Anatomical distribution and number of parathyroid glands, and parathyroid function, after total parathyroidectomy and bilateral cervical thymectomy

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Abstract
The normal distribution of parathyroid glands is well documented. However, this study aims to evaluate the efficacy of total parathyroidectomy (TPTx) and bilateral cervical thymectomy (BCTx) for the treatment of secondary hyperparathyroidism (SHPT) through identifying the location of parathyroid glands with attention to the pattern and frequency of orthotopic and ectopic glands.

Between 2013 and 2018, sixty chronic hemodialysis patients with medically refractory SHPT underwent TPTx & BCTx. The adequacy of the operation was defined by the pathological confirmation of at least 4 parathyroid glands, accompanied by an intact parathormone (iPTH) value of <60 pg/mL on postoperative day 1 (POD1). Based on their anatomical localizations, four distinct sites were identified for both the upper (Zone I–IV) and lower parathyroid glands (Zone V–VIII).

The mean follow-up was 15.2 ± 14.6 months. The mean iPTH values on POD1 were normal in 50 patients, with an average of 11.7 ± 4.4 pg/mL. Ten patients (16.6%) had persistent HPT after the operation, three of whom underwent complementary parathyroidectomy. The surgical success rates after first and second operations were both 83.3%. A total of 235 parathyroid glands were detected. Ninety-two percent of the upper parathyroids were located in Zones I and II. However, almost 28% of the lower parathyroids were ectopic and located in Zones VII and VIII.

At least one fourth of the lower parathyroids are ectopic; for this reason, Zones VII and VIII require careful investigation during surgery. For upper parathyroids not found in Zone I–III, total thyroidectomy on the same side is recommended.

Abbreviations: BCTx = bilateral cervical thymectomy, CKD = chronic kidney disease, iPTH = intact parathormone, POD1 = postoperative day 1, SHPT = secondary hyperparathyroidism, TPTx = total parathyroidectomy, TTLX = thyrothymic ligament excision.

Keywords: bilateral cervical thymectomy, end stage renal disease, location of parathyroid glands, parathyroid, secondary hyperparathyroidism, total parathyroidectomy

1. Introduction
Secondary hyperparathyroidism (SHPT) is a manifestation of chronic kidney disease (CKD) which insidiously develops in nearly all patients undergoing chronic hemodialysis treatment, with approximately 90% displaying clinical symptoms.[1] Although novel medical treatment options have emerged in recent years, this has only slightly reduced the rate of surgery for SHPT. As reported by the US Renal Data System, the annual rate of parathyroidectomy has remained steady since 2006 with 5.4 parathyroidectomies per 1000 SHPT patients.[2] The distribution of normal parathyroid glands has been well documented in anatomical studies by Akerström et al and Thompson et al.[3,4]

However, the first of these focused on autopsy results from 503 cases and the second was based on primary hyperparathyroid patients. Our study, however, has additional features in terms of detailing the anatomic location of the parathyroid glands in patients with secondary hyperparathyroidism. In this context, the aim of this prospective study is to document the number and location of hyperfunctioning parathyroid glands in various positions after “intended” total parathyroidectomy (i.e., removal of at least four glands) and bilateral cervical thymectomy; also to evaluate the parathyroid metabolism after this surgical procedure.
2. Anatomical considerations

Normal anatomic locations for the upper and lower parathyroid glands were considered to be as follows: the orthotopic superior parathyroid glands are those residing between the cricothyroid ligament and the anatomic demarcation of the recurrent laryngeal nerve and inferior thyroid artery, Zone I; the area below the position where the inferior thyroid artery enters the thyroid and crosses the inferior laryngeal nerve, Zone II; cranially and along the posterior aspect of the superior thyroid artery, Zone III; cranially and along the posterior aspect of the inferior thyroid artery, Zone III; cranially and along the posterior aspect of the superior thyroid artery, Zone IV; intrathyroidal.

Based on the anatomical localizations observed during the surgery, four different sites were defined for the upper and lower parathyroid glands (Figs. 1 and 2).

3. Materials and methods

The study included 60 consecutive hemodialysis patients with SHPT who underwent parathyroidectomy between October 2013 and December 2018. Indications for surgery were based on the 2009 KDIGO guidelines on CKD and mineral-bone disorders. All patients had previously been treated with calcimimetic agents for at least one year and were referred for initial parathyroidectomy due to medically refractory SHPT. Thus, patients who, despite maximized medical treatment, failed to maintain appropriate levels of serum calcium (8.4–10.2 mg/dL), phosphate (2.5–4.6 mg/dL) and PTH between two to nine times the normal upper limit (130–600 pg/mL), were referred for surgery.

Surgery was performed under general anesthesia and with Kocher’s neck incision. The operative procedure was bilateral neck exploration and TPTx without autotransplantation accompanied by bilateral cervical thymectomy (BCTx). All the parathyroid glands identified in the operation were photographed for archival purposes. Thus, actual anatomical locations and association with neighboring organs were clearly documented.
The adequacy of the operation was defined, as previously described by Hiramitsu et al, by the pathological confirmation of at least 4 parathyroid glands, accompanied by an iPTH value <60 pg/ml on postoperative day 1 (POD1). After discharge the patients were followed up at 6 month intervals, when iPTH, Ca, phosphorus, and Ca×P product values were measured and recorded.

The Access Intact PTH assay (Access Immunoassay Systems Intact PTH A16972, Beckman Coulter, Inc., Fullerton, CA) was used for quantitative determination of intact parathyroid hormone levels in human serum and plasma. The reference interval of this immunoassay kit is 12–88 pg/ml.

All patients who had preoperative cervical ultrasound examination and technetium-99m sestamibi (99mTc-MIBI) scintigraphy to document the enlarged parathyroid glands and ectopic and/or supernumerary parathyroids deep in the mediastinum. Imaging methods did not contribute to our decision to operate or choice of surgical approach in any way and were only used for localizing glands with adenomatous or nodular hyperplasia. Intraoperative intact iPTH monitoring was not performed with this group.

For the purpose of our study, persistent hyperparathyroidism was defined as elevated iPTH within 6 months of surgery and recurrent hyperparathyroidism denoted elevated iPTH that occurs more than 6 months after TPTx plus BCTx. All the factors that might underlie these complications, such as ectopic gland location, supernumerary glands and surgeon inexperience were investigated and recorded and necessary corrective surgical intervention was performed as long as patient consent was given.

The study protocol was approved by the Ethics Committee of the University of Health Sciences, Bozyaka Health Practice and Research Hospital. Written informed consent was obtained from all participants before surgical intervention.

4. Statistical analysis

All analyzes were performed with SPSS 15.0 (Chicago, IL) for Windows statistical package. The mean and SD of all values were calculated. A paired t test was used to calculate the difference between paired observations (e.g., iPTH values before and after TPTx & BCTx). For all the analyzes, a P value of <.05 was considered statistically significant.

5. Results

The mean age of the patients was $50.1 \pm 12.0$ years (range: 21–73 yrs). In-hospital mortality rate was nil in this series. The mean follow-up was $15.2 \pm 14.6$ (range: 1–57 mos). All, but one patient, were alive at the last follow-up. One female patient deceased during hemodialysis four years after the operation. Also, recurrent laryngeal nerve damage requiring tracheostomy occurred in one patient who was further treated with arytenoid adduction followed by speech therapy. Unilateral superior laryngeal nerve damage occurred in another patient who underwent complementary parathyroidectomy. Thus, the major morbidity rate for sixty-three operations was 3.2%. The demographics and patient characteristics are shown in Table 1.

US examination correctly established the largest parathyroid in 27 of 38 patients (71%). The mean diameter of the dominant nodule was $13.9 \pm 7.8$ mm. (5.0–45.0). MIBI scan failed to demonstrate any of the parathyroids in 38 patients (68%). Thirty patients (53.5%) required simultaneous thyroidectomy due to solitary or multinodular goiter, or because of the failure to identify the fourth parathyroid gland during bilateral neck exploration. Of these, sixteen had multinodular and three had colloid goiter, four had Hashimoto’s thyroiditis and four had micropapillary thyroid carcinoma. Three patients with normal thyroid tissue underwent thyroidectomy because of failure to identify the fourth parathyroid gland during bilateral neck exploration. iPTH values and the biochemistry profile of all SHPT patients, one day after the operation, are shown in Table 1.

During the operation, all identified glands and thyroidic ligament were sent to the pathology department in separate pots. From these materials, pathologists reported 5 parathyroid glands in 18 cases, 4 glands in 50 cases, and 3 glands in one patient. Thus, the total number of parathyroid glands detected in the pathological examination was 232. The iPTH values on POD1 were normal in 50 patients, with an average of $11.7 \pm 14.4$ pg/mL (95% CI; range: 7.8–15.7). The iPTH values obtained at the last checkup of these 50 patients were $13.3 \pm 18.8$ pg/mL. In this group, the number of pathologically confirmed parathyroid glands was five in one patient, four in forty-eight and three in one patient. 10 patients (16.7%) had persistent HPT after the operation. In these ten cases, the number of parathyroid glands reported by the pathology was four in two cases and three in 8 cases. Of these, three patients underwent complementary parathyroidectomy. Preoperative neck MR and/or 99mTc-MIBI SPECT/CT did not detect the missing parathyroid; thus, the area to be surgically explored was determined according to the pathology report of the undetected parathyroid. Reoperation was successful in all three patients. Operative results, pathological evaluation and the clinical course of these cases are shown in Table 2. Finally, 53 patients had normal iPTH levels at POD1 and at the last checkup. After pathological evaluation, the final number of parathyroid glands reported was 51 patients with four; and two patients with three and five glands each.

In conclusion, the surgical success rates after the first and second operations (TPTx plus TTLx) in our series were 83.3% and 88.3%, respectively.

6. Mapping of the parathyroids

A total of 235 parathyroid glands were detected, photographed and removed after the operations. The number of glands

| Table 1 | The characteristics and biochemistry profile of SHPT patients. |
|---------|---------------------------------------------------------------|
| All patients (n = 60) | Mean ± SD | Range |
| Age (years) | $50.1 \pm 12.0$ | 21–73 |
| Gender (male/female) | 36/24 |
| Duration of hemodialysis (months) | $136 \pm 53$ | 48–288 |
| Calcium (mg/dl) | $11.7 \pm 1.35$ | 7.7–12.0 |
| Phosphorus (mg/dl) | $5.9 \pm 1.5$ | 2.2–8.9 |
| Albumin (g/dl) | $4.1 \pm 0.4$ | 3.2–5.1 |
| Calcium-phosphorus product (mg²/dL²) | $54.7 \pm 16.7$ | 25–118 |
| Alkaline phosphatase (UL) | $502.7 \pm 440$ | 75–2069 |
| Intact PTH (pg/ml) (preoperative) | $1702.5 \pm 802.8$ | 220–3298 |
| Hemoglobin (g/dl) | $11.8 \pm 1.7$ | 8.3–17.3 |
| One day postoperative Calcium (mg/dl) | $8.5 \pm 1.1$ | 5.0–10.3 |
| Phosphorus (mg/dl) | $3.5 \pm 1.3$ | 1.2–7.3 |
| Calcium-phosphorus product (mg²/dL²) | $29.7 \pm 11.5$ | 9.7–59 |
| Alkaline phosphatase (UL) | $452.3 \pm 695.5$ | 86–2260 |
| Intact PTH (pg/ml) | $53.9 \pm 136.6$ | 0.3–788.0 |

iPTH = parathormone.
harvested and their distribution according to anatomical zones are shown in Table 3.

More than ninety percent of the upper parathyroids were located in Zones I and II (107/117: 92%). In addition, it was observed that more than ninety percent of the upper parathyroids located in these two zones were symmetrical. On the contrary, approximately 28% (33/118) of the lower parathyroids were ectopic and located in the thyrothymic ligament or between the carotid sheath and tracheoesophageal groove, below the lower pole of the thyroid. The ectopic left inferior parathyroid was commonly identified in Zone VII and the right inferior in Zones VII and VIII. The patient with five parathyroid glands had two right inferior glands located at Zones VII and VIII.

7. Discussion

Herein, we presented a series of SHPT patients who underwent total parathyroidectomy with thyrothymic ligament excision. All of the patients in this series have complete follow-up data, including the last postoperative checkup. Surgical success rates following the first and second operations were 83.3% and 88.3%, respectively. The total number of parathyroid glands excised from sixty patients was 235. Ectopic gland(s) were not identified in only seven cases.

As found in a previous pathological study, upper parathyroids in our series almost always settled in Zones I and II (107/117: 92%); however, roughly 30% of the lower parathyroids were found in Zones VII and VIII, which were not adjacent to the thyroid gland. Interestingly, we have not recorded any superior parathyroid gland below the anatomic demarcation of the recurrent laryngeal nerve and inferior thyroid artery.

In two studies concerning complementary parathyroidectomy, the missed glands were commonly located in the thyromental notch (24%–28.4%). This popular location was more pronounced in another study of 902 patients with SHPT. In this study, the incidence of intrathymic glands either as inferior parathyroid, supernumerary glands or parathyromatosis was 45.3%, indicating the importance of removing the thymic tongue on both sides of the neck. In another group of 570 patients who underwent TPTx with forearm autografting, the incidence of supernumerary glands was 16.5%. Of these, 51.0% were located in the thymic tongue and almost 60% of them were identified only microscopically (in parathyromatosis pattern). This clearly indicates that postoperative hyperparathyroidism may persist over a remarkable range due to parathyromatosis, even if four parathyroid glands are excised in the initial operation. However, since all of the cases in our series had undergone TTLx, there is no possibility that the intra-operatively non-detected glands were in this location. Although none of our imaging methods revealed the missing glands, we consider the mediastinum to be their possible location. In another retrospective study, the location of missed glands was investigated in 82 patients who underwent TPTx with the resection of the thyromental notch. In support of our conjecture, 93 missed glands were identified during reoperation and were most commonly located in the mediastinum (22/93: 24%).

In our study group, we preferred total parathyroidectomy (TPTX) and thyrothymic ligament excision (TTLX) due to its'

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Table 2
Clinical course of ten patients with persistent hyperparathyroidism.

| Patient no. | Pathologically confirmed (PTs) (n) | Undetected PTs | Postoperative iPTH | Reoperation | Final iPTH | Result |
|------------|-----------------------------------|----------------|-------------------|-------------|-------------|--------|
| 1          | 3                                 | Left inferior  | 184.4             | NO          | 447.6       | Retained left inferior parathyroid |
| 2          | 3                                 | Left superior  | 483.1             | NO          | 2077        | Retained left superior parathyroid |
| 3          | 4                                 | Unknown        | 269               | NO          | 615.8       | Ectopic gland was not detected by MIBI scan or cervical MRI. Probably located at mediastinum |
| 4          | 4                                 | Unknown        | 1726              | NO          | 1918        | Ectopic gland was not detected by MIBI scan or cervical MRI. Probably located at mediastinum |
| 5          | 3                                 | Left superior  | 113               | NO          | 618.5       | Retained left superior parathyroid |
| 6          | 3                                 | Left superior  | 325.5             | NO          | 328         | Retained left superior parathyroid |
| 7          | 3                                 | Right superior | 798               | YES         | 10.4        | Right sup. gland at Zone III removed |
| 8          | 3                                 | Right inferior | 741               | YES         | 6.1         | Right inf. gland at Zone VIII removed |
| 9          | 3                                 | Left inferior  | 422               | NO          | 261         | Retained left inferior parathyroid |
| 10         | 3                                 | Left inferior  | 757               | YES         | 4.6         | Left inf. gland at Zone VIII removed |

PTH = parathormone, PTs = parathyroids.

**Table 3**
The distribution of parathyroids by zone.

| Zone    | Right superior parathyroids (60) | Left superior parathyroids (57) |
|---------|----------------------------------|----------------------------------|
| Zone I  | 37 & 62%                         | 37 & 65%                         |
| Zone II | 18 & 30%                         | 15 & 26%                         |
| Zone III| 3 & 5.0%                         | 4 & 7.0%                         |
| Zone IV | 2 & 3.0%                         | 1 & 2%                           |
| Zone V  | 33 & 55%                         | 32 & 55%                         |
| Zone VI | 11 & 18%                         | 9 & 16%                          |
| Zone VII| 7 & 12%                          | 11 & 19%                         |
| Zone VIII| 9 & 15%                          | 6 & 10%                          |
significant lower rate of recurrent or persistent hyperparathyroidism when compared with other procedures. Indeed, recurrence rates after TPTX are almost always less than 5%; while recurrence rates between 5%–80% have been reported in cases of TPTX and autotransplantation. In two recent review articles comparing TPTX and autotransplantation with TPTX, the absolute risk reduction for recurrence and recurrence or persistence of SHPT was almost 20% less in patients who underwent TPTX.

Meanwhile, with respect to surgical approach for secondary hyperparathyroidism, the possibility of removing at least four parathyroids with a hundred percent success in a single operation seems unrealistic. In a recent study, a total success in nine cases with SHPT was achieved with a novel surgical method termed “prune parathyroidectomy,” a more extensive method than the classical TPTx+TTLx. However, this technique has not been tested in other studies and a similar success has yet to be repeated. When we look at the literature, the percentage of cases where four glands are resected together is reported as between 72% and 98.5% according to the series. Furthermore, even if four parathyroids are excised, surgical and biochemical success cannot be achieved due to the supernumerary glands found in 13% to 20% of random autopsies and 20% to 30% of patient cohorts.

According to data from the “Parathyroid Surgeons’ Society of Japan,” 2016 patients with SHPT underwent parathyroidectomy between 2009 and 2013. In this group, the percentage of cases where four or more glands were removed during the first operation was 77.7%. Persistent or recurrent hyperparathyroidism developed in 12.7% of the study group. Therefore, while we have not achieved a biochemical and surgical success rate above 90% in our own study, it is evident that even in a series handled by endocrine surgeons specialized in the subject, such a rate is likely unattainable. In this study, an intact PTH value of <60 pg/mL in POD1, accompanied by four or more pathologically confirmed parathyroid glands, constituted surgical success. This concept was first described by Schneider et al. as an indicator of successful removal of parathyroid glands.

8. Limitations

However, the most important factor overlooked in the methodology of this study is the omission of frozen section during the operation. Had frozen section been applied, we probably could have detected the fourth gland in most of the eight patients whose pathological evaluation revealed only three glands. The second limitation of our study is that we did not perform Sestamibi scans plus single photon emission computed tomography to detect the missing parathyroid and subsequent computed tomography scans of chest to precisely localize the anatomic position of the gland. The high efficacy of this method in the detection of retained glands (100%) has been demonstrated in a current study.

So far, this study draws attention to the anatomic localization of parathyroids in SHPT cases. Unfortunately, the number of studies on this subject is very low; with the majority emphasizing parathyroid anatomy only after secondary operations to determine the location of orthotopic or ectopic missing glands in cases with recurrent or persistent SHPT. In the majority of studies, ectopic thymic parathyroids are almost always inferior and the incidence is reported as between 18%–28%. In our study, we detected 18 parathyroids (15%) in the thyrothymic ligament.

In conclusion, in our patients with SHPT, the proportion of upper parathyroid glands located in zones III and IV is 8.5% (10/117). For the upper parathyroid gland which cannot be found intraoperatively, it is a suitable approach for dissecting Zone III dissection on the same side initially and switching to ipsilateral total thyroidectomy if the parathyroid gland is not found. However, approximately 30% of lower parathyroids have an anatomic position not adjacent to the thyroid gland. Therefore, in SHPT patients, thyrothymic ligament excision should be an essential and indispensable part of surgical treatment. Moreover, in cases where there is a missing inferior parathyroid during TPTX and TTLx, bilateral Zone VIII explorations should be performed. Additionally, the use of frozen section to verify parathyroid glands removed during surgery should be an essential part of the treatment and mandatory.

Acknowledgments

Native-speaker English editing has been carried out by Claire Olmez MEd, MSc, ELT. We thank Claire for her valuable contribution.

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