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THE ROLE OF FLUORESCENCE-GUIDED SURGERY FOR THE VERIFICATION OF PARATHYROID GLANDS AND RECURRENT LARYNGEAL NERVES IN THE NEAR INFRARED SPECTRUM

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Recurrent laryngeal nerve (RLN) pulsity and damage to the parathyroid glands are considered as major complications that might be seen during surgical interventions for benign and malignant thyroid and parathyroid neoplasms. Fluorescence-guided surgery is a method that involves the use of contrast agents and fluorophore signal identification devices for intraoperative visualization of anatomical structures. A little is known about fluorescence-guided surgery for the verification of RLN and parathyroid glands.

The aim of the study was to evaluate the role of fluorescence-guided surgery using two different imaging systems and indocyanine green to identify the RLN and parathyroid glands during operations for benign and malignant thyroid and parathyroid tumors.

A 131 patients were indentified for the study. All operations were performed using the capsule dissection technique. Parathyroid glands were identified with the naked eye, followed by analysis of the operative field using one of the available imaging systems to detect near infrared (NIRF) signal. Indocyanine green (ICG) was used as a fluorophore.

Identification of parathyroid glands using NIRAF determination was combined with indocyanine green fluorophore administration in 102 (78%) patients. In all these cases, a uniform distribution of the signal in the NIRAF was determined. RLN did not show a signal without ICG. But after the injection of contrast ICG, a clear signal was obtained in all 131 (100%) cases from RLN.

Fluorescent guided surgery is promising and novel approach for the confirmation of both RLN and parathyroid gland, which is better to apply with fluorophore such as ICG. Fluorescent guided surgery is considered as an additional tool to the naked eye conformation of the RLN and parathyroid glands.

Key words: parathyroid glands, recurrent laryngeal nerve; fluorescence-guided surgery; near-infrared fluorescence; indocyanine green.

Connection of the publication with planned research works.

The work does not have connection with planned research works.

Introduction.

One of the important stages of thyroid surgery for management of benign and malignant tumors of the thyroid gland is the identification and preservation of the recurrent laryngeal nerve (RLN) and parathyroid glands [1-3]. Damage to the RLN is a significant disabling complication resulting in temporary paresis or permanent paralysis of the vocal cords and narrowing of the larynx, which is associated with dysphonia, stridor, and respiratory failure. The risk of RLN pulsity is increased in cases of neck dissection, which is a routine step in the

surgical management of thyroid cancer [4]. Identification of the RLN is usually performed by the naked eye during the a dissection of the tracheoesophageal groove in the area of the projection of the tubercle of Zukerkandl. Intraoperative neuromonitoring (IONM) is an additional and routine method for verifying RLN to be routinely used by endocrine surgeons, helping to avoid damage to the RLN and is often [5-7]. It worth to mention that for an high-experienced endocrine surgeon, the identification of the RLN is a task that is solved in most cases under the condition of primary surgical intervention in the area of the thyroid gland [1, 5]. However, the identification of the RLN is a clinical challenge in case of neck reoperations for recurrence of thyroid cancer, for retrosternal goiters, as well as in the anatomical varieties of the RLN, such as

presence of its additional branches [8]. As mentioned above, it is also important task to identify and preserve parathyroid glands [4, 9]. However, it is also difficult task to identify and verify parathyroid glands in case of reoperations for recurrent thyroid cancer or in cases of hyperparathyroidism as well as in patients with multiglandular parathyroid gland disease or intrathyroidal localization or other anatomical variations [8, 10-12]. The routinely applied preoperative identification of parathyroid adenomas is performed by using scintigraphy with ^{99m}Tc -sestamibi or by computed tomography [10]. The last, but not the list surgical challenge for parathyroid preservation is discussed for the patients with thyroid cancer metastases to the local lymph nodes of central compartment which is difficult to discriminate from the lower parathyroid glands [13].

Given the possible difficulties in identifying RLN and parathyroid glands when these structures were determined by the naked eye and IONM, the use of fluorescence-guided surgical intervention is promising. Fluorescence-guided surgery is a method that involves the use of contrast agents (fluorophores) and fluorophore signal identification devices for intraoperative visualization of anatomical structures and tissue differentiation [14, 15]. A promising option for fluorescence-guided surgery for RLN verification can be the use of indocyanine green (ICG) and determination of its signal in the near infrared spectrum, which can be an additional method of RLN and parathyroid identification and verification [1, 16]. It is worth to mention that, parathyroid gland is exhibited autofluorescence in near infrared spectrum (NIRAF) with wave length of 800-820 nm with a detectable signal in comparison to thyroid tissue [9]. Such a unique feature of parathyroid tissue was also reported for both vascularized and unintentionally excised entities [13]. Available devices for detecting signals in NIRAF (wave length 800-820 nm) can increase the sensitivity and specificity of RLN and parathyroid identification in addition to the their detection by naked-eye or with application of IONM for RLN [1, 17].

The aim of the study.

To evaluate the role of fluorescence-guided surgery using two different imaging systems and ICG to identify the RLN and parathyroid glands during operations for benign and malignant thyroid and parathyroid tumors.

Object and research methods.

The study involved 131 patients who underwent surgical treatment at the Verum Expert Center, which is the clinical base of the Department of Surgery of the NSC «Institute of Biology and Medicine» at Taras Shevchenko National University of Kyiv (Kyiv, Ukraine) during 2019-2022. There were following diagnosis: papillary thyroid carcinoma (PTC) in 91 patients; medullary carcinoma of the thyroid gland in 4 patients; follicular thyroid adenoma in 8 patients; diffuse toxic goiter in 4 patients; toxic thyroid adenoma in 8 patients; parathyroid adenoma (oxyphilic cell) in 16 patients. At the preoperative period, all patients underwent a hormone testing, clinical chemistry blood tests, biochemical tests, and ionized calcium. Ultrasound examination of the thyroid gland and neck compartments were also performed in all patients. Fine needle aspiration biopsy (FNAB) was carried out for the suspicious nodules of the thyroid gland, followed by cytological verification of punctate FNAB according to the classification Bethesda. Calcitonin was determined in

patients with suspected medullary thyroid carcinoma. All operations were performed using the capsule dissection technique. Parathyroid glands were identified with the naked eye, followed by analysis of the operative field using one of the available imaging systems: Fluobeam 800 or Fluobeam LX (Fluoptics, France). Indocyanine green (ICG) was used as a fluorophore, administered intravenously. Angiographic evaluation of ICG signal in the parathyroid glands was carried out 1-2 minutes after intravenous administration. Near infrared autofluorescence (NIRAF) evaluation of the parathyroid glands was carried out by manually pointing the near infrared (NIR) camera over the area of the operative field at a distance of 20 cm. Written informed consent was obtained from the the patients to participate in the study.

Non-parametric statistical methods were used for statistical data processing by applying Mann-Whitney test, Fisher's exact test (two-tailed) by using statistical software GraphPad by Dotmatics. The difference between the studied groups was considered significant with $p < 0.05$.

Research results and their discussion.

Analysis of the clinical data from 131 patients showed that malignant neoplasms predominated in the cohort, which was diagnosed in 95 (73%) patients (91 patients with PTC and 4 patients with medullary thyroid cancer), 36 (27%) patients had benign neoplasms of the thyroid and parathyroid glands. Among the studied cohort, there were 89 (68%) female, 42 (32%) men ($p < 0.05$). The mean age of the patients was 45.5 years (range

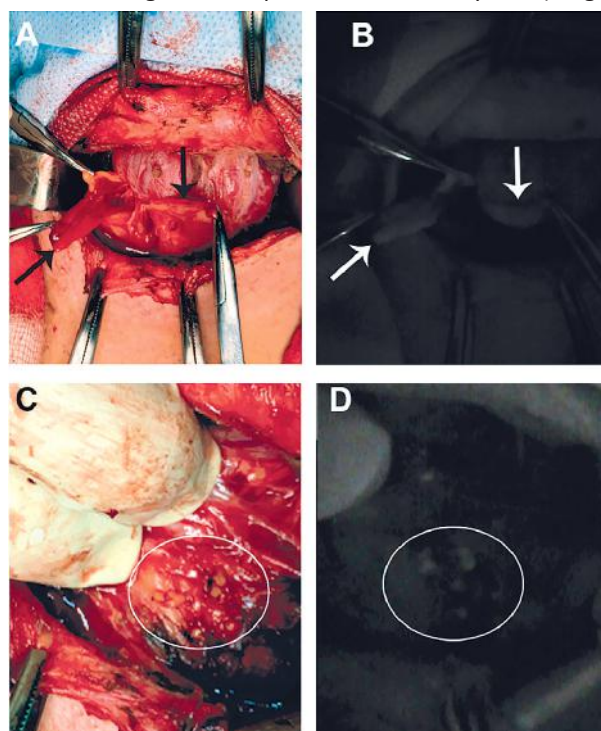


Figure 1 – Intraoperative photograph during removal of parathyroid adenoma in patient with relapse of hyperparathyroidism (repeated surgical intervention for parathyroid adenoma): A – Two parathyroid adenomas of right parathyroid glands (upper and lower glands) indicated with arrows; B – The right parathyroid adenoma showing autofluorescence in near infrared spectrum (indicated with arrow); C -Normal left lower parathyroid gland was identified as multiple fragments of tissue after surgical interventions on the left side (indicated with circle); D – Illustration of autofluorescence signal in near infrared spectrum of fragmented left lower parathyroid gland (marked with circle).

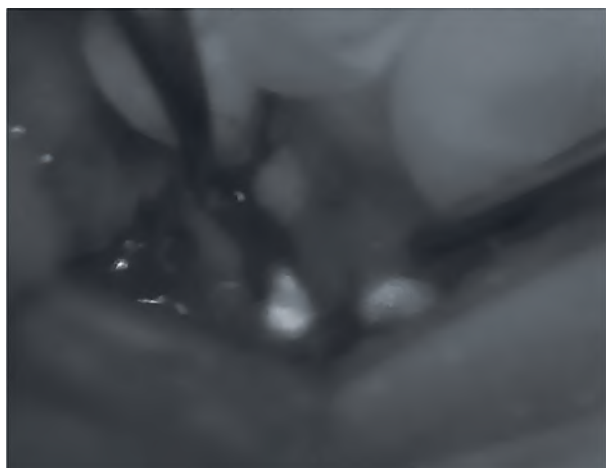


Figure 2 – Intraoperative photograph illustrating two signals in near infrared spectrum. The signal to the left is stronger indication good vascularization of parathyroid gland, whereas signal to the right is weak, indication possible decrease of blood supply to the parathyroid gland. The tissues from the thyroid gland does not exhibit near infrared signal.

20-77 years). Recurrent surgeries were performed in 4 cases due to hyperparathyroidism, and in 2 cases of PTC. Normal parathyroid glands were accurately identified and mobilized under visual control (naked eye) in 117 (89%) cases, which was subsequently confirmed by NIRAF of the parathyroid glands. Distinct identification of the parathyroid glands by visual assessment with the naked eye was unsure in 22 (17%) patients. There was no ambiguity in the visual identification of the lower parathyroid glands in 14 patients due to neck dissection for PTC. In these cases, to verify all the lower parathyroid glands and their differentiation from lymph nodes, they were determined by NIRAF using one of the available imaging systems.

In 4 (3%) patients, parathyroid glands were identified in the area of the previous surgical intervention in the presence of pronounced scarring, followed by NIRAF application using one of the available imaging systems, but not by visual identification with the naked eye (**figure 1**). Identification of parathyroid glands using NIRAF determination was combined with indocyanine green fluorophore administration in 102 (78%) patients. In all these cases, a uniform distribution of the signal in the NIRAF was determined, both in the thyroid gland and in the parathyroid adenoma. However, in 15-20 minutes after the administration of indocyanine green, the tissue of the thyroid gland showed a stronger signal as compared to the parathyroid adenoma. These results indicate that the determination of NIRAF in combination with the administration of the fluorophore indocyanine green is associated with better visualization of the parathyroid tissue, in particular it can help to identify occult intrathyroidal adenomas of the parathyroid gland, which may be located in atypical anatomical areas, but within a limited time period of up to 20 minutes after exposure to the contrast agent (*i.e.* ICG).

It should be noted the presence of a weak signal from normal thyroid tissue, as well as from benign neoplasms of the parathyroid glands and thyroid gland using fluorescence with and without the use of indocyanine green fluorophore contrast. Weaker signal intensity in the NIR spectrum was also detected in 12 cases of parathyroid glands, during operations for benign thyroid tumors, indicating possible devascularization of the parathyroid glands (**figure 2**), which was subsequently confirmed by a lower level of parathyroid hormone (PTH) in the early postoperative period (2 days after the surgery).

It is worth to mention that during the postoperative follow-up of these patients, the level of PTH returned



Figure 3 – Illustration of fluorescent-guided surgery for confirmation of recurrent laryngeal nerve (RLN). At the monitor to the left, a fork-like probe from intraoperative monitoring (IONM) confirms the linear structure (within the circle) as RLN, showing good signal after the injection of fluorophore indocyanine green. The signal from IONM is showed as a distinct curve at the IONM monitor to the right part of the figure.

to normal range in a period of 6 weeks. The use of the fluorophore indocyanine green to improve the identification parathyroid glands in 64 (49%) cases after their mobilization. Before doing fluorescence-guided surgery, all parathyroid glands were identified by naked eye. A more intense NIR signal was determined in the parathyroid glands after contrasting with indocyanine green.

The RLNs were mobilized during all surgical interventions. RLN identification was carried out by the naked eye, and it was subsequently verified using intraoperative neuromonitoring (IONM). It should be noted that when applying NIR camera during the verification of the parathyroid glands, the area of the RLN did not show a signal. But after the injection of contrast ICG, we obtained a clear signal in all 131 (100%) cases from RLN. A linear structure judged to be the recurrent laryngeal nerve confirmed by IONM demonstrated accumulation of indocyanine green (indicating presence of fluorophore perfusion in the vessels of the recurrent laryngeal nerve), and this structure was also verified by IONM during contrast administration (figure 3).

Unexpectedly for us, a more intense signal from the parathyroid glands was noted after changing the angle of inclination to 45-65° of the NIR of the Fluobeam 800 camera in relation to the area of localization of the parathyroid gland in the operative field in all 131 (100%) patients, compared to the location of the NIR camera under at an angle of 90°. When performing such a maneuver, we identified parathyroid glands next to the thyroid gland, as well as parathyroid glands in the

thymus tissue in 13 patients. However, when using the Fluobeam LX device, a good NIR signal was determined in all cases without specific changes in the angle of the NIR camera, which is associated with better confidence in the recognition of the NIRAF signal by the surgical team.

Conclusions.

In summary, the use of NIRAF in parathyroid glands increases their better identification and preservation. The application of NIRAF of the parathyroid glands is considered as a useful, but additional method to their visual evaluation in the case of primary surgical intervention. The practical value of NIRAF visualization of the parathyroid glands increases with repeated surgical interventions due to the displacement of anatomical landmarks, the increased risk of inadvertent removal of the parathyroid glands, and their differentiation from lymph nodes in malignant processes. Indocyanine green contrast does not improve the identification of parathyroid glands. When using different imaging systems, the change in camera angle must be considered. Our study has fully supported the importance of the employment of the apparatus tools, such as IONM for the verification of the RLN, as well ICG angiography and NIRAF for the parathyroid gland confirmation.

Prospects for further research.

Results from this study indicate the importance of further investigations of fluorescence-guided surgery for identification of RLN and parathyroid glands. It is important to establish and evaluate cohorts of patients in other endocrine surgery centers to test our hypothesis and reproducibility of our results.

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РОЛЬ ФЛУОРЕСЦЕНТ-КЕРОВАНОЇ ХІРУРГІЇ ДЛЯ ВЕРИФІКАЦІЇ ПРИЩИТОПОДІБНИХ ЗАЛОЗ ТА ЗВОРОТНИХ ГОРТАНИХ НЕРВІВ В ІНФРАЧЕРВОНОМУ СПЕКТРІ

Дінець А. В., Горобейко М. Б.

Резюме. Вступ. Пошкодження зворотнього гортанного нерва (ЗГН) і прищитоподібних залоз вважаються важкими ускладненнями операцій на щитоподібній та прищитоподібній залозах. Флуоресцент-керована хірургія – це метод, який передбачає використання контрастних речовин і пристроїв для ідентифікації флуорофорного сигналу для інтраопераційної візуалізації анатомічних структур. Наразі актуальним є розробка та впровадження апаратних методик для покращення інтраопераційної ідентифікації та верифікації прищитоподібних залоз та ЗГН.

Мета дослідження – оцінити роль флуоресцент-керованої хірургії з використанням двох різних систем візуалізації та індоціаніну зеленого для ідентифікації ЗГТ і прищитоподібних залоз під час операцій з приводу доброякісних і злоякісних пухлин щитоподібної та прищитоподібної залоз.

Об'єкт і методи дослідження. Для дослідження були ідентифіковані 131 пацієнтів з доброякісними та злоякісними новоутвореннями щитоподібної та прищитоподібної залоз. Всі операції проводили методом капсульної дисекції. Прищитоподібні залози та ЗГТ ідентифікували неозброєним оком з подальшим аналізом операційного поля за допомогою однієї з доступних систем візуалізації для виявлення флуоресцентного сигналу у ближній інфрачервоній ділянці (АФБІЧ). Як флуорофор використовувався індоціаніновий зелений (ICG).

Результати. Ідентифікацію прищитоподібних залоз за допомогою АФБІЧ поєднували з введенням флуорофора індоціаніну зеленого у 102 (78%) пацієнтів. У всіх цих випадках було визначено рівномірний розподіл сигналу в АФБІЧ. ЗГТ не показував сигнал без ICG. Але після введення ICG отримали чіткий сигнал у всіх 131 (100%) випадків від ЗГТ.

Висновки. Флуоресцент-керована хірургія є перспективним і новим підходом для підтвердження як ЗГТ, так і прищитоподібних залоз, який краще застосовувати з таким флуорофором, як ICG. Флуоресцент-керована хірургія розглядається як додатковий інструмент до підтвердження неозброєним оком ЗГТ і прищитоподібних залоз.

Ключові слова: прищитоподібні залози, зворотній гортанний нерв; флуоресцент-керована хірургія; флуоресценція в ближній інфрачервоній ділянці; індоціанін зелений.

THE ROLE OF FLUORESCENCE-GUIDED SURGERY FOR THE VERIFICATION OF PARATHYROID GLANDS AND RECURRENT LARYNGEAL NERVES IN THE NEAR INFRARED SPECTRUM

Dinets A. V., Gorobeiko M. B.

Abstract. *Introduction.* Damage to the recurrent laryngeal nerve (RLN) and parathyroid glands might be a severe complication of thyroid and parathyroid surgeries. Fluorescence-guided surgery is a method that involves the use of contrast agents and fluorophore signal identification devices for intraoperative visualization of anatomical structures. Currently, there is a need to develop and to implement in routine clinical practice new tools for improvement of the intraoperative identification and verification of parathyroid glands and RLN.

Aim. The aim of the study was to evaluate the role of fluorescence-guided surgery using two different imaging systems and indocyanine green to identify the RLN and parathyroid glands during operations for benign and malignant thyroid and parathyroid tumors.

Object and methods. A 131 patients were identified for the study with benign and malignant neoplasms of the thyroid and parathyroid glands. All operations were performed using the capsule dissection technique. Parathyroid glands were identified with the naked eye, followed by analysis of the operative field using one of the available imaging systems to detect near infrared (NIRF) signal. Indocyanine green was used as a fluorophore.

Results. Identification of parathyroid glands by using NIRAF signals was combined with indocyanine green fluorophore intravenous administration in 102 (78%) patients. In all these cases, a uniform distribution of the signal in the NIRAF was determined. RLN did not show a signal without indocyanine green. But after the injection of contrast indocyanine green, a clear signal was obtained in all 131 (100%) cases from RLN.

Conclusions. Fluorescent-guided surgery is promising and novel approach for the confirmation of both RLN and parathyroid gland, which is better to apply with fluorophore such as indocyanine green. Fluorescent-guided surgery is considered as an additional tool to the naked eye conformation of the RLN and parathyroid glands.

Key words: parathyroid glands, recurrent laryngeal nerve; fluorescence-guided surgery; near-infrared fluorescence; indocyanine green.

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