Clinical Application of Near-infrared Autofluorescence Detection of Parathyroid Glands in Thyroid Surgery

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Abstract: The purpose of this paper is to compare and analyze the advantages and disadvantages of near-infrared autofluorescence (NIRAF) imaging, nano-carbon negative imaging and conventional naked eye recognition technology in recognizing parathyroid glands during surgery, and to preliminary evaluate the effectiveness, safety and feasibility of NIRAF imaging in the recognition of parathyroid glands. The clinical trials retrospectively analyzed 36 patients, including 12 patients in Group A (NIRAF imaging group), 12 patients in Group B (nano-carbon negative imaging group), and 12 patients in Group C (conventional naked eye recognition group). The number of parathyroid glands recognized during surgery in Group A was more than that in Group B and C. The time taken to recognize parathyroid glands in Group A was shorter than that in Group B and C. There was no significant difference in the average calcium ion levels of the three groups A, B and C before surgery. After surgery, the average calcium ion level of Group A was not significantly different from that of Group B, but it was higher than that of Group C, and the difference was significant. The incidence of postoperative hypocalcemia in Group A was not significantly different from that in Group B, but it was lower than that in Group C. The incidence of postoperative symptomatic hypocalcemia in Group A was lower than that in Group B and C. Compared with traditional technology, NIRAF can recognize more parathyroid glands during surgery, take less time, and make the probability of postoperative hypocalcemia and symptomatic hypocalcemia lower during thyroid surgery. It needs noninvasive and simple operation. It is a kind of safe, effective and feasible parathyroid gland recognition technology, which is worth further exploration.
Keywords: parathyroid glands, NIRAF, nano-carbon, naked eye recognition

1. Introduction

Thyroid carcinoma is the most common malignant tumor of the endocrine system, accounting for about 3.1% of all malignant tumors [1]. Through effective surgical treatment, the prognosis of thyroid carcinoma is usually good. However, due to the particularity of the anatomy, location and color of the parathyroid glands, the parathyroid glands are easy to be damaged or mistakenly cut during surgery, causing complications such as secondary hypoparathyroidism or hypocalcemia [2]. For example, limb numbness, acmesthesia, muscle cramps, etc. will seriously affect the patient's quality of life. Therefore, the exact protection of the parathyroid glands during surgery is particularly important.

The purpose of this research is to evaluate the feasibility, safety, and effectiveness of NIRAF in recognizing parathyroid glands during surgery, and to initially explore the advantages and disadvantages of NIRAF compared with traditional parathyroid gland recognition technology during surgery.

2. Materials and methods

2.1 Research objects

A total of 36 patients who underwent radical thyroid malignant tumor surgery at the Thyroid and Breast Surgery Department of the First Affiliated Hospital of University of South China from January 2019 to December 2019 were selected and divided into three groups. There were 12 patients in the experimental group (Group A, NIRAF imaging group), 12 patients in the the control group (Group B, nano-carbon negative imaging group), and 12 patients in the control group (Group C, conventional naked eye recognition group). The patients' postoperative results of paraffin pathological examination were all thyroid papillary carcinoma, and the doctors obtained the patients' informed consent before carrying out all operations. (See Table 1 for basic information)

2.2 Parathyroid gland recognition steps

Group A: After fully exposing the thyroid, the area around the thyroid and the thyroid tumor, use a hand-held NIRAF detector to scan the side where the thyroid tumor needs to be removed for about 2 to 3 minutes (Figure 1), and capture the image information. Then use the infrared imaging instrument to receive the signals captured by the detector, and present the parathyroid glands displayed on the infrared imaging instrument (the parathyroid glands appear as strong light spots on the infrared imaging instrument) (Figure 2 and Figure 3).

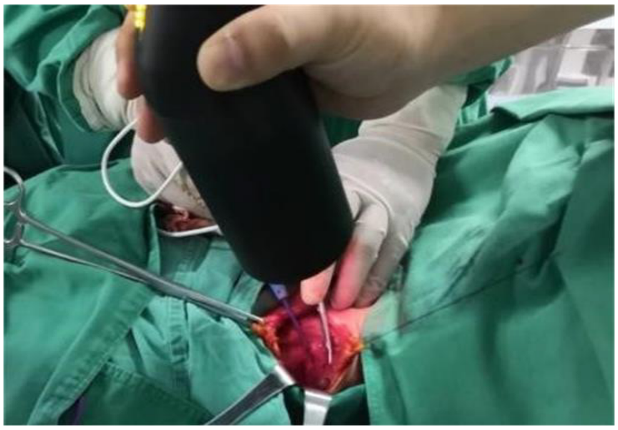

Figure 1. Scan the thyroid with a hand-held NIRAF detector

Figure 2. The superior parathyroid gland recognized by NIRAF

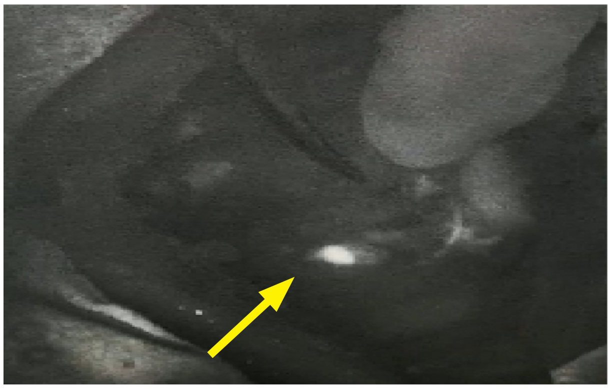

Figure 3. The inferior parathyroid gland recognized by NIRAF

Group B: After full exposure of the thyroid and the thyroid tumor, use a 1 ml skin test syringe to take out an appropriate amount of nano-carbon suspension and then inject 0.1-0.3 ml of nano-carbon suspension around the thyroid tumor tissue. Pay attention to withdrawing the plunger of the syringe before injection to avoid injecting the nano-carbon suspension into the blood vessel. After removing the syringe, use gauze to press the injection point for about 1 minute to avoid the overflow of the nano-carbon suspension, which will pollute the surgical field. After about 5-minute wait, unstained parathyroid gland imaging can be seen (Figure 4).

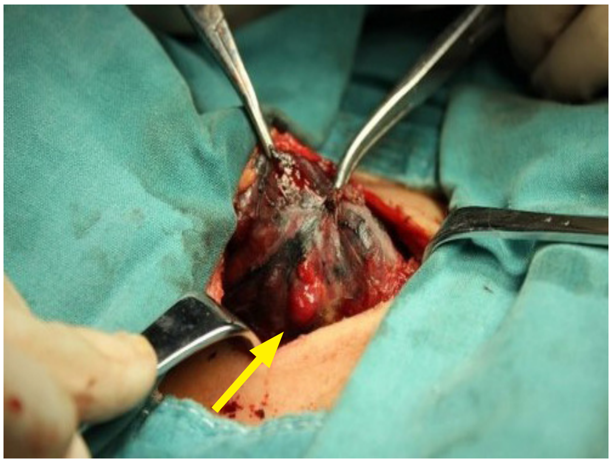

Figure 4. The negative imaging parathyroid gland after using nano-carbon suspension

Group C: After exposing the thyroid and the thyroid tumor, open the back of the thyroid and look for the parathyroid glands. The normal parathyroid glands are flat and elliptical, with a smooth and flat surface and a yellow color. They are generally 3-6 mm long and 1-3 mm thick (Figure 5). When it is not possible to judge whether it is a parathyroid gland, the sinking and floating test can be used to help. That is, cut off the suspicious tissue and put it into normal saline. The parathyroid gland generally sinks (Figure 6), while fat floats up. If it is still impossible to distinguish between parathyroid tissue and other tissues, an intraoperative frozen pathological examination should be performed to assist in the judgment.

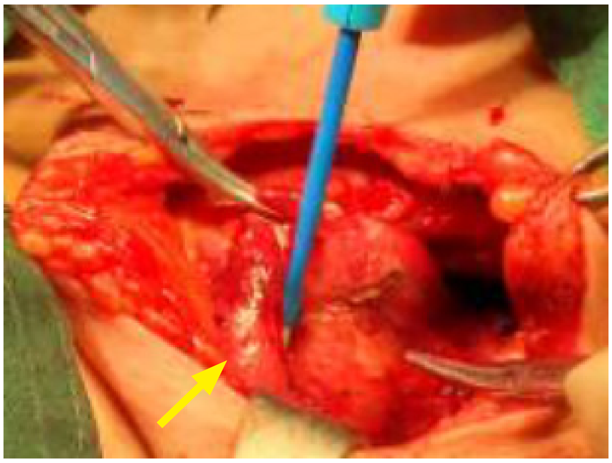

Figure 5. The parathyroid gland conventionally recognized with the naked eye

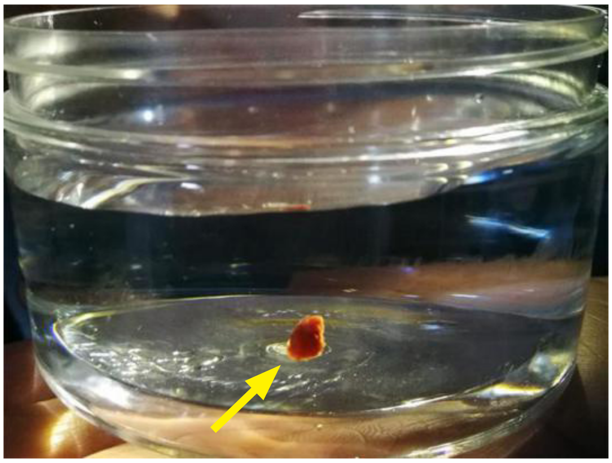

Figure 6. The parathyroid gland recognized by the sinking and floating test

The statistical software used for the collected experimental data is IBM SPSS Statistics 21.0, and the measurement data is expressed by (x ± s). Student's t test is used for comparison between independent groups; paired sample t test is used for comparison between paired groups; one-way ANOVA is used for comparison among multiple groups; LSD method is used for pairwise comparison afterwards. The count data is represented by n (%), and chi-square analysis is used for comparison between groups. According to the test level, $P < 0.05$, suggesting the difference is statistically significant.

3. Results

3.1 Comparison results of the general data of the research objects

A total of 36 patients with thyroid tumors were enrolled in clinical trials. The basic data is shown in Table 1. All patients had no other serious organic diseases and required thyroid surgery. And they voluntarily joined the group. All patients' postoperative results of paraffin pathological examination were thyroid papillary carcinoma. Comparing the gender, age and tumor size of the three groups of patients, the difference is not statistically significant ($P > 0.05$) (Table 1).

Table 1. Comparison of general data of the three groups of patients

	Group A	Group B	Group C	P
Gender（male/female）	2/10	3/9	1/11	0.536
Age（year old）	46.33±13.49	40.25±8.28	44.5±12.33	0.428
Tumor size（cm）	2.71±0.62	2.04±0.69	2.25±0.78	0.073

3.2 Comparison results of the numbers of parathyroid glands recognized during surgery in each group

The average number of parathyroid glands recognized in Group A was 3.33 ± 0.49; the average number of parathyroid glands recognized in Group B was 2.67 ± 0.49; the average number of parathyroid glands recognized in Group C was 2.00 ± 0.6. Through the comparison of the number of parathyroid glands recognized during surgery among the three groups, it was found that the number of parathyroid glands recognized in Group A was the largest. And the difference was significant ($P < 0.05$). (Table 2)

Table 2. Comparison of the numbers of parathyroid glands recognized in the three groups of patients during surgery

	The number	P
Group A	3.33±0.49	
Group B	2.67±0.49a	< 0.001
Group C	2.00±0.6ab	

Note: One-way ANOVA is used for comparison among multiple groups; LSD method is used for comparison between groups. a means $P < 0.05$ compared with Group A; b means $P < 0.05$ compared with Group B.

3.3 Comparison results of time taken to recognize parathyroid glands during surgery in each group

Length of time (minutes): Group A took an average of 4.470.84 minutes; Group B took an average of 10.571.12 minutes; Group C took an average of 9.781.32 minutes. Comparison of three groups: the average time of Group A was the shortest and the difference was statistically significant ($P <0.05$). (Table 3)

Table 3. Comparison of time taken to recognize parathyroid glands during surgery among three groups of patients

	Time (minutes)	P
Group A	4.47±0.84	
Group B	10.57±1.12a	<0.001
Group C	9.78±1.32a	

Note: One-way ANOVA is used for comparison among multiple groups; LSD method is used for comparison between groups. a means $P < 0.05$ compared with Group A.

3.4 Comparison results of patients' calcium ion levels before and after surgery in each group

The average calcium ion level before surgery in Group A was 2.280.17 mmol / L; the average calcium ion level before surgery in Group B was 2.280.10 mmol / L; the average calcium level before surgery in Group C was 2.220.11 mmol / L. The comparison of calcium ion levels before surgery among the three groups was not statistically significant (P > 0.05), which indicated that the calcium ion levels before surgery in the three groups were consistent and not comparable.

The average calcium ion level after surgery in Group A was 2.090.15 mmol / L; the average calcium ion level after surgery in Group B was 2.010.13 mmol / L; the average calcium ion level after surgery in Group C was 1.880.20 mmol / L. The calcium ion levels after surgery in the three groups were lower than those before surgery, and the difference was significant (P < 0.05). The average calcium ion level after surgery in Group A was not significantly different from that in Group B (P > 0.05). The average calcium ion level after surgery in Group A was higher than that in Group C, with a significant difference (P < 0.05). (Table 4)

Table 4. Comparison of calcium ion levels before and after surgery in the three groups of patients

	The calcium ion level		P
	Before surgery	After surgery	
Group A	2.28±0.17	2.09±0.15	0.000
Group B	2.28±0.10	2.01±0.23	0.007
Group C	2.22±0.11	1.88±0.20a	0.002
P	0.479	0.040	

Note: One-way ANOVA is used for comparison among multiple groups; LSD method is used for pairwise comparison. a means P < 0.05 compared with Group A. Paired sample t test is used for comparison before and after surgery.

3.5 Comparison results of the incidence of postoperative hypocalcemia among patients in each group in clinical trials

In Group A, there was 1 patient with postoperative hypocalcemia, and the incidence of postoperative hypocalcemia was 8.3%. In Group B, there were 4 patients with postoperative hypocalcemia, and the incidence of postoperative hypocalcemia was 33.3%. In Group C, there were 7 patients with postoperative hypocalcemia, and the incidence of postoperative hypocalcemia was 58.3%. The incidence of postoperative hypocalcemia in the three groups was statistically different (P < 0.05). The incidence of postoperative hypocalcemia in Group A was lower than that in Group C, and the difference was significant (P < 0.05). The incidence of postoperative hypocalcemia in Group A was not significantly different from that in Group B (P > 0.05). (Table 5)

Table 5. Comparison of the incidence of postoperative hypocalcemia among the three groups of patients

	Postoperative hypocalcemia		P
	Yes	No	
Group A	1（8.3）	11（91.7）	
Group B	4（33.3）	8（66.7）	0.025
Group C	7（58.3）	5（41.7）	

Note: Chi-square test is used.

3.6 Comparison results of the incidence of postoperative symptomatic hypocalcemia among patients in each group

There were 1 patient with symptomatic hypocalcemia after surgery in Group A, with an incidence of 8.3%; 4 patients with symptomatic hypocalcemia after surgery in Group B, with an incidence of 33.3%; 4 patients with symptomatic hypocalcemia after surgery in Group C, with an incidence of 33.3%. The difference among the three groups was not significant (P > 0.05), but the incidence of postoperative symptomatic hypocalcemia in Group A was slightly lower. (Table 6)

Table 6. Comparison of the incidence of postoperative symptomatic hypocalcemia among the three groups of patients

	Postoperative symptomatic hypocalcemia		P
	Yes	No	
Group A	1 (8.3)	11 (91.7)	
Group B	4 (33.3)	8 (66.7)	0.217
Group C	4 (33.3)	8 (66.7)	

Note: Chi-square test is used.

4. Discussion

Hypoparathyroidism is one of the common complications after total thyroidectomy. It mainly manifests as perioral numbness and finger (toe) end numbness. In severe cases, hand and foot convulsions can occur, or larynx and diaphragm spasm can be caused, resulting in asphyxia and even death [3]. At present, the main prevention method of the disease is to carefully dissect the posterior capsule of the thyroid and carefully protect the parathyroid glands.

It was the Das team [4] who first proposed that the parathyroid gland has autofluorescence. They used a 830 nm near-infrared laser to excite the parathyroid gland to cause it to produce fluorescence in 2006. It was the Para team [5] who further confirmed this phenomenon, and their research showed that the parathyroid gland has autofluorescence properties in the near infrared region. For example, the Ladurner team [6] used NIRAF to recognize a total of 35 parathyroid glands in 25 patients and recognized 27 parathyroid glands. And they found that there was no significant difference in fluorescence intensity among parathyroid adenoma, parathyroid hyperplasia or normal parathyroid gland, so they proposed the autofluorescence of parathyroid tissue in near-infrared spectroscopy could be used to distinguish the parathyroid gland from other tissues. Another example is the research of the Kahramangil team [7], which shows whether applying indocyanine green (ICG) contrast agent does not affect the intraoperative imaging of the parathyroid gland. NIRAF was approved for clinical application by the US Food and Drug Administration (FDA) as a new kind of parathyroid gland recognition technology on November 2, 2018.

In this study, the clinical data of patients were analyzed retrospectively, and the preliminary clinical applications of NIRAF, nano-carbon negative imaging and conventional naked eye recognition technology were compared from the aspects of time taken to recognize parathyroid glands during surgery, the number of parathyroid glands that can be recognized during surgery and the incidence of postoperative hypocalcemia. And the following conclusions were drawn.

(1) NIRAF makes the recognition of parathyroid glands take less time during surgery. The application of near-infrared autofluorescence imaging technology does not require the injection of exogenous contrast agents or lymphatic tracers. It can shorten the time of drug injection and waiting for drug diffusion.

(2) NIRAF increases the number of parathyroid glands recognized during surgery. The number of parathyroid glands recognized in Group A is larger than that in the other two groups. Similarly, the research of the Falco [8] team also showed that near-infrared autofluorescence imaging technology can display parathyroid glands as strong fluorescent spots during surgery. The number of parathyroid glands recognized by NIRAF is significantly larger than that recognized with the naked eye, and the fluorescence intensity of parathyroid glands is not affected by age, gender, and histopathology.

(3) NIRAF reduces the incidence of postoperative hypocalcemia. Similarly, Dip's [9] research also shows that NIRAF can reduce the incidence of postoperative hypocalcemia, and improve the recognition rate of parathyroid glands.

In summary, NIRAF imaging technology has many advantages. It shortens the time taken to recognize parathyroid glands during surgery, and it can help recognize more parathyroid glands. It reduces the incidence of postoperative hypocalcemia, and the operation is simple and relatively noninvasive. Although NIRAF has been gradually improved and applied to the clinic, the mechanism of parathyroid gland autofluorescence is still unclear. And there will be some factors that affect the fluorescence intensity of parathyroid glands, such as preoperative calcium level and vitamin D level and so on. These influencing factors indicate the potential clinical application value of optical guidance in the detection of parathyroid glands [10]. This also gives us an opportunity for further research, and its application prospects will be broad.

5. Acknowledgments

This article belongs to the Research Project for Young and Middle-aged Thyroid Doctors in 2019 — Intraoperative parathyroid recognition: comparison of near-infrared autofluorescence imaging with other techniques (supported by China Health Promotion Foundation 2019018).

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