspicion. Fine-needle aspiration is typically not recommended due to the limitations of cytology and the possibility of tumor seeding [2, 6, 7]. Additionally, up to 40% of cases may exhibit artifact due to fine-needle aspiration changes that can confound the pathologist during histopathologic examination [9]. Intraoperative frozen section also may be inaccurate, with 1 series reporting a correct diagnosis in only 15% of cases [6].

An emerging diagnostic tool for detecting parathyroid glands is near-infrared (NIR) autofluorescence. This technique, initially described in 2011 [10], has not been studied yet in parathyroid carcinoma. The parathyroid gland has intrinsic autofluorescence due to the presence of endogenous fluorophore(s) that emit a fluorescent signal in the NIR wavelength range at a peak near 820 nm. Cameras designed to image indocyanine green can detect autofluorescence to intraoperatively identify normal parathyroid glands and benign parathyroid disease [11]. Hyperfunctioning parathyroid glands have heterogeneous and, in general, lower autofluorescence intensity than normal parathyroid glands, but autofluorescence characteristics of parathyroid carcinoma has never been reported [12].

We present 3 cases of autofluorescence assessment of parathyroid carcinomas.

Methods

Patients with preoperative suspicion for parathyroid carcinoma were prospectively identified from ongoing studies...
examining parathyroid autofluorescence in neck surgery with NIR technology at 2 separate institutions [Institutional Review Board–approved protocol 2017H0172 at The Ohio State University (OSU) and 20-33106 at University of California San Francisco (UCSF)]. Preoperative suspicion was based upon clinical factors such elevated serum calcium levels > 14 mg/dL, pronounced symptoms of hypercalcemia, and/or PTH of 3 to 10 times the normal range in 2 of the cases and on genetic testing in 1 case.

All suspected parathyroid carcinomas were imaged ex vivo, and 1 (from UCSF) was also imaged in vivo. Our prior studies have found the ex vivo autofluorescence to be higher than the in vivo autofluorescence. We therefore utilized the ex vivo numbers for comparison [11]. This was done using the handheld NIR camera (PDE-Neo II; Hamamatsu, Mitaka USA, Denver, CO, USA) as previously described at OSU and using the PTeye™ (Medtronic) and the Fluobeam® (FLUOPTICS©) at UCSF [11, 13]. Fluorescent activity was recorded, and the specimens were imaged in both white light and in the NIR range. Parathyroid carcinoma was confirmed by final pathology.

All images were then analyzed using Image J software, version 1.52a (National Institutes of Health, National Center for Microscopy and Imaging Research: ImageJ Mosaic Plug-ins, RRID:SCR_001935) as previously described [11] to quantify the fluorescence values of the thyroid and parathyroid carcinoma. These values were then compared to a previously analyzed cohort of parathyroid adenomas. This prior cohort included 69 parathyroid glands from 59 patients with hyperparathyroidism. Sixty-three of the glands were hypercellular while the remaining were normal glands. Ex vivo autofluorescence levels of parathyroid glands and available thyroid glands were used as the comparison group for the current study [11].

The in vivo and ex vivo images of the case from UCSF were compared intraoperatively with the values obtained from the PTeye, a parathyroid autofluorescence detection device that uses a disposable fiber-optic probe that emits a NIR 785-nm laser source and detects the autofluorescence on a photo detector. A baseline autofluorescence value is taken from thyroid tissue or muscle, and then ratio and absolute values of autofluorescence are used to sample tissues suspected to be parathyroid [13].

The operative case at UCSF was an exploration of an ectopic parathyroid gland in an anterior mediastinal location and did not involve extensive surgical exposure of the thyroid, and so an image of the parathyroid carcinoma with the thyroid as a background for comparison was not performed. Enough of the thyroid was exposed to allow for assessment with the PTeye probe.

### Results

Three patients with preoperative suspicion of parathyroid carcinoma were included, patients 1 and 2 from OSU and patient 3 from UCSF. Patient details are included in Table 1.

All parathyroid specimens were examined ex vivo with the handheld NIR camera and the specimen from UCSF also with the PTeye. Compared to normal parathyroid tissue (Fig. 1), there was relatively no fluorescent activity detected in any of the 3 parathyroid carcinomas (Figs. 2-4). Pathology did

Table 1. Patient details

|                | Patient 1 | Patient 2 | Patient 3 |
|----------------|-----------|-----------|-----------|
| Age            | 42        | 47        | 27        |
| Sex            | M         | F         | M         |
| Clinical Symptoms | Fatigue, abdominal pain, constipation | Myalgias, memory loss, fatigue, depression, anxiety, poor concentration, headaches, frequent urination, increased thirst, nausea, and reflux | None |
| Preop Ca mg/dL | 16.5      | 15.5      | 11.0      |
| Preop PTH pg/mL| 1160      | 936       | 688       |
| Maximum size of parathyroid carcinoma | 3.4 cm | 6.0 cm | 3.2 cm |
| Presence of germline mutation | Not performed | Not performed | Yes, CDC73 |
| Operation performed | Total thyroidectomy with en bloc parathyroidectomy and resection of adjacent strap muscles | Right thyroid lobectomy en bloc with parathyroidectomy | Parathyroidectomy of ectopic gland in anterior mediastinum |
| Frozen section | NA        | Hypercellular parathyroid tissue | NA |
| Final pathology | Multifocal papillary thyroid carcinoma in both lobes of the thyroid gland (largest focus 1.5 cm) and parathyroid carcinoma, 3.4 cm, with lymphovascular invasion but no perineural or thyroid invasion | Low-grade parathyroid carcinoma measuring 6.0 x 4.3 x 2.8 cm with lymphovascular invasion but no perineural invasion or extrathyroidal extension, 0/10 lymph nodes positive | Parathyroid carcinoma, 3.2 cm, with vascular invasion. Staining was negative for parafibromin in neoplastic cells but retained in scant peripheral benign parathyroid. |

Abbreviations: Ca, calcium; F, female; M, male; NA, not available; preop, preoperative; PTH, parathyroid hormone.
confirm the diagnosis of parathyroid carcinoma in all 3 cases and specifically showed vascular invasion in all cases.

The relative fluorescence was calculated for each parathyroid carcinoma from OSU (n = 2), which were 40.53 for case 1 and 46.1 for case 2. Thyroid fluorescence values were 50.43 for case 1 and 46.23 for case 2. The mean value of fluorescence for ex vivo parathyroid carcinoma was 43.3(±3.9) compared to 89.7 (± 29.0) in our comparison series detailed in the methods of previously reported 69 ex vivo glands from 59 primary hyperparathyroidism patients. The mean ratio of parathyroid carcinoma to thyroid ex vivo fluorescence was 0.90 (±0.14) whereas mean parathyroid adenoma ex vivo to thyroid ex vivo ratio was 1.54 (±0.53).

The relative fluorescence of the parathyroid gland of patient 3 from UCSF was assessed with the PEye probe as well as the saved image. Using the PEye probe, the autofluorescence ratio between the parathyroid carcinoma and normal thyroid as baseline was 0.2 to 0.5. The standardized threshold to confirm tissue as parathyroid is for the ratio to be >1.2 compared to thyroid. A normal left lower parathyroid gland was also encountered in this case, and the autofluorescence ratio was 7 for the normal gland as compared to baseline thyroid. Analyzing the saved image with ImageJ software, the relative fluorescence was 37.5 for the parathyroid carcinoma compared to 65.0 for the normal parathyroid gland. We did not have a picture of the thyroid
for comparison but the background fluorescence of adjacent muscle was 51.8 (Table 2).

Discussion

Parathyroid carcinoma is a very rare tumor. Its diagnosis may be suggested by its clinical presentation but can be elusive even upon histological examination of the primary tumor. Preoperatively, one must have a high suspicion from clinical features such as a significantly elevated calcium, markedly elevated PTH level, large palpable mass, evidence of surrounding invasion on imaging, hoarseness from involvement of the recurrent laryngeal nerve, presence of metastasis, or known genetic predisposition such as our third case [3, 6, 7]. Personal and family history of jaw tumor increases the risk of parathyroid cancer [14, 15].

Simple parathyroidectomy for parathyroid carcinoma has been associated with recurrence rates as high as 100% [16]. Therefore, when a parathyroid carcinoma is suspected, parathyroidectomy should be performed with an en bloc resection of the ipsilateral thyroid lobe and of adherent soft tissues and any clearly involved lymph nodes [7]. Because of the increased risk of tumor recurrence with an inadequate resection, a confident intraoperative identification of parathyroid carcinoma would be beneficial, especially when evidence of a malignancy is equivocal. Intraoperative frozen sections, unfortunately, have not been valuable in distinguishing benign from malignant disease [3, 17, 18]. For example, in 1 series of parathyroid carcinomas, only 17 of 113 frozen sections were confirmatory [3].

NIR autofluorescence was first applied to identify parathyroid glands in 2011 [10] and has been used at multiple institutions across the world [11, 19]. Two randomized controlled studies found that NIR reduced the likelihood of short-term postthyroidectomy hypoparathyroidism [20]. Hyperfunctioning glands have been shown to have lower fluorescence activity than normal parathyroid glands [12]. This finding may be useful when trying to determine which parathyroid glands are abnormal in multiple-gland primary hyperparathyroidism.

Some surgeons have found little or no fluorescence in the large glands discovered in renal hyperparathyroidism [21]. In that setting, parathyroid glands tend to be larger and more biochemically active than the glands seen in primary hyperparathyroidism. Considering the impact of adenomatous changes upon parathyroid tissue, one might also expect diminished fluorescence in hyperfunctioning parathyroid carcinomas. However, no prior studies have examined NIR fluorescence in parathyroid cancer, likely due to the rarity of the disease. This report is the first to describe the NIR fluorescence pattern of parathyroid carcinoma. Malignancy was preoperatively suspected in all 3 patients based upon clinical and laboratory factors. None of the tumors had any appreciable NIR fluorescence detected by the PDE-Neo II camera used Parathyroid carcinoma AF ex vivo Parathyroid carcinoma: thyroid AF ratio Parathyroid carcinoma: normal parathyroid AF ratio

| Procedure | Camera used | Parathyroid carcinoma AF ex vivo | Parathyroid carcinoma: thyroid AF ratio | Parathyroid carcinoma: normal parathyroid AF ratio |
|-----------|-------------|---------------------------------|----------------------------------------|-----------------------------------------------|
| Benign hyperparathyroid tissue (previously reported) [9] | PDE-Neo II | 89.7 (not cancer) | 1.54 | NA |
| Case 1 | PDE-Neo II | 40.5 | 0.80 | NA |
| Case 2 | PDE-Neo II | 46.1 | 1.00 | NA |
| Case 3 | Fluobeam | 37.5 | NA | 0.58 |

Abbreviations: AF, autofluorescence; NA, not available.

Table 2. Relative values of autofluorescence using Image J

Figure 4. Autofluorescence features of case 3. (A) Parathyroid carcinoma specimen with small focus of normal parathyroid. *Small area of normal parathyroid. **Parathyroid carcinoma. (B) Near-infrared autofluorescence of parathyroid carcinoma with small focus of normal parathyroid. *Small area of normal parathyroid with autofluorescence. **Parathyroid carcinoma with no autofluorescence.
and Fluobeam cameras. While this finding is not completely surprising given prior results comparing hyperfunctioning and normocellular glands, these are the first reported applications of autofluorescence to parathyroid cancers. Notably, frozen section in the second case did not confirm diagnosis of carcinoma.

One major limitation of our study is the small number of parathyroid carcinomas due to the rarity of the disease. Despite this, our findings were reproducible in 3 cases and lay the groundwork for future studies. Another limitation is the use of 2 different cameras; however, these are the 2 commercial systems that have been used the most in prior publications. There are no studies directly comparing their sensitivities to our knowledge, but several studies have shown them to be comparable in the detection of parathyroid autofluorescence [22, 23].

This finding may have implications for the use of NIR autofluorescence in the intraoperative detection of parathyroid carcinoma. While obvious features of a malignancy, such as adhesions and fibrosis, will prompt the surgeon to resect parathyroid cancer en bloc with the ipsilateral thyroid lobe and affected soft tissues, the absence of NIR autofluorescence might increase the suspicion of malignancy in equivocal cases.

Future studies are warranted to confirm the lack of NIR autofluorescence in parathyroid cancer.

Conclusion
Parathyroid carcinoma is a rare cancer. We reported the relative absence of NIR autofluorescence in 3 cases of parathyroid carcinoma. Further studies are needed to confirm this finding.

Disclosures
J.E.P. is part of the Vanderbilt University patent that was licensed to AiBiomed and served as a consultant for the company. All other authors have no conflict of interest to disclose related to this manuscript.

Data Availability
Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in the references.

References
1. Silva-Figueroa AM, Bassett R Jr., Christakis I, et al. Using a novel diagnostic nomogram to differentiate malignant from benign parathyroid neoplasms. *Endocr Pathol.* 2019;30(4):285-296. Doi: 10.1007/s12022-019-09592-3
2. Ferraro V, Gsaramella LI, Di Meo G, et al. Current concepts in parathyroid carcinoma: a single Centre experience. *BMC Endocr Disord.* 2019;19(suppl 1):46. Doi: 10.1186/s12902-019-0368-1
3. Wang P, Xue S, Wang S, et al. Clinical characteristics and treatment outcomes of parathyroid carcinoma: a retrospective review of 234 cases. *Oncol Lett.* 2017;14(6):7276-7282. Doi: 10.3892/ol.2017.7076
4. Goswamy J, Lei M, Simo R. Parathyroid carcinoma. *Curr Opin Otolaryngol Head Neck Surg.* 2016;24(2):155-162. Doi: 10.1097/ MOO.0000000000000234
5. Mohebati A, Shaha A, Shah J. Parathyroid carcinoma: challenges in diagnosis and treatment. *Hematol Oncol Clin North Am.* 2012;26(6):1221-1238. Doi: 10.1016/j.hoc.2012.08.009
6. Wei CH, Harari A. Parathyroid carcinoma: update and guidelines for management. *Curr Treat Options Oncol.* 2012;13(1):11-23. Doi: 10.1007/s11864-011-0171-3
7. Kassahun WT, Jonas S. Focus on parathyroid carcinoma. *Int J Surg (London, England).* 2011;9(1):13-19. Doi: 10.1016/j.ijsu.2010.09.003
8. Erickson LA, Mete O, Juhlin CC, Perren A, Gill AJ. Overview of the 2022 WHO classification of parathyroid tumors. *Endocr Pathol.* 2022;33(1):64-89. Doi: 10.1007/s12022-022-09709-1
9. Steen S, Hysek M, Zedeenius J, Falhammer H, Juhlin CC. Cyto-morphological features of parathyroid lesions: fine-needle aspiration cytology series from an endocrine tumor referral center. *Diagn Cytopathol.* 2022;50(2):75-83. Doi: 10.1002/dc.24923
10. Paras C, Keller M, White I, Phay J, Mahadevan-Jansen A. Near-infrared autofluorescence for the detection of parathyroid glands. *J Biomed Opt.* 2011;16(6):067012. Doi: 10.1117/1.3583571
11. Squires MH, Jarvis R, Shirley LA, Phay JE. Intraoperative parathyroid autofluorescence detection in patients with primary hyperparathyroidism. *Ann Surg Oncol.* 2019;26(4):1142-1148. Doi: 10.1245/s10434-019-07161-w
12. Kose E, Kahramangil B, Aydin H, Donmez M, Berber E. Heterogeneous and low-intensity parathyroid autofluorescence: patterns suggesting hyperfunction at parathyroid exploration. *Surgery.* 2019;165(2):431-437. Doi: 10.1016/j.surg.2018.08.006
13. Thomas G, McAide MA, Nguyen QJ, et al. Innovative surgical guidance for label-free real-time parathyroid identification. *Surgery.* 2019;165(1):114-123. Doi: 10.1016/j.surg.2018.04.079
14. Torresan E, Iacobone M. Clinical features, treatment, and surveillance of hyperparathyroidism-jaw tumor syndrome: an up-to-date and review of the literature. *Int J Endocrinol.* 2019;2019:1761030. Doi: 10.1155/2019/1761030
15. Harari A, Waring A, Fernandez-Ranvier G, et al. Parathyroid carcinoma: a 43-year outcome and survival analysis. *J Clin Endocrinol Metab.* 2011;96(12):3679-3686. Doi: 10.1210/jc.2011-1571
16. Givi B, Shah JP. Parathyroid carcinoma. *Clin Oncol (R Coll Radiol).* 2010;22(6):498-507. Doi: 10.1016/j.clon.2010.04.007
17. Shane E. Clinical review 122: parathyroid carcinoma. *J Clin Endocrinol Metab.* 2001;86(2):485-493. Doi: 10.1210/jcem.86.2.7207
18. Duan K, Mete O. Parathyroid carcinoma: diagnosis and clinical implications. *Turk Patoloji Dergisi.* 2015;31(suppl 1):80-97. Doi: 10.5146/tipath.2015.01316
19. Solozano CC, Thomas G, Bargegiamian N, Mahadevan-Jansen A. Detecting the near infrared autofluorescence of the human parathyroid: hype or opportunity? *Ann Surg.* 2020;272(6):973-985. Doi: 10.1097/ sla.0000000000003700
20. Dip E, Falco J, Verna S, et al. Randomized controlled trial comparing white light with near-infrared autofluorescence for parathyroid gland identification during total thyroidectomy. *J Am Coll Surg.* 2019;228(5):744-751. Doi: 10.1016/j.jacollsurg.2018.12.044
21. DiMarco A, Chotalia R, Bloxham R, McIntyre C, Tolley N, Palazzo FF. Autofluorescence in parathyroidectomy: signal intensity correlates with serum calcium and parathyroid hormone but routine clinical use is not justified. *World J Surg.* 2019;43(6):1532-1537. Doi: 10.1007/s00268-019-04929-9
22. Takahashi T, Yamazaki K, Takeuchi M, et al. Detection of parathyroid gland auto-fluorescence using a near infrared fluorescence imaging: comparison with pde-neo® and FLUOBED imaging. *Jpn Soc Head Neck Surg.* 2020;30(3):277-283. Doi: 10.5106/jjshns.30.277
23. DSouza AV, Lin H, Henderson ER, Samkoe KS, Pogue BW. Review of fluorescence guided surgery systems: identification of key performance capabilities beyond indocyanine green imaging. *J Biomed Opt.* 2016;21(8):80901. Doi: 10.1117/1.JBO.21.8.080901