Autofluorescence pattern of parathyroid adenomas

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Abstract

Background: Primary hyperparathyroidism (pHPT) is a common endocrine pathology, and it is due to a single parathyroid adenoma in 80–85 per cent of patients. Near-infrared autofluorescence (NIRAF) has recently been used in endocrine surgery to help in the identification of parathyroid tissue, although there is currently no consensus on whether this technique can differentiate between normal and abnormal parathyroid glands. The aim of this study was to describe the autofluorescence pattern of parathyroid adenoma in pHPT.

Methods: Between January and June 2019, patients with pHPT who underwent surgical treatment for parathyroid adenoma were enrolled. Parathyroid autofluorescence was measured.

Results: Twenty-three patients with histologically confirmed parathyroid adenomas were included. Parathyroid adenomas showed a heterogeneous fluorescence pattern, and a well defined autofluorescent ‘cap’ region was observed in 17 of 23 specimens. This region was on average 28 per cent more fluorescent than the rest of the adenoma, and corresponded to a rim of normal histological parathyroid tissue (sensitivity and specificity 88 and 67 per cent respectively). After resection, all patients were treated successfully, with normal postoperative values of calcium and parathyroid hormone documented.

Conclusion: Parathyroid adenomas show a heterogeneous autofluorescence pattern. Using NIRAF imaging, the majority of specimens showed a well defined autofluorescent portion corresponding to a rim of normal parathyroid tissue. Further studies should be conducted to validate these findings.

Introduction

Primary hyperparathyroidism (pHPT) is a frequent endocrine pathology that affects 0.3 per cent of the general population, and results from the inappropriate production of parathyroid hormone (PTH) from one or more parathyroid glands 1. This condition is linked to single parathyroid adenoma in 80–85 per cent of patients 2. Surgery has been widely recognized as the standard treatment in the management of pHPT 3, which has led to the development of minimally invasive surgical procedures 4,5. Different imaging modalities, such as ultrasonography and 99mTc-labelled sestamibi (sestamibi) scintigraphy, have been used to identify and localize diseased parathyroid tissue before surgery, with sensitivities of 69–75 and 49–70 per cent respectively 6,7. Other emerging techniques, such as multiphase four-dimensional CT and 18F-labelled choline PET–CT, are increasingly being used to facilitate accurate preoperative localization, particularly in the setting of negative or discordant ultrasonography and sestamibi imaging 8,9.

In some centres, intraoperative PTH testing is used after excision to confirm that the correct parathyroid adenoma has been removed adequately, which offers the benefits of minimizing the extent of surgical dissection and rate of persistence/recurrence 9.

Despite significant advances in imaging, intraoperative detection of normal and abnormal parathyroid glands remains a major challenge for both expert and non-expert endocrine surgeons 10. A novel imaging modality that uses near-infrared autofluorescence (NIRAF) has recently been described for the intraoperative localization of normal parathyroid glands 11–15; however, whether NIRAF can be used to differentiate between hyperfunctioning and normal functioning parathyroid tissue remains unclear, with inconsistent results published across studies 16,17.

The purpose of this study was to describe the autofluorescence patterns exhibited by hyperfunctioning parathyroid tissue in patients with pHPT undergoing parathyroidectomy, and to explore associations between NIRAF patterns and histopathological findings.

Methods

Patients with a confirmed diagnosis of pHPT who underwent parathyroidectomy at Geneva University Hospital between January and June 2019 were reviewed retrospectively. The
patients included were aged over 18 years, and were diagnosed with pHPT using laboratory and preoperative imaging studies. Patients with secondary or tertiary hyperparathyroidism were excluded.

For each patient, the following data were recorded: medical history, demographic characteristics, preoperative laboratory evaluation (serum calcium and PTH levels), and results of imaging studies (including ultrasound examination, sestamibi scintigraphy, and 18F-labelled choline PET–CT, when performed). Samples were also collected for measurement of serum calcium and PTH levels 4 h after surgery, and on postoperative days 1 and 10. Calcium and PTH were measured at the Central Clinical Laboratory of the Geneva University Hospital.

This study was approved by the Institutional Review Board of Geneva University Hospitals, and all subjects signed written consent before enrolment.

**Surgical treatment and autofluorescence**

All operations were performed by an experienced endocrine surgeon using the typical standard of care in a high-volume hospital. Unilateral neck exploration was chosen for adenomas that had been clearly localized before operation, whereas bilateral neck exploration was performed in patients with discordant or inconclusive preoperative imaging findings or multiple lesions. All patients underwent parathyroidectomy for pHPT using autofluorescence imaging. A Fluobeam LX device (Fluoptics, Grenoble, France) was used to capture intraoperative and resected specimen images.

During surgery, the Fluobeam LX device was used to help in the identification of parathyroid glands (Fig. 1). Once the parathyroidectomy had been completed, the resected specimen was exposed to near-infrared light, and the resulting fluorescence was captured using the same device held at a distance of 20 cm from the specimen, under the same ambient light as that used in the operating room (Figs 2 and 3).

The NIRAF images were analysed using ImageJ software (National Institutes of Health, Bethesda, Maryland USA). The autofluorescence patterns observed visually were classified as either homogeneous, when the adenoma fluorescence detected by the device was uniform, or heterogeneous, when a mixture of bright and dim areas was recorded. The presence of a single well-defined bright autofluorescent region was defined as the ‘cap’ (Fig. 2), and indicated with a surgical marker for pathological assessment (Fig. 4 and Video S1); of note, the ink emits a reproducible standardized fluorescence signal.

Variables collected from the NIRAF image of the resected specimens included the size of the area resected, the mean fluorescence measurements for the whole adenoma, and both the brightest (most autofluorescent) and dimmest regions of the resected specimen, which were used to calculate the fluorescence score. This score was created to normalize and establish uniformity among the measurements, and was calculated by dividing the mean fluorescence intensity for the three measurements by the mean fluorescence intensity of the same 1 x 1-cm-square region marked on a white surgical ruler with the purple surgical marker used for marking the surgical specimen (Fig. 5).

The resected specimens were then transferred to the pathology department of the University of Geneva for histological analysis, including the marked and unmarked areas of the specimen.

**Statistical analysis**

Continuous variables are expressed as mean(SD.). Statistical analysis was performed using XLSTAT (Addinsoft, PARIS, France) for Excel (Microsoft, Redmond, Washington, USA). Student’s t-test was used to test the statistical significance of differences and \( P < 0.050 \) was considered statistically significant.

**Results**

Of 29 patients who underwent parathyroidectomy during the study interval, 23 were selected according to the study criteria. All included patients underwent parathyroid adenoma resection for pHPT (mean(SD) preoperative PTH 13.65(SD 6.31) pmol/l); however, two of these patients also underwent thyroid lobectomy because of the simultaneous presence of thyroid disease. The adenoma was confirmed and localized before surgery by both ultrasonography and sestamibi scintigraphy in 18 patients. The adenoma was detected only by sestamibi scintigraphy in 18 patients. The adenoma was detected only by sestamibi scintigraphy in three patients, and by neither of these modalities in two. Finally, four patients were investigated using 18F-labelled choline PET–CT.
Patient demographics, clinical characteristics, and interventions are summarized in Table 1. The median age of the cohort was 56 (range 40–83) and median BMI was 25.1 (18–35) kg/m². Preoperative and postoperative serum calcium and PTH levels are shown in Table S1. A single parathyroid adenoma was resected in 22 patients, and two adenomas were resected in one patient, who underwent bilateral cervical exploration; this was based on negative preoperative localization studies and intraoperative finding of increased size of the two parathyroid glands (intraoperative PTH measurement is not performed routinely in this institution) (Fig. 6).

All patients were treated successfully, and had normal calcium (2.42(0.15) mmol/l) and PTH (1.87(0.84) pmol/l) values on postoperative day 1, and 10 days after the intervention (calcium 2.32(0.11) mmol/l, PTH 3.65(1.13) pmol/l) (Table S1). The pathological reports confirmed the diagnosis of parathyroid adenoma.

Fluorescence pattern
All adenomas showed a heterogeneous pattern of autofluorescence. Adenomas in 17 of the 23 patients exhibited a cap region, showing a clear, well defined bright autofluorescent region within the gland (Fig. 2). The remaining six patients had either low or heterogeneous signals (Fig. 3). Specifically, in two patients the adenomas displayed a very low heterogeneous signal and did not have an appreciably more autofluorescent part, so were not marked (Fig. 7); on the other hand, the remaining four adenomas had an autofluorescent region, although this was not sufficiently bright to be defined as a cap.

The difference in mean enhancement of fluorescence scores between the most and least fluorescent regions was 1.51(0.65) fold (Table 2); this corresponded to a mean 28 per cent difference in fluorescence intensity (Fig. 8). On the other hand, normal parathyroid tissue, documented on histopathological examination, had a 1.39(0.2)-fold (27 per cent) higher NIRAF signal compared with adenomas.

Of note, the fold increase between fluorescence scores was 1.59(0.73) (31 per cent mean difference) for adenomas presenting with a cap and 1.26(0.17) (19 per cent mean difference) for those without a cap.

The performance of the cap in detecting normal parathyroid tissue in a suspected adenomatous parathyroid was assessed (Table 3). Presence of a cap could detect normal parathyroid tissue in a parathyroid adenoma with a sensitivity of 88 per cent and a specificity of 67 per cent.

Histological analysis
Histological findings are summarized Table 4. Briefly, all resected specimens fulfilled the criteria for diagnosis of parathyroid adenoma. In 17 of 21 specimens marked by the surgeon following the NIRAF evaluation, a rim of normal parathyroid was observed.
Fig. 4 Near-infrared autofluorescence image, operative specimen, and histology of parathyroid adenoma

a Near-infrared autofluorescence (NIRAF) image showing cap of parathyroid adenoma; b surgical specimen showing cap marked with surgical ink; and c histological analysis showing a conserved rim of normal parathyroid tissue (green ink) corresponding to the marked area in b (haematoxylin and eosin stain; original magnification c × 20).

Fig. 5 Zones of fluorescence intensity

Zones of fluorescence intensity detected with ImageJ software are outlined in yellow: a no zone marked; b most intense part of resected specimen (cap); c dimmest part of resected specimen; and d whole adenoma. A 1 × 1-cm-square is indicated on a surgical ruler (in colour, the marking is purple on a white ruler).

Table 1 Patient characteristics, results of preoperative imaging, interventions, and resected gland(s)

| Patient no. | Sex | BMI (kg/m²) | Age (years) | Ultrasound localization | Scintigraphy localization | Localization concordance | PET localization | Intervention | Resected gland |
|-------------|-----|-------------|-------------|-------------------------|--------------------------|-------------------------|----------------|-------------|----------------|
| 1           | F   | 26.8        | 48          | NO                      | NO                       | NO                      | NP             | Bilateral exploration | SUP R         |
| 2           | M   | 22.7        | 59          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF L         |
| 3           | F   | 22.8        | 51          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF L         |
| 4           | M   | 29          | 71          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF L         |
| 5           | F   | 26.1        | 45          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF R         |
| 6           | F   | 19.9        | 52          | NO                      | YES                      | NO                      | NO             | Unilateral parathyroidectomy | INF L         |
| 7           | F   | 30.1        | 43          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF L         |
| 8           | F   | 18.1        | 56          | YES                     | NO                       | NO                      | YES            | Bilateral exploration | INF R         |
| 9           | F   | 22.3        | 49          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | SUP R         |
| 10          | F   | 28.6        | 54          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | SUP L         |
| 11          | F   | 22.1        | 48          | NO                      | YES                      | NO                      | NO             | Unilateral parathyroidectomy | SUP R         |
| 12          | F   | 26.9        | 75          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF R         |
| 13          | F   | 35          | 55          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF L         |
| 14          | F   | 22.5        | 55          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF L         |
| 15          | F   | 25.4        | 40          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | SUP R         |
| 16          | F   | 25.1        | 75          | YES                     | NO                       | NO                      | YES            | Bilateral exploration | INF L + SUP R |
| 17          | F   | 22.2        | 62          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF L         |
| 18          | F   | 23.8        | 75          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | SUP R         |
| 19          | M   | 28          | 63          | NO                      | YES                      | NO                      | NO             | Bilateral exploration | SUP L         |
| 20          | F   | 18          | 83          | YES                     | NO                       | NO                      | NO             | Unilateral parathyroidectomy | SUP L         |
| 21          | F   | 21          | 68          | NO                      | YES                      | NO                      | NO             | Unilateral parathyroidectomy | INF R         |
| 22          | F   | 30          | 59          | YES                     | YES                      | YES                     | YES            | Unilateral parathyroidectomy | INF R         |
| 23          | F   | 29          | 59          | NO                      | NO                       | NO                      | YES            | Unilateral parathyroidectomy | Ectopic SUP L |

SUP, superior; INF, inferior; R, right; L, left; NP, not performed.
Adipose tissue was noted in two instances, one with white and the other with brown adipose tissue (Figs 9 and 10), which is a well known cause of false-positive NIRAF findings in non-parathyroid tissues. One specimen showed mild oncocytic (oxyphilic) foci of the parathyroid adenoma, and in another the mark was wrongly placed on the adenomatous part of the specimen. In 15 of 17 specimens exhibiting a well defined more autofluorescent cap on NIRAF imaging, the rim of normal residual parathyroid tissue was marked correctly.

**Discussion**

Localization of parathyroid glands during parathyroidectomy remains one of the major challenges faced by endocrine surgeons, who must otherwise rely on visual assessment to identify the gland. Frozen-section analysis and intraoperative parathyroid aspiration (and intraoperative PTH measurement of the aspirate) have been used to identify parathyroid tissue and confirm removal of the correct parathyroid gland. However, there remains a clinical need for better identification of normal and diseased parathyroid glands, including instantaneous, intraoperative identification. Even with the use of preoperative imaging, such as ultrasound examination and MIBI scintigraphy which, when combined, are considered to be the most sensitive preoperative imaging examinations (sensitivity approximately 90 per cent), intraoperative localization of the diseased glands or adenoma with a high level of certainty is still difficult.

Paras and colleagues first described the intrinsic autofluorescence properties of the parathyroid glands in the near-infrared range. This technique is based on the fact that, when parathyroid glands are stimulated by near infrared light (at about 780 nm), they re-emit significantly more autofluorescent light (at about 825 nm) than the surrounding tissues (such as thyroid, fat, vessels, lymph nodes), which enables their detection and identification by means of a label-free imaging system. A near-infrared autofluorescence signal has been used progressively in endocrine surgery for identification of parathyroid gland during thyroidectomy and parathyroidectomy, with the potential to provide real-time intraoperative feedback.

Until now, discriminant traits in NIRAF signals between normal and abnormal parathyroid gland have not been demonstrated clearly, with conflicting results between authors. Therefore, no consensus exists regarding whether NIRAF imaging of parathyroid tissue can differentiate a normally functioning parathyroid gland from a diseased one.

A previous study found that diseased parathyroid glands show weaker NIRAF intensity than normal glands, and another reported that hyperfunctioning parathyroid glands exhibit lower autofluorescence intensity than normally functioning glands. However, other studies found no significant difference in NIRAF intensity between healthy and diseased parathyroid glands associated with pHPT, as later confirmed by further research. Moreover, it has been reported that parathyroid adenomas in pHPT show higher NIRAF intensities than normal parathyroid glands.

In the present study, most parathyroid adenomas demonstrated a heterogeneous NIRAF pattern, with adenoma tissue documented as significantly less autofluorescent than the rim of normal parathyroid tissue. It was also observed that parathyroid adenomas expressed on average approximately 28 per cent lower fluorescence intensity than the most intense region of the adenoma. However, the reason for the discrepancy regarding the autofluorescence signal in six samples remains unclear and requires further investigation, as the present study was limited in terms of the number of patients included.

NIRAF could therefore be a useful tool during parathyroidectomy, first to locate the normal and diseased parathyroid glands, and then to confirm that a parathyroid gland is indeed an adenoma when a cap of more autofluorescent tissue is found. Further studies should be conducted to validate these findings and the accuracy of this tool.
Table 2 Near-infrared autofluorescence image scores

| Patient no. | Fluorescence score | Fold mean fluorescence | Fluorescence difference (%) |
|-------------|--------------------|------------------------|-----------------------------|
|             | Greatest NIRAF signal | Lowest NIRAF signal | greatest NIRAF signal | Lowest NIRAF signal |
| 1           | 113                | 77                     | 32                          | 1.85                  | 1.26                  |
| 2           | 67                 | 36                     | 36                          | 1.10                  | 0.59                  |
| 3           | 49                 | 48                     | 2                           | 0.80                  | 0.79                  |
| 4           | 87                 | 72                     | 17                          | 1.43                  | 1.18                  |
| 5           | 106                | 60                     | 43                          | 1.74                  | 0.98                  |
| 6           | 85                 | 61                     | 28                          | 1.39                  | 1.00                  |
| 7           | 77                 | 52                     | 32                          | 1.26                  | 0.85                  |
| 8           | 73                 | 57                     | 22                          | 1.20                  | 0.93                  |
| 9           | 69                 | 16                     | 53                          | 1.13                  | 0.26                  |
| 10          | 79                 | 67                     | 15                          | 1.30                  | 1.10                  |
| 11          | 87                 | 77                     | 11                          | 1.43                  | 1.26                  |
| 12          | 59                 | 43                     | 27                          | 0.97                  | 0.70                  |
| 13          | 90                 | 84                     | 7                           | 1.48                  | 1.38                  |
| 14          | 45                 | 39                     | 13                          | 0.74                  | 0.64                  |
| 15          | 27                 | 17                     | 37                          | 0.44                  | 0.28                  |
| 16          | 112                | 78                     | 30                          | 1.84                  | 1.28                  |
| 17          | 55                 | 34                     | 36                          | 0.87                  | 0.56                  |
| 18          | 153                | 131                    | 14                          | 2.51                  | 2.15                  |
| 19          | 83                 | 57                     | 31                          | 1.36                  | 0.93                  |
| 20          | 140                | 93                     | 34                          | 2.30                  | 1.52                  |
| 21          | 95                 | 61                     | 36                          | 1.56                  | 1.00                  |
| 22          | 114                | 94                     | 18                          | 1.87                  | 1.54                  |
| 23          | 80                 | 52                     | 28                          | 1.31                  | 0.85                  |

Mean(SD.) 84(29.6) 61(26.1) 28 1.38(0.49) 1.00(0.43) 1.51(0.65)

NIRAF, near-infrared autofluorescence scores were calculated as the normalized mean autofluorescence intensity in the most autofluorescent region (Fig. 5b) and the rest of the adenoma (Fig. 5c), with the difference in fluorescence expressed as a percentage and fold change.

Table 3 Accuracy of detection of the cap on normal parathyroid tissue

| Normal parathyroid histology | Yes | No |
|-----------------------------|-----|----|
| Cap                         | 15  | 2  |
| No cap                      | 2   | 4  |
| Total                       | 17  | 6  |

Sensitivity 88 (95 per cent c.i. 64 to 99) per cent, specificity 67 (22 to 96) per cent.

Table 4 Histological findings in resected specimens and within ink-marked zone

| Patient no. | Pathological findings on marked part | Cap | Dimension (mm) | Weight (g) |
|-------------|--------------------------------------|-----|----------------|------------|
| 1           | Ink: normal parathyroid              | Yes | 25             | 0.335      |
| 2           | Ink: normal parathyroid              | Yes | 12             | 0.207      |
| 3           | No ink                               | No  | 15             | 0.257      |
| 4           | No ink                               | No  | 18             | 0.479      |
| 5           | Ink: adipose tissue                  | Yes | 20             | 0.729      |
| 6           | Ink: normal parathyroid              | Yes | 11             | 0.200      |
| 7           | Ink: adenoma, away from normal parathyroid | No  | 20             | 1.521      |
| 8           | Ink: normal parathyroid              | Yes | 11             | 0.195      |
| 9           | Ink: brown adipose tissue            | Yes | 3              | 2.290      |
| 10          | Ink: mild oncocytic metaplasia       | No  | 2              | 0.823      |
| 11          | Ink: normal parathyroid              | Yes | 17             | 0.187      |
| 12          | Ink: normal parathyroid              | Yes | 25             | 1.950      |
| 13          | Ink: normal parathyroid              | Yes | 25             | 1.710      |
| 14          | Ink: normal parathyroid              | Yes | 15             | 0.495      |
| 15          | Ink: normal parathyroid              | Yes | 30             | 2.064      |
| 16          | Ink: normal parathyroid              | Yes | 10             | 0.220      |
| 17          | Ink: normal parathyroid              | Yes | 15             | 0.247      |
| 18          | Ink: normal parathyroid              | Yes | 16             | 0.168      |
| 19          | Ink: normal parathyroid              | No  | 19             | 1.492      |
| 20          | Ink: normal parathyroid              | Yes | 14             | 0.208      |
| 21          | Ink: normal parathyroid              | Yes | 14             | 0.169      |
| 22          | Ink: normal parathyroid              | No  | 20             | 1.020      |
| 23          | Ink: normal parathyroid              | Yes | 17             | 0.308      |

Fig. 8 Fold of mean near-infrared autofluorescence differences between the most intense and dimmest parts of the resected specimen. Median (bold line), mean (cross), i.q.r. (box), and range (error bars) excluding outlier (circle) are shown.

Disclosure. The authors declare no conflict of interest.

Supplementary material

Supplementary material is available at BJ Open online.
Fig. 9 Parathyroid adenoma with brown adipose tissue
Brown adipose tissue is marked with green ink (haematoxylin and eosin stain, original magnification a ×20, b ×200).

Fig. 10 Near-infrared false-positive autofluorescence image of a parathyroid adenoma, which was confirmed as adipose tissue on anatomopathological analysis

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