Intraoperative Real-Time Localization of Normal Parathyroid Glands with Autofluorescence Imaging

Sung Won Kim,MD,[1,2], Seo Hyun Song[2,4], Hyoung Shin Lee,MD,[1,2], Woong Jae Noh,MD,[1,2], Chulho Oak,MD[2,3], Yeh-Chan Ahn,PhD[2,4], Kang Dae Lee,MD[1,2]

[1]Department of Otolaryngology-Head and Neck Surgery, Kosin University College of Medicine, Busan, S Korea; [2]Innovative Biomedical Technology Research Center, College of Medicine, Kosin University, Busan, Korea; [3]Department of Internal Medicine, Kosin University College of Medicine, Busan, S Korea; [4]Department of Biomedical Engineering and Center for Marine-Integrated Biomedical Technology, Pukyong National University, Busan, S Korea

Context: This biomedical investigation is valuable for identification and localization of parathyroid glands during thyroidectomy, which can provide an intraoperative real-time visual guidance.

Objective: To investigate the feasibility of real-time autofluorescence imaging of the parathyroid glands without exogenous contrast dye for their localization and demonstration of relation to the background tissues.

Setting: This research was underwent at Kosin University Gospel Hospital.

Methods: Sixteen normal parathyroid glands from 8 patients with papillary thyroid carcinoma were enrolled. Photo-images of surgical field including the parathyroid and background tissues were taken with a digital camera, 780 nm LED to excite the parathyroid, and infrared (IR) illuminator to visualize the entire neck. Area-averaged autofluorescence intensity of parathyroid over area-averaged fluorescence intensity of background tissues (P/B ratio) was measured.

Main Outcome Measure: The location of parathyroid gland was verified with single image.

Results: The P/B ratio for all parathyroid glands was higher than 1 with a minimum of 1.95 and a maximum of 5.20 (average: 2.76, standard deviation: 0.79). By our technique, all 16 parathyroid glands were detected (Positive predictive value = 100%), and entire surgical field including the parathyroid and background tissues were visualized as well. The parathyroid glands that were exposed or even covered by connective tissues or blood vessels could be detected with strong emission.

Conclusions: This method showed the precise localization of the parathyroid glands and demonstrated their relation to background tissue. We believe that this simple, non-exogenous dye technique of anatomical guidance can aid surgeons to preserve parathyroid glands during thyroidectomy.

Thyroidectomy is one of the most frequently conducted endocrine surgeries and has increased in use in recent years as the incidence of thyroid cancer has risen worldwide. Survival of patients with well-differentiated thyroid cancer is excellent (1, 2). However, minimizing thyroidectomy complications to prevent lifelong disabilities such as recurrent laryngeal nerve injury or hypoparathyroidism remains a challenge for surgeons (3). While transient hypoparathyroidism is relatively common, occurring after 6.9 to 46% of thyroid surgeries, permanent hypoparathy-

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roidism occurs after total thyroidectomy at rates ranging from 0.5 to 6.6% (4, 5). The risk of causing permanent hypoparathyroidism may be even higher for less-experienced surgeons since the parathyroid gland is very small, has a color similar to surrounding tissues, and is embedded within paratracheal fat tissues or behind the thyroid gland. Even for experienced surgeons, the incidence of inadvertent parathyroid gland removal has been reported to vary from 9.1 to 15% (6–8).

Identification of the parathyroid glands during thyroid surgery may be the first step to preserve these organs and prevent loss of their function. Surgeons can confirm parathyroid tissues through frozen biopsy during surgery, but this method can lead to vascular damage as a result of cutting part of the parathyroid glands and is associated with additional cost and waiting time (9). Several exogenous dyes have been used for the identification of diseased and normal parathyroid glands. However, they may lead to side effects from the dye or injection procedure (10, 11). Suh et al (12) and Hyun et al (13) reported that indocyanine green and certain synthetic fluorophores are useful for identifying parathyroid glands in animal models. However, these exogenous agents require intravenous (IV) injection and the feasibility of these fluorophores in human thyroid surgery has not yet been verified.

McWade et al (14) presented intraoperative autofluorescence imaging of parathyroid glands with near infrared (NIR) light to identify normal parathyroid glands in patients undergoing thyroidectomy (n = 3) and parathyroidectomy (n = 3). Specifically, they demonstrated that autofluorescence imaging is a highly sensitive, label-free tool for identifying normal parathyroid gland tissue, and were successful in obtaining an excellent autofluorescence image of the parathyroid gland. However, application of their NIR autofluorescence imaging technique to thyroid surgery was associated with several limitations, since only the parathyroid gland was presented in their images without showing the surrounding background tissues. In other words, the NIR autofluorescence image was limited to ‘identification’ of the parathyroid gland. Thus, in their study, it was necessary to overlay separate bright-field and fluorescence images to identify the parathyroid gland from the surrounding tissue for ‘localization’, which required two separate images of the same surgical field.

In the present study, we hypothesized that adding an infrared (IR) illuminator to the NIR imaging system may reduce shortcomings of the method and allow to visualize immediately both the parathyroid glands and peri-parathyroidal tissues on a single image. An imaging system allowing for both ‘identification’ and ‘localization’ of the parathyroid gland during thyroid surgery can provide better guidance to surgeons with respect to preserving the parathyroid glands. To the best of our knowledge, this is the first study to report a real-time single imaging technique that presents autofluorescence of the parathyroid glands as well as surrounding tissues in patients undergoing thyroidectomy.

Materials and Methods

This study included 16 normal parathyroid glands from 8 papillary thyroid carcinoma patients (men : women, 3 : 5, between ages 34 and 73 years) who underwent surgical ablation, comprising 3 total thyroidectomies and 5 hemithyroidectomies. Central neck dissection was conducted in all patients (Table 1). The imaging system used in this study consisted of a digital single lens reflex (DSLR) camera (EOS REBEL T3, Canon, Tokyo, Japan), camera lens (Canon EF 50 mm f/1.8 II, Canon), excitation filter (#84121, Edmund Optics, Barrington, NJ, USA), emission filter (#84123, Edmund Optics), 780-nm collimated light-emitting diode (LED) (M780L3-C1, THORLABS, Newton, NJ, USA), and infrared (IR) illuminator (INFRALUX-300, Daekyung Electro Medical Co, Republic of Korea). The IR filter in front of the camera’s CCD array was removed to recognize the fluorescence emitted from the infrared wavelength range. The excitation filter consisted of a bandpass filter with a 769-nm center wavelength and 41-nm bandwidth, which was inserted in a 780-nm collimated LED body. The emission filter consisted of a bandpass filter with an 832-nm center wavelength and 37-nm bandwidth that was attached to the front of camera lens when taking images in the infrared range. Study approval was obtained from the

Table 1. Patient demographic data

Number of Patients	Age	Gender	Diagnosis	Operation Extent	Number of Parathyroid Glands	Confidence Level of Surgeon
1	34	M	PTC	Total thyroidectomy, CCND	2	high
2	38	F	PTC	Hemithyroidectomy left, CCND	2	high
3	52	M	PTC	Total thyroidectomy, CCND	2	high
4	49	M	PTC	hemithyroidectomy right, CCND	2	high
5	37	F	PTC	Total thyroidectomy, CCND	2	high
6	73	F	PTC	hemithyroidectomy left, CCND	2	high
7	55	F	PTC	hemithyroidectomy right, CCND	2	high
8	40	F	PTC	hemithyroidectomy right, CCND	2	high

PTC, papillary thyroid carcinoma; *CCND*, central compartment neck dissection.

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Institutional Review Board at Kosin University College of Medicine.

During surgery, the operator determined whether the tissue being examined was parathyroid gland by visual inspection. NIR fluorescence images were obtained after lateralization of the thyroid lobe when the parathyroid gland was exposed or even when the parathyroid gland was covered by surrounding connective tissue, fat tissue or blood vessels.

To compare visible images, autofluorescence images, and NIR-IR images in the same surgical field, photo-documentation in the following three conditions was performed. First, a visible-light image of the paratracheal area where the parathyroid was expected to be placed was taken with a DSLR camera as the baseline image (Figure 1A). Second, autofluorescence images of the parathyroid were captured from the operation area (16 cm^2). A 780-nm collimated LED light source with the excitation filter was used for excitation, and the total beam power of the LED was 210 mW. After turning off the overhead light and indoor light of the operation theater, images were taken with a camera with which the emission filter described above was attached in front of the lens. This is to accept the emitted wavelength light and block scattered excitation (Figure 1B). For the third image sequence, an IR illuminator was added to the conditions described for the second step. A broad band (700 to 1400 nm) IR light was used to illuminate and visualize the entire surgical field to demonstrate the relation between the autofluorescence of the parathyroid gland and surrounding structures (NIR-IR imaging) (Figure 1C).

Since no fluorescence agent was required, preoperative preparation was not needed for imaging. The second and third images were captured at exposure times of 4 or 8 seconds with the DSLR camera fitted with the emission filter described above. The collimated LED was used at high intensity but the illuminator was used at low intensity because the parathyroid gland has very low autofluorescence intensity. Image J software (NIH) was used to measure the fluorescence intensity and establish the parathyroid and background tissue ratio (P/B ratio). Specifically, the P/B ratio was defined as area-averaged autofluorescence intensity of parathyroid over area-averaged fluorescence intensity of surrounding tissues, such as thyroid, trachea, esophagus, and all other connective tissues.

Results

The P/B ratio was higher than 1 with a minimum of 1.95 and a maximum of 5.20 (average: 2.76, standard deviation: 0.79) in 16 parathyroid glands, which suggests that parathyroid glands can be shown as brighter signals than the background structures in NIR-IR fluorescence imag-

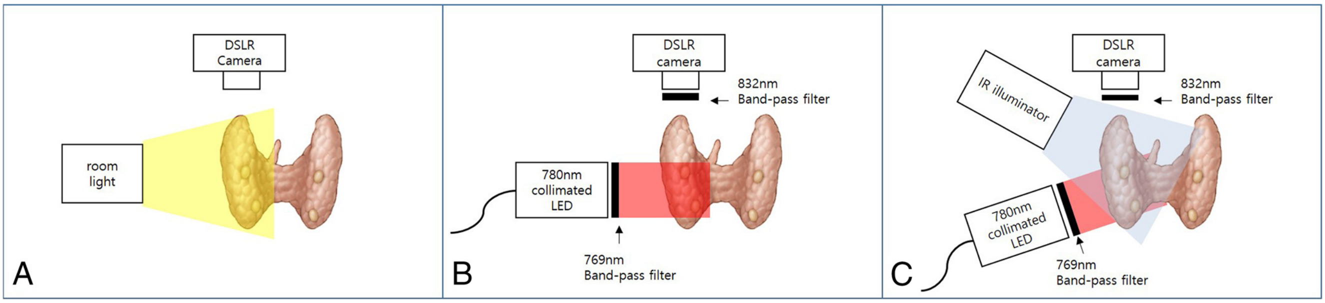

Figure 1. Schematic diagram for (A) capturing visible images, (B) Capturing autofluorescence images of parathyroid tissue, and (C) Detecting autofluorescence of parathyroid tissue with simultaneous illumination of the surrounding tissues.

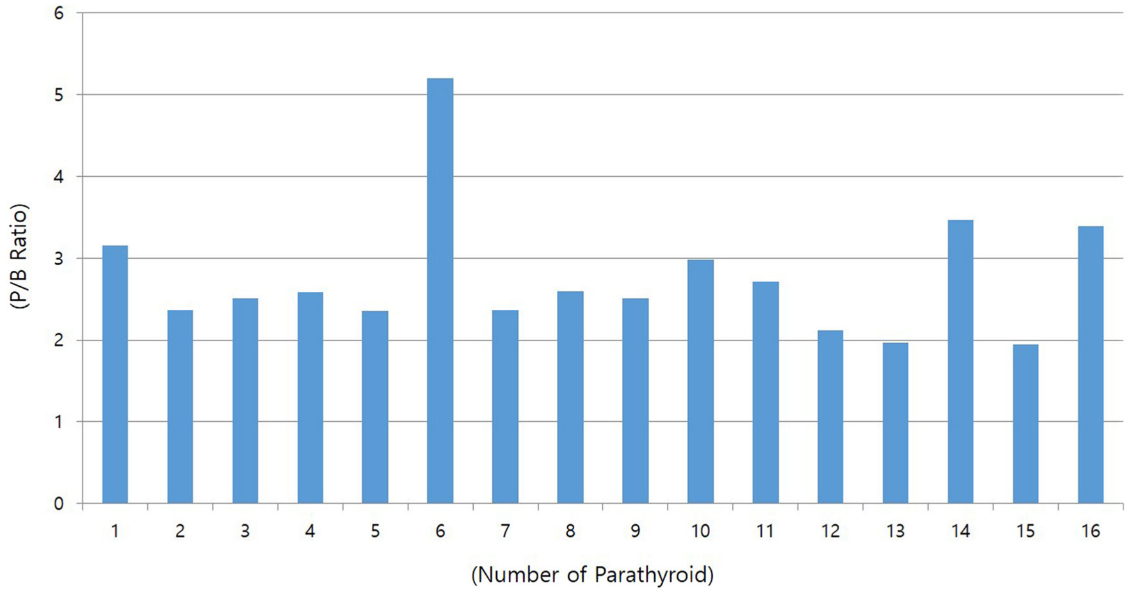

Figure 2. The autofluorescence intensity of all parathyroid glands showed higher than 1 (P/B ratio : 1.95 to 5.20).

ing (Figure 2). This means that normal parathyroid glands can be detected and distinguished from thyroid gland or other structures by our imaging technique.

The equipment set up for the examination took 5 to10 minutes. It was prepared ahead of the thyroidectomy. In addition, the total time needed for fluorescence imaging was less than 1 minute, which did not interfere with operative procedure.

Surgical Correlation of NIR-IR Fluorescence Imaging

When the paratracheal area was dissected after lateralization of the thyroid gland, 16 parathyroid glands were detected by our imaging technique. Six parathyroid glands were found in the exposed state and the other 10 were covered by blood vessels, fat, or connective tissues. Two inferior parathyroid glands were found in unusual locations. One was identified superficially and one was found very close to the superior parathyroid gland. In these cases, our imaging technique has been useful in the ways we have described below. Although histological validation of the parathyroid tissue was not possible due to ethical issues, three thyroid surgeons agreed all tissues to be tested were parathyroid glands with high confidence. Parathyroid tissue was not found in the specimen of thyroidectomy and central compartment dissection. No patients experienced postoperative hypoparathyroidism.

Detecting typical location of parathyroid gland and demonstration of surrounding structures by NIR-IR fluorescence imaging

While approaching the tracheo-esophageal (T-E) groove, the putative superior and inferior parathyroid glands were located in the paratracheal area (Figure 3A). In autofluorescence image, two bright spots with high-intensity emission of autofluorescence were found which may indicate the parathyroid glands (Figure 3B). When a low-intensity IR illuminator was added as a second light source, in addition to parathyroid gland, all structures of surgical field including the thyroid, trachea, esophagus, fat, connective tissues, skin flap, and even the surgical instruments could be visualized. The parathyroid gland emitted the strongest autofluorescence signal. The thyroid gland also showed a signal but the intensity was significantly lower than that of the parathyroid gland. Other organs such as muscle, fat and connective tissues did not produce significant autofluorescence. The two brightest autofluorescence spots exactly coincided with the superior and inferior parathyroid gland (Figure 3C). This allows us

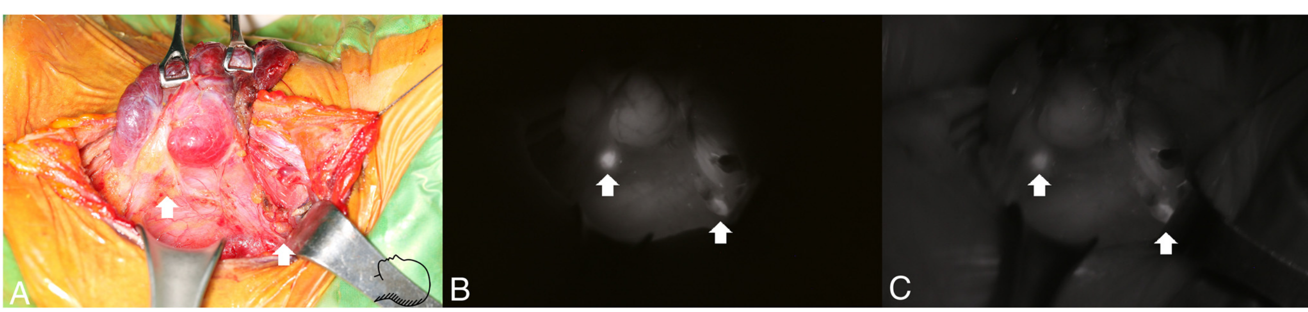

Figure 3. A, Visible image after lateral lobe mobilization. The superior and inferior parathyroid glands were identified (arrowheads). B, Autofluorescence image: two strong points of emission (arrows) from the paratracheal area were noted. C, Combined autofluorescence and IR illumination image (NIR-IR imaging): the two autofluorescence emission points (arrows) coincided with the superior and inferior parathyroid glands. The entire surgical field was visualized in their original form by adding IR illumination because other organs did not emit autofluorescence.

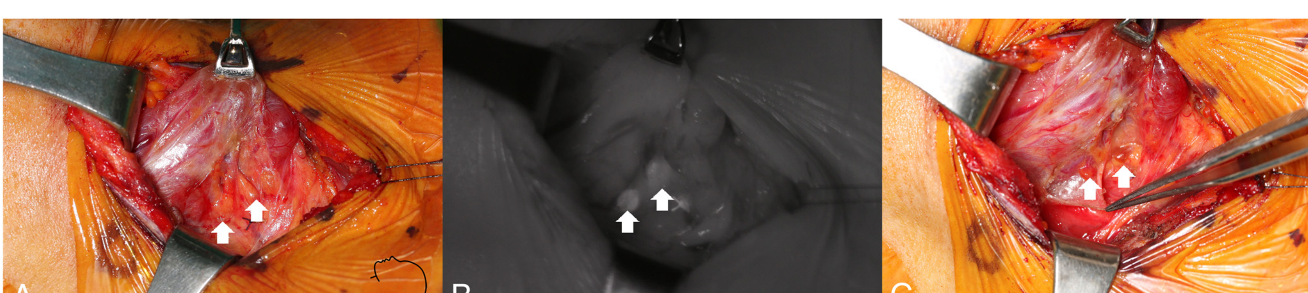

Figure 4. A, Visible image of the paratracheal area after lateral lobe mobilization. Both superior and inferior parathyroid glands are covered by connective tissue and blood vessels (arrowheads) (B) Combined autofluorescence and IR illumination image: autofluorescence (arrows) from unexposed parathyroid glands. The veins near the parathyroid gland were dimly visible, with a faint vessel overlying the parathyroid gland with low fluorescence intensity (C) Detection of the superior and inferior parathyroid glands (arrowheads) after dissection of connective tissue and ligation of blood vessels.

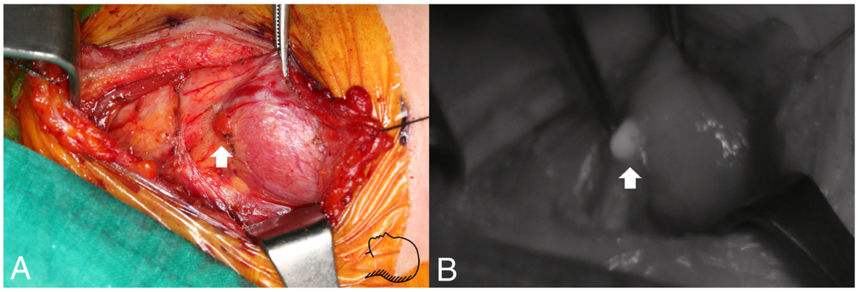

Figure 5. A, Image of an early stage of thyroid gland dissection and visible superficial location of inferior parathyroid gland (arrow). B, Localization of superficially located inferior parathyroid gland by NIR and IR illumination at early stage of paratracheal dissection.

to distinguish the parathyroid gland from surrounding structures.

Detecting parathyroid gland covered with connective tissues or blood vessels by NIR-IR fluorescence imaging

In a case where the parathyroid gland was veiled with paratracheal connective tissues or vessels (Figure 4A), when the gland was not detectable with the naked eye, it was still possible to detect the parathyroid gland by autofluorescence imaging. Specifically, when the superior parathyroid was covered with fat or connective tissues, it could be identified as high-intensity fluorescence. However, when the inferior parathyroid was hidden by blood vessels, it showed a low intensity fluorescence (Figure 4B). After ligating the overlying blood vessel at the inferior fluorescent area, we could identify the inferior parathyroid gland. Likewise, after dissection of the connective tissue in the superior fluorescent area, we were able to identify the superior parathyroid gland (Figure 4C). This suggests that unexposed parathyroid glands could be detected by our technique.

Detecting unusual location of the parathyroid gland by NIR-IR fluorescence imaging

While conducting lateral lobe mobilization and paratracheal dissection, the parathyroid may be missed if it is very superficially or unusually located (Figure 5). In this occasion, checking the paratracheal area with NIR-IR imaging immediately after lateral lobe mobilization may help to identify unusual parathyroid gland locations in the early stages of dissection (Figure 5B). In some cases, the superior and inferior parathyroid glands can be unusually close together. In this case, a small, autofluorescence signal from just inferior to the strong fluorescence emission was detected. When we dissected the overlying fatty, connective tissue on the fluorescent area, a parathyroid gland was identified, which may be an inferior parathyroid gland or a supernumerary parathyroid gland. These findings suggest that surgeons may have benefits for detecting unusual location of parathyroid glands from the use of NIR-IR imaging.

Discussion

In the present study we demonstrated the feasibility of intraoperative, label free, real-time localization of normal parathyroid glands with NIR autofluorescence imaging in patients undergoing thyroidectomy for papillary thyroid carcinoma. The procedure was simple and reproducible, and had no adverse effects on either patients or the surgical procedure. Compared with ultraviolet (UV)-visible wavelength ranges, NIR wavelengths are optimal for biomedical applications, since the NIR range with an optical window between 700 and 900 nm allows for a deeper penetration depth of several millimeters with low scattering and low absorption (15). Importantly, NIR wavelengths produce higher resolution images and can give better surgical information. Several studies have confirmed that NIR endogenous contrast excited at 785 nm and emitted at 822 nm is more concentrated in the parathyroid glands than the thyroid gland and other neck structures. Indeed, these observations led proposal of using NIR autofluorescence imaging to detect the parathyroid glands during thyroid surgery (9, 14, 16). Although several exogenous dyes (methylene blue, indocyanine green) have been used to localize the parathyroid glands, they require IV injection and may lead to untoward symptoms such as neurotoxicity, photobleaching, and pain at the infusion site (10, 12). Hyun et al (13) synthesized fluorophores that showed high uptake in the parathyroid glands and observed the glands using a dual-channel NIR imaging system in a porcine model. However, their method requires IV injection and the feasibility of using the proposed fluorophores in human has not yet been verified.

NIR autofluorescence technique introduced by previous studies (9, 14, 16) may be useful for identifying the parathyroid glands, but they need overlay of visual and autofluorescence images to indicate the anatomical location in the surgical field. This is the limitation of the previous studies. In the present study, we developed an easy way of showing the location of the parathyroid glands and related adjacent structures using an intraoperative realtime single image-based technique. Our technique was distinguished in that we used an IRilluminator to view the entire operative field with simultaneous NIR imaging. These combined NIR-IR imaging techniques allowed surgeons to localize the parathyroid glands in the surgical field. Since the devices (DSLR camera, LED, etc.,) used in the present study were much lower in cost, our system has economical benefits.

We were also able to acquire autofluorescence images of parathyroid tissue even in cases where the glands were covered with fat or connective tissue. Since this imaging method provided the possible locations of parathyroid glands before gland exposure, the operator could use this information to detect the parathyroid gland and continue the surgical procedure. This is due to the optical window of NIR (15). The autofluorescence intensity of the parathyroid covered by connective tissue (superior parathyroid gland) was higher than the intensity of the parathyroid partially covered by a blood vessel (inferior parathyroid gland) (Figure 4B, 4C). The difference in autofluorescence intensity can be caused by higher absorption of activated light energy at the wavelength range of 780 nm from a blood component, such as hemoglobin, in the vessels compared to absorption by fat or connective tissue.

Due to their embryological origin, the inferior parathyroid glands can exhibit a variable range of locations, ranging from the superior parathyroid located around the cricothyroid muscle to the thymus in the upper mediastinum. In cases where the parathyroid glands lie in unusual site, it is often difficult for the surgeon to ensure that they have been preserved during thyroidectomy. Typically, the operator must dissect the lower thyroid lobe or deeply located paratracheal tissue to find the inferior parathyroid glands; however, if the parathyroid glands are located superficially, they may be inadvertently removed due to their attachment to the resected specimen (Figure 5). Similarly, when a small autofluorescent signal is found close to the superior parathyroid gland, it can be difficult to establish whether it represents an unusual superior location of the inferior parathyroid gland or a supernumerary parathyroid gland (Figure 6). We may infer it as an inferior parathyroid gland if only two signals are detected in the central compartmental area. Specifically, NIR-IR autofluorescence imaging can be useful to prevent surgeons from missing the parathyroid gland when entering the TE groove, and helpful in detecting the parathyroid gland when it is located in unexpected areas. In the present study, we were able to easily identify and preserve unusually located parathyroid glands based on the autofluorescence images.

Parathyroid glands were accurately detected with the NIR-IR autofluorescence imaging method described in this study in 100% of patients (positive predictive rate = 100%). Furthermore, the parathyroid-to-background surrounding tissues fluorescence intensity ratio (P/B ratio) ranged from 1.95 to 5.20. These outcomes demonstrated that NIR-IR autofluorescence may be a feasible intraoperative method for accurate detection and real-time localization of parathyroid glands. In addition, none of the patients in our series showed any parathyroid gland identified from the surgical specimen. Thus, sensitivity, specificity, and negative predictive value (NPV) were 100% based on surgeons' decision.

Histological validation may be required to ensure that areas of autofluorescence are parathyroid tissue. However, tissue resection to verify parathyroid tissues may pose ethical problems, and the use of frozen section biopsies can lead to additional costs and long waiting times for results. This process also risks damaging healthy parathyroid tissue during its removal for analysis. On the other hand, we believe that expert thyroid surgeons can be confident about differentiating tissue through characteristic findings and experience. Consistently, none of the patients enrolled in this study had postoperative hypoparathyroidism.

Limitation of this study may be the limited number of enrolled patients. In addition, identification and localization of the parathyroid gland may not necessarily lead to preservation of the organ. Therefore, the usefulness of our NIR-IR autofluorescence imaging technique to prevent hypoparathyroidism should be verified by further clinical studies. Nonetheless, absence of any inadvertent removal of parathyroid gland in patients of this study may imply the feasibility of this imaging technique to preserve the organ during thyroidectomy. This is the first study to report a real-time single imaging technique that presents autofluorescence of the parathyroid glands as well as background other tissues in patients undergoing thyroidectomy.

Conclusions

We developed a method to identify and localize normal parathyroid glands in real time during thyroidectomy using a single autofluorescence imaging with a digital camera, NIR LED and IR illuminating lights. Based on the feasibility of the NIR-IR autofluorescence imaging method shown in this study, we believe that this simple, nonexogenous dye technique of anatomical guidance can aid surgeons to preserve parathyroid glands during thyroidectomy

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Address all correspondence and requests for reprints to: Kang Dae Lee, MD, PhD, Department of Otolaryngology-Head and Neck Surgery, Kosin University College of Medicine, 34, Amnam-Dong, Seo-Gu, Busan, 602–715, Republic of Korea, Tel: 82–51–990-6470 Fax: 82–51–245-8539 E-mail: kdlee59@gmail.com.

Sung Won Kim and Seo Hyun Song contributed equally to this work.

Cocorresponding author: Yeh-Chan Ahn, PhD, Department of Biomedical Engineering/Interdisciplinary Program of Medical Biotechnology Application Engineering, Pukyong National University, Busan 608–737, Republic of Korea, Tel: 82–51–990–6470, Fax: 82–51–245–8539, E-mail: ahny@pknu.ac.kr

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