The Laryngoscope

Near-Infrared Fluorescence Imaging in the Identification of Parathyroid Glands in Thyroidectomy

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Objectives/Hypothesis: To assess the ability of near-infrared fluorescence imaging (NIFI) to identify parathyroid glands (PGs) among histologically proven PG/non-PG specimens compared with a surgeon's visual acumen, and to determine NIFI sensitivity in detecting incidentally resected PGs from thyroidectomy specimens, compared to the surgeon's visual inspection.

Study Design: Prospective study.

Methods: With mean age of 61 years, 36 patients with various thyroid diseases were enrolled. Possible PGs (n = 28) and lymph nodes (n = 32) were identified by the experienced surgeon's visual inspection. Using NIFI, 15 PGs were further identified from thyroidectomy specimens. For these 75 specimens, the surgeon's judgments (PG vs. non-PG) were recorded. Histological evaluation was performed after examining the NIFI auto-fluorescence of each specimen.

Results: There were no significant differences in sensitivity, specificity, positive predictive value, and negative predictive value between the surgeon's visual inspection and NIFI in identifying PGs, with values of 100%/97.1%, 85.0%/87.5%, 85.4%/87.2%, and 100%/97.2%, respectively. The sensitivity of NIFI (82.9%) for detection of PGs from thyroidectomy specimens was significantly higher than that of the surgeon's visual inspection (61.0%). False negative specimens contained bleeding/congestion and/or encapsulation by thick tissues, whereas false positive specimens contained electrocoagulated tissues.

Conclusions: NIFI showed results comparable to the experienced surgeon's visual inspection in identifying PGs. This could benefit novice surgeons. NIFI may be useful for experienced surgeons to locate incidentally resected PGs within thyroidectomy specimens for auto-transplantation. Prevention of intra-gland bleeding and congestion, careful removal of thick capsules, and bloodless surgeries without electrocoagulation are important for reducing false positive and false negative results.

Key Words: Near-infrared fluorescence imaging, parathyroid gland, thyroidectomy.

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INTRODUCTION

In thyroid surgery, especially total thyroidectomy, failure to identify, and preserve the parathyroid glands (PGs) can lead to postoperative hypocalcemia. Intraoperative PG identification has conventionally relied on the surgeons' experience.[1–3]

In 2011, Paras et al found the PG to have intrinsic auto-fluorescence (AF). They used a 785-nm diode laser as an excitation light and detected AF using a camera with a long-pass 808-nm filter. AF from the PG was in the range of 800–950 nm, with peak emission at 822 nm.[4] These AF characteristics are similar to those of indocyanine green (ICG); therefore, near-infrared fluorescence imaging (NIFI) for ICG, which has been used for sentinel lymph node biopsy, intraoperative cholangiography, and evaluation of venous flow angiography, has been applied to detect AF from the PG.[5–8] McWade et al reported that 256 of 264 (97%) PGs were correctly identified over a wide variety of disease states using NIFI,[9] suggesting a very high sensitivity to detect PGs. In the present study, we used histology-proven specimens and examined the sensitivity, specificity, positive predictive value, and negative predictive value of NIFI-based PG identification compared with the surgeon's gross visual inspection. We also examined the power of NIFI to detect incidentally resected PGs in thyroidectomy specimens for auto-transplantation purposes.

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MATERIALS AND METHODS

This prospective study was approved by the Institutional Review Board of Niigata University Hospital (approval #2018-0305) and was conducted according to the Declaration of Helsinki. Each patient provided written informed consent.

Patients and Surgery

Thirty-six patients undergoing total thyroidectomy or thyroid lobectomy for thyroid disease between July 2019 and December 2019 at the Department of Otolaryngology Head and Neck Surgery, Niigata University Hospital, were enrolled in this study. Table I shows the demographic and clinical characteristics of the study patients.

Surgeries with usual techniques were carried out by five surgeons (T.T., K.Y., H.O., R.S., Y.U.), who had 11–18 years of experience in head and neck and thyroid surgery. PGs were treated carefully for in situ preservation. However, if the PGs had a questionable blood supply, these were removed and auto-transplanted after confirming

TABLE I.
Demographic and Clinical Characteristics of Study Patients.

Parameter	All Patients (n = 36)
Age in years, mean (SD)	61.0 (15.4)
Sex, n (%)	
Male	10 (28)
Female	26 (72)
Body mass index, kg/m^2, mean (SD)	23.4 (4.2)
Diagnosis, n (%)	
Benign thyroid nodule/MNG	14 (39)
Thyroid cancer	19 (53)
Basedow's disease	3 (8)
Type of surgery, n (%)	
Total thyroidectomy	9 (25)
Thyroid lobectomy	27 (75)

MNG = multinodular goiter; SD = standard deviation.

their histology by frozen sections. In thyroid cancer cases, all inferior PGs were removed together with central neck tissue for lymph nodes resection followed by auto-transplantations. PGs that could be preserved in situ were excluded from the study because they could not be confirmed pathologically. PGs histologically confirmed by frozen sections were auto-transplanted into the sternocleidomastoid muscle for prevention of postoperative hypocalcemia.

NIFI Using PDE-Neo Device

We used commercially available equipment for NIFI (PDE-Neo, Hamamatsu Photonics, Hamamatsu, Japan). The excitation light source was a 760-nm LED, and the light-receiving camera was a charge-coupled device that captured near infrared light of 820 nm or more. The target tissue was observed in the center of the screen, as the center of the camera captured the AF most efficiently. While the focal length could be adjusted from 50 mm to 300 mm, we used a 50-mm focus. Similarly, while the PDE-Neo has adjustable configurations, its parameters for measurement were fixed (brightness −1.0, contrast 5.0, and excitation light maximum) throughout the study.

NIFI was performed by holding the camera 50 mm from the resected tissues on a sterile drape (Fig. 1A: white light imaging, B: near infrared light imaging). AF images were seen on the monitor with all operating room lights turned off. If AF from the resected tissues was brighter than the thyroid gland, it was determined to be AF-positive.

Comparison of Predictive Capabilities Between Surgeon's Visual Inspection and NIFI

Possible PGs (n = 28) and lymph nodes (n = 32) were identified by the surgeon's visual inspection. From the resected thyroidectomy specimens, 15 possible PGs were further identified using NIFI. For the total of 75 specimens, one surgeon's (K.Y.) judgments (PG vs. non-PG) were recorded, without knowledge about the AF characteristics of the specimens. The AF of each specimen was examined by one surgeon (T.T.) with NIFI, and then the specimen (judged PG by surgeon's visual inspection and/or by AF positivity) was sent for histological evaluation under frozen section. Sensitivity, specificity, negative predictive value, and positive predictive value for predicting PGs were compared between the surgeon's visual inspection and NIFI.

Detection of Incidentally Resected PGs in Thyroidectomy Specimens for Auto-Transplantation by Surgeon's Visual Inspection or NIFI

A surgeon (K.Y.) visually searched for PGs in the thyroidectomy specimens, then NIFI inspections were performed (Fig. 2).

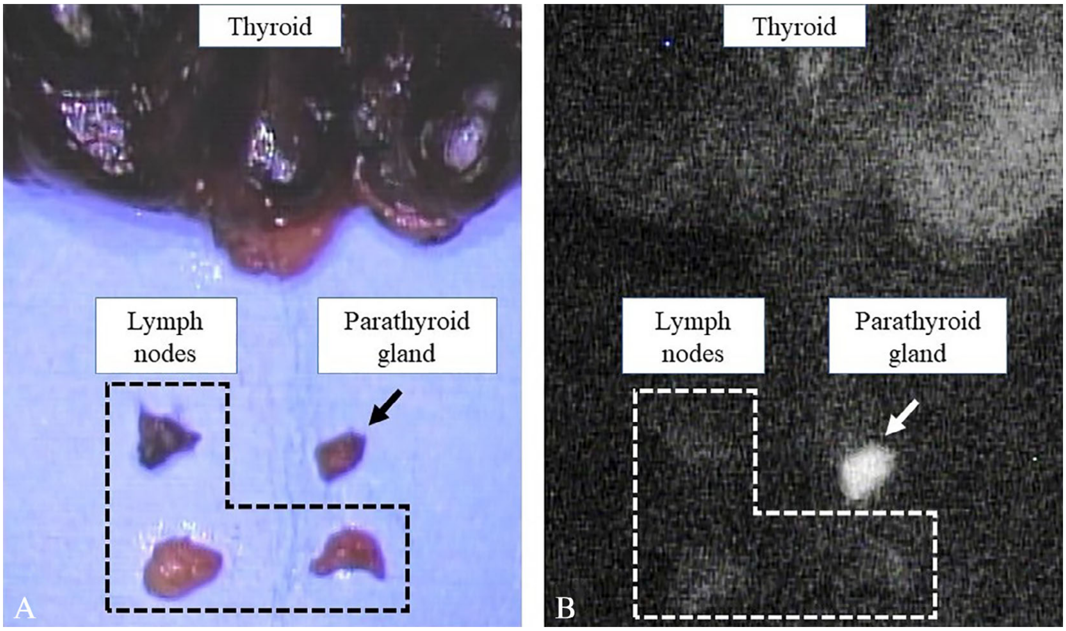

Fig. 1. Typical ex vivo measurement of the resected tissues: true negative lymph nodes and true positive parathyroid gland. (A) white light imaging, (B) near-infrared light imaging. Histological examination revealed that three AF-negative specimens and one AF-positive specimen were lymph nodes and PG, respectively. The true positive PG showed stronger AF than the normal thyroid gland.

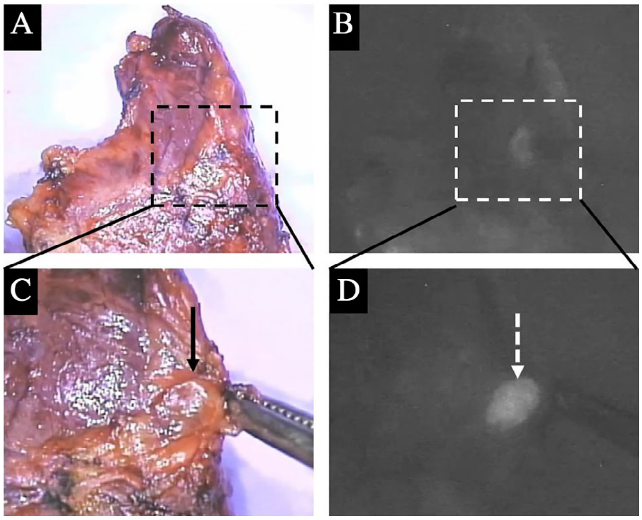

Fig. 2. The typical appearance of incidentally resected PG from thyroidectomy specimen. (A, C) white light imaging. (B, D) near infrared imaging. This was a parathyroid gland undetected by the surgeon's visual inspection (A). The small amount of AF led to the discovery of the parathyroid gland (B). AF became more pronounced when the surrounding tissue was removed (C, D).

TABLE II.
Diagnostic Capabilities of Surgeons' Visual Inspection and NIFI to Predict PGs.

	Surgeon's Visual Inspection		NIFI		
Parameter	n/N	%	n/N	%	*P* Value
Sensitivity	35/35	100.0	34/35	97.1	>.99
Specificity	34/40	85.0	35/40	87.5	>.99
Positive predictive value	35/41	85.4	34/39	87.2	>.99
Negative predictive value	34/34	100.0	35/36	97.2	.50
Accuracy	69/75	92.0	69/75	92.0	1.00
False positive	6/75	8.0	5/75	6.7	>.99
False negative	0/75	0.0	1/75	1.3	>.99

NIFI = near-infrared fluorescence imaging.

All specimens detected by visual inspection or NIFI were sent for frozen sections, and those proven as PGs were auto-transplanted into the sternocleidomastoid muscle. Finally, possible residual PGs in the remainder of the thyroidectomy specimens were examined by permanent pathology. Therefore, incidentally resected PGs were counted as a total of those noticed by the surgeon's

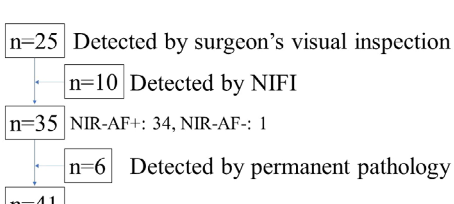

Fig. 3. Identification of incidentally resected PGs from thyroidectomy specimens by NIFI and the surgeon's visual inspection. NIFI = near infrared fluorescence imaging; NIR-AF = near infrared autofluorescence.

TABLE III.
Detection Sensitivity of Incidentally Resected PGs from Thyroidectomy Specimens.

	Surgeon's Visual Inspection	NIFI	*P* Value
Detection			
Possible	25	34	
Impossible	16	7	
Sensitivity	25/41	34/41	.0479

NIFI = near-infrared fluorescence imaging.

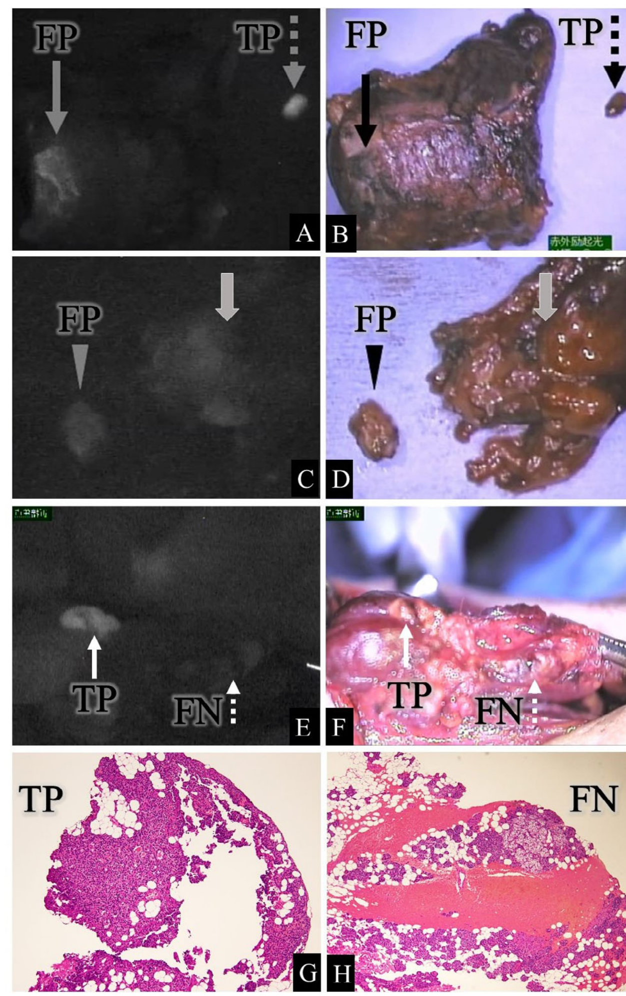

Fig. 4. Photos, NIFI images, and histology of false positive/negative specimens. (A, C, E) near-infrared imaging. (B, D, F) white light imaging. (G, H) hematoxylin and eosin staining sections. Thyroid tissue with fat (A, solid arrow) emits AF comparable to that of the PG (A, dashed arrow). This false positive thyroid tissue with fat had an electrocoagulated portion (B, solid arrow). Tissue indicated by the arrowhead (C) emits AF; however, this was electrocoagulated adipose tissue (D, arrowhead). Solid arrow in C and D is central neck tissue. Possible PGs indicated by solid arrow and dashed arrow (F) were AF-positive and AF-negative, respectively (E). Pathological examination demonstrated that the AF-positive nodule was normal PG (G) and the AF-negative nodule was also PG but contained intra-glandular bleeding (H). TP = true positive; FP = false positive; FN = false negative.

visual inspection, NIFI, and permanent pathology. False positive and false negative specimens were histologically characterized.

Statistical Analysis

The differences in diagnostic capabilities between NIFI and the surgeon's visual inspection were tested by Fisher's exact test using EZR (Easy R; the R Foundation, Vienna, Austria), which is now being distributed on the following website: http://www.jichi.ac.jp/saitama-sct/. *P* values <.05 were considered statistically significant.

RESULTS

Table I summarizes the demographic and clinical details of the 36 patients. The diagnosis was benign nodular goiter in 14 patients, thyroid cancer in 19 patients, and Basedow's disease in 3 patients. The procedures performed were total thyroidectomy in 9 patients and lobectomy in 27 patients. Twenty-nine possible PGs were preserved in situ and not subject to study, since their histology could not be proven. From the 36 patients, 75 specimens were resected and sent for histological examination. The final histological confirmation of these specimens showed 35 PGs, 34 lymph nodes, 5 adipose tissues, and 1 thyroid tissue.

Comparison of PG Identification Between Surgeon's Visual Inspection and NIFI

Fig. 1 shows the typical NIFI images of 3 true negative lymph nodes and one true positive PG. The AF of a true positive PG is stronger than that of a normal thyroid

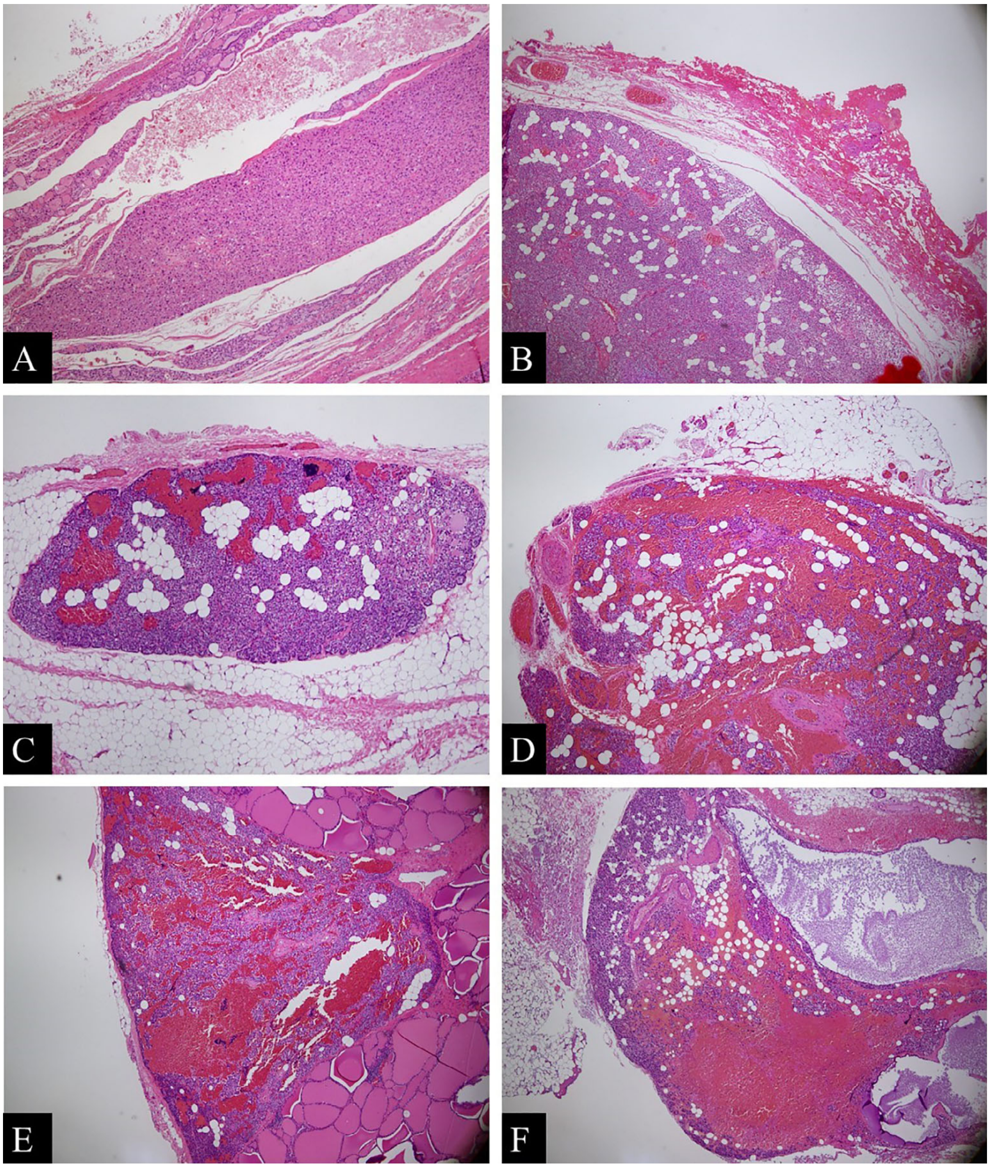

Fig. 5. Histology of false negative parathyroid glands. Pathological findings of 6 PGs that were definitively identified by permanent pathology but were unidentified by the surgeon's visual inspection and/or NIFI. Three PGs were covered with thick surrounding capsules (A, B, C), and 4 PGs had congestion and intra-glandular bleeding (C, D, E, F).

gland, while true negative lymph nodes show no significant AF (Fig. 1B). Table II shows the capabilities of the surgeon's visual inspection and NIFI to identify PGs. Out of 75 specimens, 41 and 34 specimens were judged as PGs and non-PGs, respectively, by the surgeon's visual inspection. As a result of pathological examination, out of 41 specimens judged as PGs, 35 were PGs and six were non-PGs, and 34 specimens judged as non-PGs were all correct. The six false positives by the surgeon's visual inspection included five fat tissues and one lymph node. Similarly, 39 and 36 specimens out of 75 were AF-positive and AF-negative, respectively, upon evaluation with NIFI. As a result of pathological examination, out of 39 AF-positive specimens, 34 were PGs, and five were non-PGs. Out of 36 AF-negative specimens, one was PG and 35 were non-PGs. The five false positives by NIFI included three fat tissues, one lymph node, and one small thyroid nodule. There was one false negative case by NIFI. Out of these six false cases by NIFI, only 1 fat tissue was missed by the surgeon's visual inspection.

Sensitivity, specificity, positive predictive value, and negative predictive value to identify parathyroid tissue for the surgeon's visual inspection and NIFI were 100%/97.1%, 85.0%/87.5%, 85.4%/87.2%, and 100%/97.2%, respectively. False positive and false negative rates by the surgeon's visual inspection and NIFI were 8.0%/6.7% and 0%/1.3%, respectively. There were no significant differences in all these parameters between the surgeon's visual inspection and NIFI.

Detection of Incidentally Resected PGs from Thyroidectomy Specimens by the Surgeon's Visual Inspection and NIFI for Auto-Transplantation Purposes

Fig. 2 shows the typical appearance of an incidentally resected PG in a thyroidectomy specimen. From the 36 thyroidectomy specimens, 25 PGs were detected by the surgeon's visual inspection, and an additional 10 were detected by NIFI in 9 patients. Of these 35 PGs, 34 were AF-positive. Further, 6 additional PGs were detected by permanent pathology. Therefore, a total of 41 PGs were histologically confirmed in 36 thyroidectomy specimens (Fig. 3). Sensitivity of NIFI for detection of PGs in thyroidectomy specimens (34/41, 82.9%) was significantly higher than that of the surgeon's visual inspection (25/41, 61.0%) ($P < .0479$) (Table III).

Analyses of False Positive and False Negative Tissues

Fig. 4 shows images of the false positive (A–D) and false negative (E–F) cases by NIFI. As shown in Fig. 4A, thyroid tissue with fat (solid arrow) emitted AF comparable to that of the PGs (dashed arrow). This false positive thyroid tissue with fat had an electrocoagulated portion (Fig. 4B, solid arrow). AF-positive tissue (Fig. 4C, arrowhead), however, was electrocoagulated adipose tissue (Fig. 4D, arrowhead).

The nodule indicated by the dashed arrow was AF-negative (Fig. 4E); however, two possible PGs were visually identified, as indicated by the solid and dashed arrows (Fig. 4F). Pathological examination demonstrated that the AF-positive nodule indicated by the solid arrow was a normal PG (Fig. 4G), and the AF-negative nodule as indicated by the dashed arrow was also a PG, but contained intra-gland bleeding (false negative, Fig. 4H).

Fig. 5 shows the pathological findings of 6 PGs that were detected by permanent pathology but had gone unnoticed by the surgeon's visual inspection and NIFI. Thick surrounding capsules covered 3 PGs (Fig. 5A–C), and 4 PGs had congestion and intra-glandular bleeding (Fig. 5C–F).

DISCUSSION

Role of NIFI in Identifying PGs Compared to that of the Surgeon's Visual Inspection

In this study, we compared the results of NIFI (AF-positive or AF-negative) with pathology. Sensitivity, specificity, positive predictive value, and negative predictive value of NIFI to identify parathyroid tissue were 97.1%, 87.5%, 87.2%, and 97.2%, respectively (Table II). These results are similar to those of a previous study.[10] We also compared the results of the surgeon's visual inspection (PG or non-PG) with pathology; its sensitivity, specificity, positive predictive value, and negative predictive value to identify parathyroid tissue were 100%, 85%, 85.4%, and 100%, respectively (Table III). Thus, NIFI-based predictive capabilities were not superior to those of the surgeon's visual inspection, suggesting that NIFI would not provide additional benefit to experienced head and neck surgeons in identifying PGs or other tissues. However, NIFI could benefit novice surgeons by providing them with the same capability to identify PGs as experienced surgeons.

Because the sensitivity and negative predictive value of NIFI were 97% or more, a surgeon can be confident that a tissue lacking AF is not likely PG. On the other hand, since the specificity and positive predictive value were less than 88% (87.5% and 87.2%, respectively), even AF-positive tissues may have histology other than PG. Therefore, even if NIFI is used, pathological confirmation of PG by frozen sections is necessary for auto-transplanting dissected tissue. Kose et al reported that specificity and positive predictive value were 97.2% and 95.1%, respectively based on 550 pathology specimens,[10] which were better than our results. They also reported that a learning curve of 15 to 20 cases was necessary to optimally use NIFI.[11] Since our results are based on our initial experience with NIFI, we believe that the accuracy may be improved with more use.

False Negative and False Positive Cases

Out of 35 histologically proven PGs, 1 lacked AF (sensitivity, 97.1%; Table II). Histological examination of this false negative case (Fig. 4F, dashed arrow) revealed massive bleeding in the PG (Fig. 4H), while the other 34 PGs did not show bleeding inside the specimens. Fluorescence is highly absorbed by hemoglobin in erythrocytes[12]; therefore, we supposed that AF from PGs may be attenuated in tissues with massive intra-glandular bleeding, causing false negative results. As other examples of false negatives, 6 PGs were finally identified by permanent pathology (Fig. 5), none of which had been noticed either by NIFI or the surgeon's visual inspection. Fig. 5 shows hematoxylin and eosin stained sections of these 6 PGs. They were characterized by accompanying intraglandular bleeding and congestion, or by encapsulation with thick surrounding tissue. We supposed that the AF was indistinct because of the absorption of excited fluorescence by hemoglobin, as in the false negative PGs (Fig 4F; dashed arrow) in the former cases. In the latter cases, AF was not produced since near-infrared excitation light did not reach the PG due to blockade by the thick capsules. These findings suggest that gentle handling of tissues to prevent bleeding inside the PG and careful removal of thick capsules before NIFI are important to increase sensitivity in identifying PGs from thyroidectomy specimens.

There were 5 false positive cases identified by NIFI (Table II): 1 small thyroid nodule, 1 lymph node, and 3 adipose tissues. As shown in Fig. 4B,D, false positive specimens had accompanying electrocoagulated tissue, though not in all cases. To reduce false positive results, it is important to perform bloodless surgeries and to carefully remove electrocoagulated tissue before NIFI.

Role of NIFI in Detecting Auto-Transplantation PGs from Thyroidectomy Specimens

As shown in Fig. 3, NIFI assessment of thyroidectomy specimens for PG auto-transplantation purposes revealed 10 additionally resected PGs, all unrecognized by the surgeon's visual inspection, in 9 of 36 patients. Six PGs could not be detected by the surgeon's visual inspection and NIFI, which were only found by permanent pathology. The NIFI detection rate of PGs from thyroidectomy specimens was 82.9% (34/41; Table III), which was significantly higher than that of the surgeon's visual inspection (61.0%, 25/41; Table III). Therefore, NIFI may be better able to detect PGs from thyroidectomy tissues than the surgeon's visual inspection. Kose et al demonstrated only a minor role of NIFI in identifying PGs for in situ preservation, possibly due to the poor permeability of AF through encapsulated tissues.[10] In contrast, two randomized controlled trials showed that NIFI was useful for preserving PGs in situ and preventing postoperative hypocalcemia.[13,14] Therefore, the utility of NIFI as a navigation tool to preserve PGs in situ may be controversial. Although the current study did not examine the capability of NIFI to detect PGs for in situ preservation, our results suggest that NIFI may be useful in detecting PGs in dissected thyroidectomy specimens for auto-transplantation purposes, even for experienced surgeons. NIFI is also advantageous because it requires no use of any medication. 5-aminolevulinic acid has been used to effectively detect PGs from dissected thyroidectomy specimens. However, it must be taken orally at a specific time prior to surgery.[15] The NIFI method is simple, medication-free, and non-invasive to patients.

Limitation

The judgment regarding the presence or absence of AF was dependent on the surgeon's subjective impression; therefore, the NIFI results might be biased. To eliminate this bias, real-time quantification of fluorescence intensity during surgery is desirable in future studies.

CONCLUSIONS

This prospective study revealed that NIFI showed capabilities comparable to the eyes of an experienced surgeon in identifying PGs. This could be beneficial for novice surgeons. NIFI could also be a useful navigation tool even for experienced surgeons to detect incidentally resected PGs from thyroidectomy specimens for auto-transplantation purposes. However, the specificity and positive predictive value of NIFI were less than 88%, indicating that frozen section confirmation of parathyroid tissue is necessary before auto-transplantation. Absorption of excited fluorescence by hemoglobin and its blockade by thick tissue capsules could interfere with the performance of NIFI.

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