Gauze blotting technique: a novel method to identify parathyroid glands during thyroid surgery without tissue damage

Masatoshi Yamamoto1, Naoyoshi Onoda1, Akira Miyauchi1, Makoto Fujishima1, Toshitetsu Hayashi2, and Mitsuyoshi Hirokawa2

1) Department of Surgery, Kuma Hospital, Kobe 650-0011, Japan
2) Diagnostic Pathology, Kuma Hospital, Kobe 650-0011, Japan

Abstract. Hypoparathyroidism is a major complication of thyroid surgery. To avoid this complication, visual identification of the parathyroid glands is essential. However, its effectiveness depends heavily on the surgeon’s expertise. Here, we describe a novel method, the gauze blotting technique, to immunochemically identify the parathyroid glands during thyroid surgery. Twenty-three patients who underwent thyroid lobectomy were enrolled in this study; 16 and 7 had benign and malignant thyroid diseases, respectively. After visually identifying candidate nodules for the parathyroid gland, a piece of dry gauze (5 mm × 10 mm) was applied to each tissue until it was moistened by exudates from the tissue. Pieces of gauze were also applied to the thyroid gland and adipose tissue located away from the candidate nodules. The gauze was immersed in saline, and the intact PTH (i-PTH) level of the supernatant was measured. The median PTH level for the parathyroid glands was 1,060 pg/mL, which was significantly higher than that for the thyroid gland (34 pg/mL) and adipose tissue (28 pg/mL) (p < 0.001). The cut-off value to distinguish the parathyroid gland from other tissues was 68 pg/mL with a positive predictive value, negative predictive value, sensitivity, and specificity of 84.6%, 88.8%, 86.8%, and 86.7%, respectively. A value ≥250 pg/mL yielded a 100% positive predictive value. Our novel gauze blotting technique can identify the parathyroid glands without damaging tissues during thyroid surgery.

Key words: Thyroid surgery, Parathyroid gland, Intraoperative identification, Gauze blotting

HYPOPARATHYROIDISM is a major complication of total thyroidectomy. The incidence of transient and permanent hypoparathyroidism is 25.4–83% [1] and 0.12–4.6% [2], respectively, depending on diagnostic criteria. Symptomatic postoperative hypoparathyroidism occurs in 20–30% of patients after total thyroidectomy and remains permanent in 1–5% of these patients [3, 4], who thus require lifetime treatment of supplemental calcium and/or active vitamin D to avoid associated symptoms, complications, and comorbidities [5, 6].

To avoid permanent dysfunction of the parathyroid glands, in situ preservation of the healthy glands or simultaneous autotransplantation of the parathyroid tissue is required. In clinical practice, visual evaluation by the surgeon is the most common method for this purpose. Most parathyroid glands are located in anatomically consistent areas. They are surrounded by thin fibrous membranous structures and are generally distinguished visually from the surrounding adipose tissue, thyroid tissue, or lymph nodes as tiny brownish nodules. Therefore, most parathyroid glands can be spared by careful observation and precise surgical techniques. However, the success of in situ preservation and reliable autotransplantation virtually depends on the surgeon’s expertise.

Several useful methods to help identify and confirm the parathyroid glands intraoperatively are available: (1) histopathological examination of biopsied specimens, (2) rapid PTH measurement of immersion fluid of tissue fragments or needle aspirate [7], and (3) in situ visualization techniques using fluorescent light [8-10]. In addition, Kikumori et al. [11] and Fujishima et al. [12] recently reported that an AST/LDH ratio >0.2 in the immersion fluid of a tissue fragment indicated parathyroid tissue. A recent report demonstrated an immunochromatographic test strip method to detect PTH in...
tissue [13]. However, all except for the fluorescence light technique require some form of invasive procedure, such as biopsy or puncture. A fluorescence light technique could be useful, but it requires special equipment, can only identify the parathyroid gland with its blood supply intact, and the mechanism for the autofluorescence remains to be clarified [9, 10].

Based on our recent incidental experience with extremely high levels of PTH found in the fluid surrounding a preserved parathyroid tissue, we developed a novel method to identify parathyroid glands in situ. In the present study, we devised a novel method, the gauze blotting method, to immunochemically confirm the parathyroid gland by measuring intact PTH (i-PTH) levels in the exudate from the target tissue without causing any damage to it.

**Patients and Methods**

**Patients**

In this study, we enrolled 23 patients who underwent thyroid lobectomy between December 2020 and February 2021. Those aged >20 years with normal calcium levels preoperatively, sufficient liver and renal function, without a previous history of neck surgery, massive cancerous invasion to adjacent organ(s), or distant metastasis of thyroid cancer were included. Patients who underwent total thyroidectomy were excluded because of the possibility of postoperative hypoparathyroidism due to the tissue confirmation procedure.

**Methods**

Surgery was performed under general anesthesia. A patient was placed in the thyroid position, and a neck collar incision was made. The strap muscles were dissected at the midline to expose the thyroid gland. The affected lobe of the thyroid was then mobilized so that the upper and lower parathyroid glands could be observed. After visually identifying the candidate nodules for the parathyroid gland by one of two surgeons (MY, NO), a small dry non-woven gauze piece measuring 5 mm × 10 mm was applied to the surface of the tissue for approximately 30 s to allow it to absorb exudate from the tissue until it got moistened sufficiently (gauze blotting technique). The moistened gauze was immersed immediately in a sample tube containing 1 mL of saline, and the i-PTH level in the supernatant was measured by electrochemiluminescence immunoassay method (Eclusys PTH, Roche Diagnostics, Tokyo) using a standard automatic analyzer (Cobas 8000, Roche Diagnostics). The above method was also applied to thyroid and adipose tissues located at a distance from the parathyroid tissue for comparison.

Each of the corresponding candidate nodules for the parathyroid gland was biopsied following completion of the lobectomy. The edge of the nodule, which appeared to be the parathyroid gland, was pinched, and a piece of tissue measuring 2–3 mm from the edge was sampled. Hematoxylin and eosin-stained specimens were prepared after fixation in a buffered formalin solution. Histopathological confirmation of the parathyroid gland was performed by two pathologists (TH and MH) who were not informed of the PTH measurement results. Only gauze blot immersions from tissues that were histopathologically confirmed as parathyroid tissue were included in the present analysis.

**Informed consent**

Informed consent was obtained from all individual participants included in the study.

**Ethics approval**

The study was approved by the Institutional Ethics Committee (#20201210-2).

**Statistics**

Numerical values are presented as range, median, and mean ± standard deviation. The Mann-Whitney U-test was used to compare the differences in values between the two groups. We confirmed the precision using the receiver operating characteristic (ROC) area under the curve (AUC). We used Stat Flex ver. 6.0. (Artec Co. Ltd., Osaka) software for analyses, and the results were considered statistically significant when \( p < 0.05 \).

**Results**

Among the expected 46 parathyroid glands, six were not identified during the operation. Two more glands that were not confirmed pathologically were excluded from the study. Thirty-eight glands from 23 patients (8 males, 15 females; mean age, 55.2 ± 12.5 years; age range, 26 to 77 years) were evaluated (Fig. 1). Sixteen patients underwent surgery for benign diseases: 7 right lobectomies and 9 left lobectomies. Seven other patients had papillary carcinomas and underwent right (four patients) and left lobectomies (three patients) with ipsilateral central node dissections (Table 1). No intra- or post-operative complications such as recurrent laryngeal nerve palsy, hemorrhage, or hypoparathyroidism occurred. None of the patients required vitamin D or calcium supplementation postoperatively.

PTH levels of the exudates from the adipose tissues (F group) and the thyroid gland (T group) ranged from 13 to 246 (median, 28; mean, 62.8 ± 66.6) and 13 to 228 (median, 34; mean, 44.6 ± 48.3) pg/mL, respectively. No
Significant difference was observed between the PTH levels of the F and T groups. The PTH levels of the exudates from the parathyroid glands (P group) ranged from 15 to >5,000 pg/mL (median, 1060; mean, 1,943 ± 1,972) pg/mL. PTH levels of the P group were significantly higher than those of both groups (p < 0.001 for each comparison) (Fig. 2). The statistically optimal cut-off value for the PTH level was 68 pg/mL using the ROC analysis (Fig. 3a). Using this cut-off value, the positive predictive value, negative predictive value, sensitivity, and specificity were 84.6%, 88.8%, 86.8%, and 86.7%, respectively.

Five specimens in the P group demonstrated PTH levels below the cut-off value (false negative). Contrastingly, two and four samples in F and T groups, respectively, demonstrated PTH levels higher than 68 pg/mL (false positive). We thought these false-positive results might have been caused by contamination of the exudate from the parathyroid gland. Samples from the F and T groups were collected before thyroid mobilization in the last two patients. The PTH level was as low as 12–68 pg/mL in these two patients. Coincidentally, this result shows that 68 pg/mL was the ideal cutoff value.

Among all 84 specimens obtained in the present study, irrespective of the sites sampled, all 28 specimens demonstrating PTH levels >250 pg/mL were obtained from the site of the parathyroid gland (Fig. 3b).

### Discussion

Here, we verified our novel method for concise confirmation of the parathyroid glands in situ without damaging the tissue during surgery. Using this gauze blotting technique to measure the PTH level of the exudate from the target tissue, we were able to identify the parathyroid gland with high sensitivity and specificity.

The results clearly demonstrated that the exudate on the parathyroid gland contained considerably high levels of PTH, which could be measured by the assay commonly used in clinical practice. Moreover, exudation occurred without causing any damage to the parathyroid gland. We also could distinguish the parathyroid gland from adjacent adipose tissues or thyroid gland with a high probability by measuring the level of PTH in the exudate of the target area.

Several specimens showed higher PTH values than the cut-off value in the F and T groups, which were to serve as negative controls. Because the absolute values were not very high, the phenomenon may be due to contamination from exudate of the parathyroid gland on the surface of the surgical gloves or instruments. Furthermore,
when the parathyroid glands were exposed to a negative control, PTH levels did not exceed 68 pg/mL. In this study, the highest PTH level in the thyroid and adipose tissues was 246 pg/mL. All samples showing PTH levels >250 pg/mL were obtained from parathyroid sites. Thus, when clinically applying the gauze blotting technique, we recommend a practical cut-off value of >250 pg/mL for the accurate identification of the parathyroid.

In contrast, some of the specimens from the P group showed PTH levels lower than the cut-off value possibly because the tissue surface was dry. Therefore, the gauze applied was not sufficiently moistened, which may have resulted in inadequate exudate sampling. It is also possible that PTH was not actually exuding from the tissue surface of the parathyroid gland due to thick surrounding fatty connective tissue. These are important points to consider for accurate sampling. In addition, samples for pathological examination to confirm the parathyroid gland were collected after the completion of thyroidec- tomy. Therefore, the site where the tissue was taken may have been different from the site where the gauze was applied.

The limitations of this study include the following. The results cannot be obtained intraoperatively in the absence of facilities for rapid PTH measurement. The identified tissue can be confirmed to be a parathyroid gland by this technique, but its functional status cannot be confirmed. Gauze fragments must be carefully handled so that they are not left behind in the surgical field. Furthermore, gauze blotting cannot be used unless the tissue is exposed.

In conclusion, our novel gauze blotting technique will be beneficial to patients who undergo thyroid surgery because accidental removal of the parathyroids can be avoided and reliable autotransplantation of parathyroid tissue can be performed.

Acknowledgements

This study was not funded by any grant.

Disclosure

The authors declare that they have no potential conflicts of interest associated with this research.

References

1. Page C, Strunski V (2007) Parathyroid risk in total thyroidec- tomy for bilateral, benign, multinodular goitre: report of 351 surgical cases. J Laryngol Otol 121: 237–241.
2. Asari R, Passler C, Kaczirek K, Schruba C, Niederle B (2008) Hypoparathyroidism after total thyroidec- tomy: a prospective study. Arch Surg 143: 132–137; discussion 138.
3. Duclos A, Peix JL, Colin C, Kraimps JL, Menegaux F, et al. (2012) Influence of experience on performance of individual surgeons in thyroid surgery: prospective cross sectional multicentre study. BMJ 344: d8041.
4. Coimbra C, Monteiro F, Olivira P, Ribeiro L, de Almeida
MG, et al. (2017) Hypoparathyroidism following thyroidectomy: predictive factors. *Acta Otorrinolaringol Esp* 68: 106–111.

5. Clarke BL, Brown EM, Collins MT, Jupner H, Lakatos P, et al. (2016) Epidemiology and diagnosis of hypoparathyroidism. *J Clin Endocrinol Metab* 101: 2284–2299.

6. Shaha AR, Burnett C, Jaffe BM (1991) Parathyroid autotransplantation during thyroid surgery. *J Surg Oncol* 46: 21–24.

7. Pelizzo MR, Losi A, Boschin IM, Toniato A, Pennelli G, et al. (2010) Rapid intraoperative parathyroid hormone assay in fine needle aspiration for differential diagnosis in thyroid and parathyroid surgery. *Clin Chem Lab Med* 48: 1313–1317.

8. McWade MA, Sanders ME, Broome JT, Solorzano CC, Mahadvan-Jansen A (2016) Establishing the clinical utility of autofluorescence spectroscopy for parathyroid detection. *Surgery* 159: 193–202.

9. Fortuny JV, Sadowski SM, Belfontali V, Guigard S, Poncet, A, et al. (2018) Randomized clinical trial of intraoperative parathyroid gland angiography with indocyanine green fluorescence predicting parathyroid function after thyroid surgery. *Br J Surg* 105: 350–357.

10. Benmiloud F, Godiris-Petit G, Gras R, Gillot JC, Turrin N, et al. (2020) Association of autofluorescence-based detection of the parathyroid glands during total thyroidectomy with postoperative hypocalcemia risk: results of the PARAFLUO multicenter randomized clinical trial. *JAMA Surg* 155: 106–112.

11. Kikumori T, Ichikawa T, Inaishi T, Miyajima N, Shibata M, et al. (2020) Measurement of the AST to LD ratio in parathyroid tissue suspension can precisely differentiate a hyperfunctioning parathyroid. *J Clin Endocrinol Metab* 105: dgaa264.

12. Fujishima M, Miyauchi A, Ito Y, Kudo T, Noda T, et al. (2021) Evaluation of the diagnostic utility of the aminotransferase/lactate dehydrogenase ratio for the suspension of tissue specimens during thyroid surgery for the identification of parathyroid tissue. *Endocr J* 68: 1303–1308.

13. Xia W, Zhang J, Shen W, Zhu Z, Yang Z, et al. (2020) A rapid intraoperative parathyroid hormone assay based on the immune colloidal gold technique for parathyroid identification in thyroid surgery. *Front Endocrinol (Lausanne)* 11: 594745.