# Applicability of rapid intraoperative parathyroid hormone assay through fine needle aspiration to identify parathyroid tissue in thyroid surgery

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**Abstract.** Hypoparathyroidism is a frequent and serious complication of thyroid surgery. Identification and preservation of the parathyroid glands are key factors in managing hypoparathyroidism. The aim of the present study was to investigate the efficacy of rapid intraoperative parathyroid hormone (rIO-PTH) assay levels through fine needle aspiration (FNA) in identifying parathyroids as a parameter in thyroid surgery. rIO-PTH assay through FNA and frozen section examination were performed on 194 suspected parathyroids anatomical structures from 50 consecutive patients undergoing thyroidectomy (rIO-PTH group). The association between the rIO-PTH values and histological results were analyzed. Clinical effects were compared between the rIO-PTH and control groups from 50 patients undergoing a similar standard surgery. rIO-PTH levels from 93/194 aspirated anatomical structures certified as parathyroid tissues by histological analysis were demonstrated to have a mean of 3,369 pg/ml (range, 145.2-5,000 pg/ml). These values were significantly increased compared with the mean value of 25.7 pg/ml from non-parathyroids tissues significantly ($P<0.001$). The mean number of 3.76 on the recognized parathyroids was obtained by naked eye measurements combined with rIO-PTH assay through FNA, was significantly higher than compared with only naked eye measurements ($P<0.05$). Postoperative permanent or transient hypoparathyroidism was not detected in the rIO-PTH groups. The difference between the postoperative serum calcium level and blood PTH values of rIO-PTH and control groups was not statistically significant ($P>0.05$). The value of rIO-PTH assay through FNA demonstrated that it is a good parameter for differentiating parathyroids and non-parathyroids tissues. The technique is a highly reliable, quick, simple and non-invasive method with a short learning curve in thyroid surgery, which is particularly valuable for inexperienced surgeons. This method may replace frozen section examination, which relies on a surgeon's personal experience on the basis of topographic or morphologic criteria for recognizing parathyroids.

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*Abbreviations:* rIO-PTH, rapid intraoperative parathyroid hormone; FNA, fine needle aspiration; PTH, parathyroid hormone

*Key words:* rapid intraoperative parathyroid hormone, parathyroid, thyroid, hypocalcaemia, hypoparathyroidism, thyroidectomy

## Introduction

Hypoparathyroidism is a frequent and serious complication of thyroid surgery. The reported incidence of postoperative hypoparathyroidism ranges from 1.7 to 68% (1-3), and clinically, mild to severe symptoms of hypocalcemia are observed. Hypocalcemia may lead to significant morbidity and impaired quality of life. Hypocalcemia post-thyroidectomy has been attributed to the extent of disease, previous thyroid operations, and other factors, but more notably, to insufficient understanding of parathyroid anatomy (4), particularly in inexperienced surgeons examining parathyroid identification (5).

Currently, parathyroid recognition depends primarily on a surgeon's personal experience and their interpretation of the morphological and topographical criteria (6). Routine frozen section examination of a parathyroid biopsy is performed to confirm the presence of parathyroid tissue and to provide pathological tissue confirmation for potential parathyroid autotransplantation (7). To date, there has been no more reliable, simpler, minimally invasive approach or technique proposed to replace frozen section examination and a surgeon's personal experience.

Parathyroid hormone (PTH) is secreted by the chief cells of the parathyroid glands to balance blood calcium. Recent reports have stated that rapid intraoperative parathyroid hormone (rIO-PTH) levels from tissue fine needle aspiration (FNA) may predict the presence of parathyroid tissue during parathyroidectomies for primary or secondary hyperparathyroidism (8-12) as a highly effective method to differentiate parathyroid and nonparathyroid tissue during parathyroidectomy (13).

rIO-PTH assay of suspected parathyroid tissue was performed using FNA to identify the parathyroids during thyroidectomy. The sensitivity and specificity of this technique was investigated to recognize and protect the parathyroids with the aim of elucidating whether this technique may replace frozen section analysis in patients undergoing thyroid surgery.

## Materials and methods

*Patients*. A total of 100 consecutive patients undergoing thyroid surgery in the Department of Thyroid Surgery, China-Japan Union Hospital of Jilin University (Changchun, China) between October 2012 and February 2013 were enrolled in the present study. Patients were randomly divided into two groups, an rIO-PTH group and a control group, with 50 cases in each group. All patients underwent the same surgical protocols and level. rIO-PTH was performed using FNA and histological examination on 194 anatomical structures from the rIO-PTH group and the relationship between the rIO-PTH values and histological results was analyzed. FNA and histological examination was not performed on all of the normal parathyroids recognized by visualization intraoperatively, in the present study. Only those anatomical structures that were suspected as parathyroid tissue were sampled. The present study was approved by the Ethics Committee of the China-Japan Union Hospital of Jilin University and written informed consent was obtained from all patients who participated in the study.

*Intraoperative FNA and surgical management*. A 26-gauge needle and a 1-ml syringe containing 0.2 ml saline solution were used to sample the specimens for rIO-PTH analysis. A total of 3-5 aspirations were performed with each puncture of the suspect tissue while maintaining appropriate negative pressure (Fig. 1A and B). The sample was diluted with 1 ml saline solution and immediately transferred to the waiting laboratory. rIO-PTH levels were analyzed using a solid-phase chemiluminescent immunometric assay (Roche Diagnostics GmbH, Mannheim, Germany). rIO-PTH concentrations were available within 15 min of receiving the sample.

To analyze the sensitivity and specificity of rIO-PTH via FNA to identify parathyroids compared with pathological diagnosis, frozen section examinations of the aspirated tissue were performed to confirm the histological source. The smallest possible tissue sample was collected for the frozen section examinations and results were available within 30 min.

Patients with benign lesions underwent subtotal or total thyroidectomy. Patients with papillary thyroid carcinoma lesions confined to a single lobe underwent lobectomy plus isthmectomy with ipsilateral central cervical lymph node dissection. If benign nodules were found in the contralateral lobe, ipsilateral lobectomy plus contralateral subtotal lobectomy with ipsilateral or bilateral central cervical lymph node dissection was performed. If papillary thyroid carcinoma lesions were found in both lobes, total thyroidectomy with bilateral central cervical lymph node dissection was performed. The above procedures involved the identification and preservation of the parathyroids.

*Statistical analysis*. Analyses were performed using the $\chi^2$, Student's t-test, or Mann-Whitney tests, as appropriate. P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed with SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA).

## Results

*Clinical characteristics*. The mean age of the rIO-PTH group was 42.5 years (range, 16-56 years); 14 were male and 36 female (ratio, 1:2.6). Subtotal or total thyroidectomy was performed in 19 patients with benign lesions, whereas 14/31 patients with papillary thyroid carcinoma lesions underwent total thyroidectomy, lobectomy plus contralateral subtotal lobectomy, or isthmectomy (n=17). The age of control group participants ranged from 20 to 62 years with a mean of 42.4 years; 13 were male and 37 female (ratio, 1:2.8). A total of 21 patients with benign lesions underwent subtotal or total thyroidectomy. Of 29 patients with papillary thyroid carcinoma lesions, total thyroidectomy was performed in 11 patients, and lobectomy plus contralateral subtotal lobectomy, or isthmectomy was performed in 18 patients. No differences in clinical characteristics were detected between the two groups.

In the rIO-PTH group, 93/194 aspirated anatomical structures were confirmed as parathyroid tissue, histologically. A total of 45 of the suspected parathyroid tissue samples were confirmed to be normal lymph nodes, with only four metastatic lymph nodes, out of a total of 49. The other non-parathyroid tissues included 13 thyroid tissue and 39 adipose tissue samples.

*rIO-PTH values in parathyroid and non-parathyroid tissue*. A total of 194 tissue samples from the rIO-PTH group were subdivided into two groups according to the pathological diagnosis: 93 parathyroid tissues and 101 non-parathyroid tissues. A mean rIO-PTH value of 3,369 pg/ml (range, 145.2-5000 pg/ml) was obtained from the aspirated confirmed parathyroids. rIO-PTH values from the non-parathyroid tissues ranged from 4.05 to 39.5 pg/ml, with a mean of 25.7 pg/ml. Using a box plot distribution, it was demonstrated that the rIO-PTH values obtained from the parathyroid tissues did not overlap with the rIO-PTH values obtained from non-parathyroid tissues (P<0.0001; Fig. 2). The present results showed that the rIO-PTH median was significantly higher in parathyroid tissue compared with non-parathyroid tissue.

*Parathyroid glands identified and postoperative effects in the rIO-PTH vs. control group*. A total of 3.76 parathyroid glands were correctly identified based on visualization and rIO-PTH assay through FNA in the rIO-PTH group, compared with 2.41 parathyroid glands in the controls detected by visualization alone. Mann-Whitney testing confirmed a significant difference between the two groups (P<0.05; Table I). The difference between the postoperative serum calcium level and blood PTH values in the two groups was not statistically significant (P>0.05; Table I).

No patient in either group experienced postoperative permanent hypoparathyroidism or other serious complications of thyroid surgery. The frequencies of transient hypocalcemia were 0% (0/50) and 12% (6/50), in the rIO-PTH and control groups, respectively (P<0.05; Table II). Patients with transient hypocalcemia complained of only minor symptoms, with

Table I. Clinical effects of rIO-PTH treatment.

| Parameter | Group (n=50 each) | | P-value |
|---|---|---|---|
| | rIO-PTH | Control | |
| Parathyroid glands (n) | 188 | 122 | <0.05 |
| Serum calcium (mmol/l) | 2.25 | 2.21 | >0.05 |
| Blood PTH levels (ng/ml) | 48.3 | 39.5 | >0.05 |

rIO-PTH, rapid intraoperative parathyroid hormone.

Table II. Incidence of postoperative transient hypocalcaemia in rIO-PTH.

| Group | Cases, n | Postoperative transient hypocalcaemia | | $\chi^2$ | P-value |
|---|---|---|---|---|---|
| | | Yes, n (%) | No, n (%) | | |
| rIO-PTH | 50 | 0 (0) | 50 (100) | 4.43 | P<0.05 |
| Control | 50 | 6 (12) | 44 (88) | | |

rIO-PTH, rapid intraoperative parathyroid hormone.

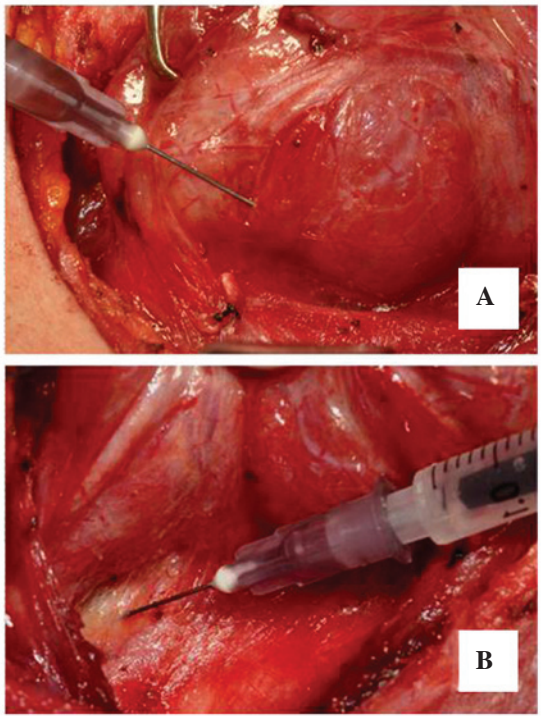


Figure 1. (A and B) Two examples of a rapid intraoperative parathyroid hormone assay with fine needle aspiration fine needle aspiration of suspected parathyroid tissue to identify the parathyroids during thyroidectomy.

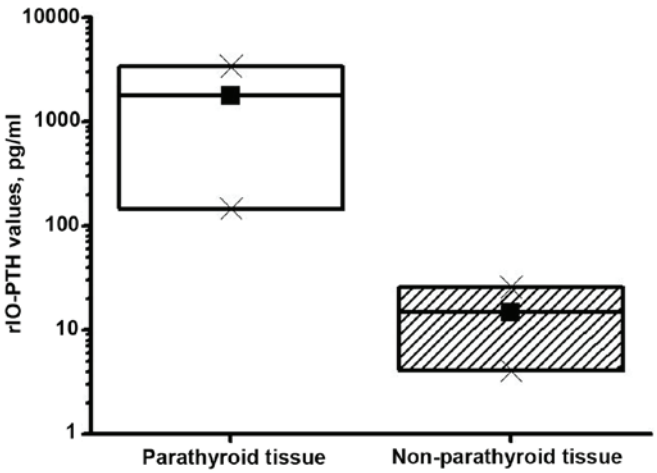


Figure 2. Box plot distribution of the rIO-PTH values obtained from parathyroid and non-parathyroid tissue. rIO-PTH, rapid intraoperative parathyroid hormone.

serum calcium levels returning to normal values <6 months after surgery.

## Discussion

Hypoparathyroidism is one of the most common complications of thyroidectomy and serious symptoms of hypocalcemia include paresthesia, numbness, or muscle cramps, which may be permanent lasting >6 months after surgery (1). Hypocalcemia may also lead to significant morbidity and impaired quality of life. Postoperative transient hypoparathyroidism (resolved <6 months after surgery) can be caused by temporary poor parathyroid blood supply, whereas permanent hypoparathyroidism may result from ischemic necrosis of the parathyroids or inadvertent removal. To reduce morbidity associated with postoperative hypoparathyroidism, surgeons strive for parathyroid protection, thus effective and improved methods of parathyroid identification are critical.

Currently, parathyroid identification relies on a surgeon's personal experience to examine the tissue based on morphological and topographical criteria (6). Therefore, inexperience of the surgeon is a significant risk factor for permanent hypoparathyroidism. A review by Paek *et al* (5) reported that permanent hypoparathyroidism occurred in 6.5% of patients treated by surgeons in the first two years of practice, decreasing to 1.8% in the second two years. Therefore, surgeons continually search for accurate, reliable, and simple methods for distinguishing parathyroid from non-parathyroid tissue. Several experimental techniques have been used previously to identify parathyroid tissue, including carbon nanoparticle suspension negative imaging (14), visible red fluorescence using aminolevulinic acid (15), visible staining by methylene blue (16) or antiparathyroid antibody BB5-G1 conjugated to Cibacron® blue (17), and gamma probe identification (18). Although these techniques may greatly benefit patients, they are predominantly experimental or controversial and the clinical applications remain limited. Frozen section examination can be used to accurately and definitively distinguish parathyroid from non-parathyroid tissue; however, histopathological examination may prolong the operative time and increase the invasiveness of surgery (19).

The intraoperative use of serum PTH assays to determine the satisfactory removal of all hyperfunctional parathyroid tissue was first reported in 1988 (20) and serum PTH is now recognized as the gold standard in the diagnosis of hyperfunctional parathyroid tissue. PTH reflects the function of the parathyroids and rIO-PTH levels from tissue FNA predicts the presence of parathyroid tissue during parathyroidectomies for primary or secondary hyperparathyroidism (8,9,12). To our knowledge, the present study details the first use of rIO-PTH assay for FNA of suspected parathyroid tissue to distinguish parathyroid and non-parathyroid tissues during thyroidectomy. To analyze the sensitivity and the specificity of this technique, the aspirated tissue underwent frozen section examinations. The results demonstrated that the rIO-PTH mean value of 3,369 pg/ml was significantly higher in parathyroid tissues that were confirmed by pathology compared with the rIO-PTH mean of 25.7 pg/ml in non-parathyroid tissue. The rIO-PTH lower limit of 145.2 pg/ml in parathyroid tissue was markedly higher than the maximal rIO-PTH value of 39.5 pg/ml obtained from non-parathyroid tissues, demonstrating that this technique correctly predicted parathyroid tissue in every case with higher sensitivity and specificity compared with frozen section examination.

Even in routine cases, frozen section examinations are often recommended to verify the identity and confirm the presence of parathyroid tissue, and to provide pathological tissue confirmation for potential parathyroid autotransplantation (7,21,22). However, frozen section examination can be time-consuming, costly, and require the excision of a significant portion of tissue, rendering it impractical in various settings (13). Compared with frozen section examinations, it was demonstrated in the present study that the rIO-PTH assay through FNA was more reliable, simple, minimally invasive, and required a reduced surgical duration. rIO-PTH assay through FNA helped successfully identify parathyroid and non-parathyroid tissues with the above advantages; thus this technique may replace frozen section examination.

No serious complications were detected in either group following the thyroid surgery, including postoperative hemorrhage and vocal cord paralysis, indicating that this novel rIO-PTH technology did not increase the surgical risks. Postoperative transient hypoparathyroidism did not occur in the rIO-PTH group compared with six cases in the control group, showing that this technique was an effective method of avoiding transient hypocalcemia. Notably, a significantly increased number of parathyroids were detected in the visualization combined with rIO-PTH assay through FNA method in the rIO-PTH group, compared visualization alone. The security and practicality of this technology were effective for protecting parathyroids that are difficult to identify in subsequent surgery, and for improving parathyroid identification by inexperienced surgeons. There was no statistically significant difference in the postoperative serum calcium level and blood PTH values between the rIO-PTH group and control group, which indicated that to avoid postoperative hypocalcemia, surgeons should stress intraoperative *in-situ* conservation and autotransplantation of the parathyroids, as well as improve parathyroid identification.

In conclusion, the present study demonstrated the value of rIO-PTH assay through FNA as an effective method for differentiating parathyroid and non-parathyroid tissue. The technique is highly reliable, quick, simple, and non-invasive and may replace frozen section examination and a reliance on surgeons' parathyroid visualization using topographic or morphologic criteria. The clinical significance of this novel technique and the reference value index of rIO-PTH require further investigation with larger numbers of cases across multiple institutions.

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