Endocrine (2011) 40:452–461
DOI 10.1007/s12020-011-9499-5

ORIGINAL ARTICLE

Intraoperative multiple-staged resection and tumor tissue identification using frozen sections provide the best result for the accurate localization and complete resection of tumors in Cushing's disease

Jung Soo Lim · Seung Ku Lee · Se Hoon Kim · Eun Jig Lee · Sun Ho Kim

Received: 13 April 2011 / Accepted: 3 June 2011 / Published online: 19 June 2011

Abstract The treatment of choice in Cushing's disease (CD) is surgical removal; however, most tumors are too small to be detected. The objective was to establish a method to achieve the complete removal of tumors on the basis of the results of high-resolution magnetic resonance imaging (MRI), inferior petrosal sinus sampling (IPSS), and a surgical resection technique using frozen biopsy. Eighteen patients who underwent transsphenoidal surgery from 2004 to 2010 were included. High-resolution MRI and IPSS, multiple-staged resection, and tumor tissue identification in frozen sections (surgical and histological identification, SHI) were performed. All patients achieved surgical remission, as confirmed by 24 h urinary free cortisol excretion tests. Visible microlesions were identified on the initial MRI in 11 patients (61%). The SHI findings agreed with the MRI findings in 10 of the 11 patients (90.9%) and with IPSS lateralization in 6 of the 11 patients (54.5%). In the 7 patients whose lesions were not visible on the initial MRI, only 1 (14.3%) showed an agreement between IPSS and SHI. In 3 of the 7 patients, the microlesions were identified by additional MRI. The rate of concordance with SHI was 77.8% for the overall MRI and 38.9% for IPSS. High-resolution MRI is better than IPSS for localizing corticotroph adenomas. In patients with lesions not visible on the initial MRI, additional MRI should be performed using a different protocol. Although high-resolution MRI is better for localizing tumors, SHI remains an important approach for removing the tumors completely.

Keywords Cushing's disease · Localization · High-resolution MRI · Inferior petrosal sinus sampling · Surgical and histological identification

J. S. Lim · E. J. Lee (✉)
Endocrinology, Brain Korea 21 Project for Medical Science, and Severance Integrative Research Institute for Cerebral & Cardiovascular Disease, Internal Medicine, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul 120-752, Korea
e-mail: ejlee423@yuhs.ac

J. S. Lim · E. J. Lee · S. H. Kim
Institute of Endocrine Research, Yonsei University College of Medicine, Seoul 120-752, Korea

S. K. Lee
Department of Radiology, Yonsei University College of Medicine, Seoul 120-752, Korea

S. H. Kim
Department of Pathology, Yonsei University College of Medicine, Seoul 120-752, Korea

S. H. Kim (✉)
Neurosurgery & Yonsei Brain Research Institute, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul 120-752, South Korea
e-mail: sunkim@yuhs.ac

Introduction

Cushing's disease (CD) is a rare disease caused by ACTH-secreting pituitary tumors, which cause the overproduction of glucocorticoid by the adrenal cortex [1]. Because the overall mortality of patients with uncontrolled hypercortisolism is 4–5 times greater than that in the general population [1], appropriate treatment of CD is important. Surgical removal of the pituitary adenomas is the treatment of choice in patients with CD [2]; however, until now there have been limitations for diagnosis and localization of the tumor because about 40–50% of corticotroph adenomas are too small to be detected even though by dynamic MR imaging [3–6].

As van Aken et al. [7] reported, improved preoperative localization of ACTH-secreting hypophyseal microadenomas increases the success and cure rate of transsphenoidal surgery (TSS). Two principal preoperative examinations, inferior petrosal sinus sampling (IPSS) and pituitary imaging are used to identify the site of ACTH secretion [5, 7]. IPSS can provide important information to help the neurosurgeon to determine the type of operation needed [8]; however, the value of IPSS for the preoperative localization of pituitary microadenomas that are not visible in magnetic resonance imaging (MRI) is controversial [9, 10]. In addition, although the resolution of MRI has made remarkable progress, most of the studies cover the role of MRI based on the results performed with 0.5–1.5 Tesla (T) magnetic field strength [11].

None of the tests used in the diagnosis of CD is 100% reliable [12, 13], and the best method for localizing pituitary microadenomas in a patient with CD remains a challenge. Many studies have addressed the diagnostic usefulness of each examination, including IPSS, high-dose dexamethasone-suppression test (DST), and pituitary imaging. However, these tests focus mainly on the preoperative process. According to Hoybye et al. [14], the surgical outcome depends on both the surgeon's experience and careful preoperative evaluation. If there was discrepancy between IPSS and MRI, especially, in case of no definite visible mass on the MR imaging, it is difficult to decide between the two methods, hemi-hypophysectomy or total hypophysectomy during the operation. Therefore, particularly in patients with discordant results between IPSS and MRI, the outcome of patients is primarily influenced by the neurosurgeon's ability to distinguish characteristic features of tumor tissues (e.g., color, consistency) from normal pituitary gland.

In this study, we evaluated the usefulness of high-resolution MRI and IPSS for localizing pituitary adenomas by comparing their results with the operative findings. On the basis of these results, we suggest a new concept, surgical identification of tumors by a neurosurgeon and histological identification (SHI), as a crucial approach for the accurate localization and complete resection of pituitary adenomas in patients with CD.

Patients and methods

Patients

Eighteen patients, 17 women and 1 man, mean age 38.56 ± 10.23 year (range 18–52 year), with CD underwent TSS performed by a single neurosurgeon (Kim) in our hospital from 2004 to 2010. In all patients, high-resolution MRI and IPSS were performed before TSS. Three patients had already undergone TSS in other hospitals and were referred to our hospital to remove remnants of the pituitary tumors. The preoperative diagnosis of CD was established on the basis of clinical features and the results of biochemical tests including 24 h urinary free cortisol (UFC) excretion, low- and/or high-dose DST, and abdominal MR imaging. The tumor location was categorized as right-sided, left-sided, bilateral, or midline.

In all patients, serum cortisol levels were checked every 6 h during the first day, at 48 and 72 h after TSS. Within 24 h after TSS, immediate therapeutic outcome was determined by postoperative serum cortisol levels. If the serum cortisol level is less than 2 μg/dl or if signs and symptoms of adrenal insufficiency are present, glucocorticoid replacement therapy was started. If the serum cortisol value is reduced to levels between 2 and 5 μg/dl, the patient was monitored for the development of clinical signs of adrenal insufficiency until 72 h after TSS and thereafter, glucocorticoid replacement therapy was initiated. In case of serum cortisol levels of more than 5 μg/dl, reoperation was considered.

Remission was confirmed by 24 h UFC excretion test, including non-suppressed serum cortisol and ACTH levels, which were regularly assessed in all patients during follow-up. Moreover, these results were interpreted with caution, particularly in patients with serum cortisol levels more than 2 μg/dl in the early postoperative phase. If 24 h UFC is elevated beyond a normal range (20–90 μg/day, radioimmunoassay) or serum cortisol levels of more than 5 μg/dl are detected during this process, we performed the test evaluating suppression to dexamethasone, such as overnight or low DST, and determined whether cure is achieved. A combined pituitary function test (CPFT) was performed more than 6 months after TSS to assess the hormonal status more accurately. The result of CPFT was interpreted by previous report [15]. Whether to maintain glucocorticoid replacement therapy was determined according to the result of CPFT. All patients were monitored for a minimum of 1 year and a maximum of 7 years, and achieved remission by surgery.

High-resolution MRI

Sella MRI was performed using a 3-T MRI unit (MR Systems Achieva, release 3.1, Philips, The Netherlands) in our hospital in all patients including 12 patients whose MRI had been performed at a different center. The standard protocol for sella MRI included T1- and T2-weighted turbo spin echo sequences in the sagittal plane, a T2-weighted turbo spin echo sequence in the axial plane, and high-resolution images in a T2-weighted turbo spin echo sequence in the coronal plane. Coronal dynamic acquisition with a slice thickness of 2 mm was started simultaneously with

intravenous gadolinium injection (0.2 ml/kg), followed by a T1-weighted turbo spin echo sequence in the sagittal and coronal planes. In 7 patients who had tumors that were not visible on the initial MRI or whose IPSS and an initial MRI finding results were discordant or suspicious of bilateral lesions, MRI was performed again using an additional protocol, such as a T1-weighted sagittal dynamic view after gadolinium administration and a thin-section navigation T2-weighted axial view with a slice thickness of 1 mm. Moreover, a half dose gadolinium (0.1 ml/kg) enhancement sella dynamic imaging was performed in three cases. The MRI images were reviewed by a single neuroradiologist who was blinded to the results of the IPSS.

IPSS

IPSS was successful and no major complications were noted in any patient. The catheterization was performed via both femoral veins. After optimal catheter placement, blood samples were obtained simultaneously from a peripheral vein and both sides of the inferior petrosal sinus 0, 5, and 10 min after administration of a 100 μg iv bolus of CRH. The data from the IPSS were interpreted according to the criteria described by Oldfield et al. [16]. The central to peripheral ACTH ratio (*C*/*P* ratio) and the ACTH ratio between the right and left inferior petrosal sinuses, called the interpetrosal ratio (IPS ratio), were calculated at baseline and at each time after CRH injection. The highest post-CRH stimulation ratio was used for interpretation. A *C*/*P* ratio $\geq$ 2 at baseline or $\geq$ 3 after CRH injection was regarded as evidence of pituitary ACTH secretion, and an IPS ratio $\geq$ 1.4 at baseline or after CRH injection was used to indicate the existence of lateralization.

Surgical and histological identification (SHI)

SHI was defined as the intraoperative process used to detect tumor tissues within the normal pituitary using surgical microscopy and multiple-stage resection followed by identification of tumor tissue in frozen sections. SHI is a practical method to determine the resection margin during the operation. Intraoperatively, a dural opening was made to expose both the medial margins of the cavernous sinus to directly visualize the medial wall of the cavernous sinus bilaterally. The surgeon first explored the suspicious lesion site on the MRI, and then, also investigated the site showing IPSS lateralization. If normal glandular tissue covered the anterior surface of the intrasellar content, a small vertical incision was made at the suspected location of the tumor. At that time, a neuronavigation system was helpful for localizing tiny lesions. After making an incision, if any suspicious tumor tissue was identified, a small fragment of tissue was obtained and sent to the pathology laboratory, where frozen serial sections were cut. At that time, the surgeon made a drawing of the biopsy site with a surgical marker pen. When the suspicious tumor tissue was removed completely, a biopsy was obtained from the underlying normal-looking tissue to confirm the total resection of the tumor. If a bilateral lesion was suspected, multiple biopsy was also performed at the anterior surface of pituitary gland as well as on the other side based on additional informations obtained from MRI, such as the sagittal dynamic view.

Results

IPSS and SHI in patients with positive findings on the initial MRI

Table 1 shows the results of the IPSS in all patients. As shown in Table 2, visible microlesions were identified in 11 patients (61.1%, all women; age range 19–48 year) on the initial MRI; 2 were on the right side, 6 on the left side, and 3 on both sides or on the midline of the gland. Three patients with previous TSS, whose results can affect the accuracy of IPSS, were all included in the MRI-positive group (refer to * patients in Table 2). Histologic confirmation of a pituitary adenoma was achieved in 10 of these 11 patients (90.9%); tumor tissues were not found in the other patient. In 6 of the 11 patients (54.5%) whose MRI results were positive, the MRI findings agreed with the IPSS lateralization, and these findings were confirmed by SHI. The positive findings on the initial MRI were consistent with SHI in 10 of 11 patients (90.9%). IPSS lateralization was consistent with the SHI findings in 6 (54.5%); 2 tumors were on the right side, 3 on the left side, and 1 in the midline of the gland. A unilateral IPS ratio $\geq$ 1.4 at baseline or after CRH stimulation (range 1.46–15.22) was found in all patients; 1 patient with a false-negative IPSS result was excluded from the lateralization data because the *C*/*P* ratio failed to meet the diagnostic criteria despite successful sampling (patient #4). A discrepancy between the pre-CRH IPS ratio and post-CRH IPS ratio, showing right-sided and left-sided lateralization, respectively, was found in 1 patient (patient #1) who had a midline pituitary adenoma. The highest IPS ratio (15.22, patient #2) was observed in a patient who had no histologic evidence of a pituitary adenoma; however, this patient achieved remission after TSS using SHI.

IPSS and SHI in patients with negative findings on the initial MRI

Seven patients (38.9%, 6 women and 1 man; age range 18–52 year) had no visible lesions on the initial MRI

Table 1 The results of IPSS in 18 patients with Cushing's disease

Patient no.	Sex	Age	ACTH, peripheral (pg/ml)			ACTH, right IPSS (pg/ml)			ACTH, left IPSS (pg/ml)		
			0 min	5 min	10 min	0 min	5 min	10 min	0 min	5 min	10 min
1	F	40	60.9	20.3	23.2	45.5	85.7	85.7	17.1	310.0	70.8
2	F	30	85.2	141.7	138.9	122.4	222.5	251.6	1863.3	2500.0	1687.4
3	F	38	81.7	224.9	303.9	1195.3	2270.0	2270.0	2270.0	2270.0	2270.0
4	F	35	25.8	43.7	64.4	42.7	122.6	112.2	25.9	83.67	89.9
5	F	19	69.2	77.3	78.8	637.6	1108.4	712.9	304.6	987.4	739.9
6	F	38	57.2	273.8	532.6	794.2	1890.0	1890.0	422.0	589.9	1023.1
7	F	28	48.4	98.3	188.0	52.6	961.0	389.0	131.0	8940.0	595.0
8	F	46	44.1	62.4	118.9	649.3	2000.0	2000.0	238.4	1318.0	2000.0
9	F	29	30.5	14.2	31.2	530.1	1519.0	768.1	176.9	674.5	774.3
10	F	46	79.9	114.0	118.5	807.9	405.2	543.5	120.2	127.5	126.0
11	F	48	108.9	106.5	129.0	1257.0	2000.0	2000.0	207.4	192.6	246.1
12	F	48	19.3	69.3	73.8	38.3	179.9	211.3	796.9	142.6	175.4
13	F	18	322.4	955.4	1032.4	2270.0	2270.0	2270.0	394.7	2270.0	2270.0
14	M	47	24.1	233.3	219.2	1183.5	2420.0	2420.0	51.1	812.1	593.9
15	F	52	189.3	208.7	198.0	2000.0	1890.0	937.6	224.1	239.3	222.1
16	F	38	103.0	141.0	165.0	122.0	371.0	354.0	113.0	172.0	239.0
17	F	47	27.9	41.7	59.1	236.6	1672.0	839.4	31.5	65.6	71.6
18	F	47	85.2	208.1	265.5	645.6	1890.0	1890.0	174.9	266.9	1890.0

IPSS inferior petrosal sinus sampling

(Table 2). The IPSS and SHI results agreed in only 1 of the 7 patients (14.3%); in the other 6 patients, the bilateral lesions were incorrectly lateralized by IPSS as right-sided lesions. Of the 5 patients whose MRI was performed again using additional protocols, a suspected pituitary adenoma lesion was discovered in 4 patients (3 with bilateral lesions and 1 with a lesion on the right side); the identified microlesions were consistent with SHI in 3 patients. After the SHI during TSS, bilateral lesions were identified in 6 patients, and unilateral lesions were identified in 1 patient (on the left side). In all patients, unilateral tumor with an IPS ratio $\geq$ 1.4 at baseline or after CRH stimulation (range 3.19–25.49) was observed, and these were proven to be bilateral lesions by SHI except 1 patient. The highest IPS ratio (25.49, patient #17) was observed in a patient with a bilateral pituitary adenoma.

Agreement between SHI, MRI, and IPSS results

As shown in Table 3, the findings of the overall MRI agreed with the SHI results for tumor in 14 patients (77.8%), and the overall lateralization results by IPSS agreed with the SHI results in 7 patients (38.9%). The data for 9 of 14 patients (64.3%) in the overall-positive MRI group disagreed with the IPSS lateralization results. In 3 patients who had no visible lesions on overall MRI, the lateralization by IPSS agreed with the SHI results in 1 (33.3%). Only 6 patients (33.3%) showed an agreement of all findings. Additional MRIs were performed in 7 patients from both the MRI-positive group and the MRI-negative group. In six of these patients, the microlesions were detected by these protocols, and the overall MRI findings were consistent with the SHI results in five patients (83.3%).

In permanent pathological specimen, the presence of an ACTH-secreting pituitary adenoma was confirmed in 16 of 18 patients; the other 2 patients showed no tumor tissues in permanent pathological specimen. To avoid confusion in the interpretation, two patients without tumor tissue identification were classified as discordant cases. Among the 12 patients whose lesions were analyzed using ACTH immunochemical staining, 10 (83.3%) showed positive staining for ACTH, confirming a corticotroph adenoma. All patients achieved remission by TSS, and the remission was confirmed by a 24 h UFC excretion test.

Results of the CPFT after TSS

Table 4 shows preoperative and postoperative CPFT results as well as the duration of follow-up in all patients. The CPFT result presented in this table puts emphasis on description of abnormal findings. CPFTs were performed to evaluate anterior pituitary function before TSS, and 6 months to 1 year after TSS. In 12 of 18 patients, both preoperative and postoperative CPFTs were performed. In spite of short-term follow-up, 9 (64%) of 14 patients in whom CPFTs were performed showed normal response or

Table 2 Comparison between the results of MRI, IPSS, SHI in all patients

Patient no.	Initial MRI findings	Second MRI findings	IPSS (ACTH, pg/ml)						IPSS lateralization	SHI
			Central/peripheral ratio				Interpetrosal ratio			
			Basal		Post-CRH		Basal	Post-CRH		
			Rt.	Lt.	Rt.	Lt.				
1	B		0.75	0.28	9.89	15.27	2.66 (R)	3.62 (L)	B	B
*2	L		1.44	21.88	1.81	17.65	15.22 (L)	11.24 (L)	L	L
3	L		14.63	27.79	10.09	10.10	1.89 (L)	1.00	L	L
4	B		1.65	1.01	2.80	1.39	1.64	1.46	X	B
5	B		9.22	4.40	14.34	12.77	2.09 (R)	1.12	R	B
6	L		13.88	7.38	6.90	2.15	1.88 (R)	3.20 (R)	R	L
*7	L		1.09	2.71	9.78	90.95	2.49 (L)	9.30 (L)	L	L
*8	L		14.73	5.41	32.0	16.82	2.72 (R)	1.52 (R)	R	B
9	L		17.40	5.81	106.82	47.43	2.99 (R)	2.25 (R)	R	L
10	R		10.12	1.50	4.59	1.12	6.72 (R)	4.31 (R)	R	R
11	R		11.54	1.90	18.80	1.90	6.06 (R)	10.38 (R)	R	R
12	In		1.98	41.25	2.86	2.38	20.84 (L)	1.20	L	L
13	In	B	7.04	1.22	2.38	2.38	5.75 (R)	1.00	R	B
14	In	In	49.11	2.12	10.37	3.48	23.17 (R)	4.07 (R)	R	B
15	In	B	10.57	1.18	9.06	1.15	8.92 (R)	7.89 (R)	R	B
16	In	B	1.18	1.09	5.07	1.48	1.08	3.19 (R)	R	B
17	In		8.45	1.12	40.09	1.57	7.52 (R)	25.49 (R)	R	B
18	In	L	7.58	2.05	9.08	7.12	3.69 (R)	7.08 (R)	R	B

SHI surgical and histological identification; *IPSS* inferior petrosal sinus sampling

R right; *L* left; *B* bilateral; *X* not lateralized; *In* invisible

* The patient who underwent reoperation

improved hypopituitarism in postoperative CPFTs (patient #1, 5, 6, 9, 10, 12, 13, 14, and 16). Except patient #17, 2 patients had no preoperative CPFT results; however, they all showed normal response except ACTH and cortisol in postoperative CPFTs. In case of three patients who underwent reoperation due to recurrence of the tumor, hypopituitarism has been persisted. Prednisolone is currently being administered in nine patients according to the result of postoperative CPFT.

Discussion

IPSS with CRH stimulation is commonly considered the gold standard for differentiating pituitary from ectopic sources of ACTH and is based on the proximity of the inferior petrosal sinus to the pituitary gland [17–20]. In addition, IPSS has a high sensitivity (88–100%) and specificity (67–100%) in the diagnosis of CD [8, 10, 16, 21–23] and may be recommended in all patients with negative or equivocal radiological findings and in those with discordant findings on biochemistry or imaging studies [20, 24]. However, IPSS is less reliable for predicting the lateralization of pituitary adenomas and has a sensitivity of 50–65% [9, 10, 25–28]. Colao et al. [10] stated that although radiologic imaging is less sensitive than IPSS in diagnosing CD, it is more accurate than IPSS in localizing the intrapituitary site of a tumor. Other studies of patients with CD but not Cushing's syndrome found that MRI findings correlated with the localization of the adenoma in 87–93% of patients but IPSS results correlated in only 68–73% of patients [29, 30].

High-resolution MRI may perfectly detect both the extension of the adenoma and its invasiveness into adjacent structures, major prognostic factors that predict the surgical outcome [31]. Considering that the pituitary gland is small, high-resolution MRI is essential for evaluating the pituitary gland satisfactorily [32]. Dynamic MRI, which is reported to be better for detecting a pituitary lesion in CD than is nondynamic MRI [33], is recommended in any patient with a suspected microadenoma [34]. High-resolution MRI, including a dynamic MRI, remains a significant tool for both the diagnosis and localization of pituitary tumors.

In this study, high-resolution MRI was better than IPSS for localizing corticotroph adenomas in patients with CD. As shown in Table 3, the results of IPSS lateralization

Table 3 Comparison between overall MRI findings and results of IPSS according to SHI

Patient no.	Overall MRI findings	IPSS lateralization	SHI
1*	*B*	*B*	B
2*	*L*	*L*	L
3*	*L*	*L*	L
4	*B*	X	B
5	*B*	R	B
6	*L*	R	L
7*	*L*	*L*	L
8	*B*	R	B
9	*L*	R	L
10*	*R*	*R*	R
11*	*R*	*R*	R
12	In	*L*	L
13	*B*	R	B
14	In	R	B
15	*B*	R	B
16	*B*	R	B
17	In	R	B
18	L	R	B

The letters in italics show the results that showed an agreement with SHI

SHI surgical and histological identification; *R* right; *L* left; *B* bilateral; *In* invisible; *X* not lateralized

* The patients that showed an agreement of all findings

disagreed with those of SHI in 61.1% of patients. When the results of the overall MRI and IPSS disagreed, IPSS lateralization showed poor concordance with the findings during surgery. According to Portocarrero et al. [35], MRI using a half dose of the contrast increases the sensitivity for detecting ACTH-secreting microadenomas because strong generalized glandular enhancement can mask the microadenoma in CD [36]. In this study, MRI performed using an additional protocol, such as sagittal dynamic MRI combined with a half dose enhancement technique, improved the accuracy of localization by identifying tumors in patients with lesions that were not visible on the initial MRI or by detecting the opposite lesion, even in those with a unilateral adenoma initially (5/7, 71.4%). The reason why the accuracy of IPSS lateralization was lower than expected is that IPSS might not be accurate for the precise localization of bilateral pituitary adenomas, which were observed in 85.7% the initial MRI-negative patients and in 55.6% of all patients.

Three of our patients with an IPS ratio of >20, including the highest IPS ratio, had a bilateral corticotroph adenoma, although all were included in the MRI-negative group (Table 2). One patient with a false-negative IPSS result (patient #4) was also proven by SHI to have a dumbbell-shaped ACTH-secreting tumor. Corticotrophs are located primarily in the median wedge of the adenophysis, and many adenomas arise in this central location [17]. Lefournier et al. [28] reported that the lateralization ratio was significantly more accurate in predicting a lateral tumor than a midline tumor. However, the importance of precise localization of bilateral or midline tumors has not been established.

A previous study suggested that all patients with a *C*/*P* ratio > 2.1 at baseline or > 2.15 after CRH injection are regarded as true positives [10], unlike the criteria described by Oldfield et al. [16]. The appropriate cutoff values for the *C*/*P* ratio and IPS ratio are still debated, especially in patients with negative findings on MRI or discordant results, for whom the IPS ratio should be interpreted carefully [17, 37]. The existence of bilateral tumors should always be considered in patients with a high IPS ratio. Further investigation of the significance of bilateral corticotroph adenomas in CD is needed.

No existing test has sufficient diagnostic accuracy when used alone [24], and the localization of corticotroph adenomas remains a challenge. TSS is the most effective and the only definite treatment for CD when used by an experienced surgeon [38]. A change in diagnostic strategy is needed to improve the cure rate of CD. Particularly, if young female patients who want to conceive show discordant results between MRI and IPSS in CD, it is very important to determine whether or not total hypophysectomy is performed to preserve the pituitary function as much as possible. SHI can reduce the need of hemihypophysectomy or total hypophysectomy in bilateral lesion or discrepancy of the lesion.

As an example, a 52-year-old female patient #15 had no visible lesions on the initial MRI, and IPSS lateralization was right (Table 2). We performed additional MRIs, as a result, the bilateral tumor was suspected in images obtained from the T1-weighted sagittal dynamic view (Fig. 1a, b). And then, intraoperative SHI confirmed the presence of bilateral tumors by detecting tumor tissues in multiple sites and we could resect the dumbbell-shaped tumor effectively. It suggests that SHI is a crucial approach for accurate localization and complete resection of ACTH-secreting tumors.

The role of pathologic findings has been limited to diagnosis of CD. However, because frozen sections enable the neurosurgeon to identify the presence of a tumor intraoperatively, the process to determine the resection margin becomes easier and more accurate. One study recommended that TSS should be considered in patients with a negative IPSS when no ectopic source can be found, especially where the pituitary MRI is positive or suspicious, or there is a robust increase in peripheral ACTH levels after CRH injection [37]. According to Kaskarelis

Table 4 Results of the combined pituitary function test and follow-up data in all patients

Patient no.	Preoperative CPFT (basal level → peak level)	Postoperative CPFT (basal level → peak level)	Duration of follow-up (years)
1	NA	fT4 1.20, TSH(3.33→13.68), GH(0.17→5.52), PRL(11.86→93.72), LH(1.59→10.07), FSH(1.01→4.38), ACTH(<1.0→11.74), Cortisol(12.8→35.92)	7
*2	fT4 1.26, TSH(0.42→3.14), GH(1.78→4.42), PRL(14.98→91.91), LH(2.89→9.74), FSH(2.90→6.37), ACTH(106.7→69.63), Cortisol(35.15→35.85)	fT4 1.29, TSH(2.68→15.06), GH(0.69→2.54), PRL(13.17→30.46), LH(4.74→37.15), FSH(3.58→8.24), ACTH(12.31→39.15), Cortisol(13.61→20.85)	4.3
3	fT4 0.82, TSH(0.58→3.97), GH(0.09→0.35), PRL(7.56→24.29), LH(1.48→2.55), FSH(2.29→6.72), ACTH(100.8→41.93), Cortisol(47.59→35.13)	NA	3
4	fT4 1.17, TSH(0.63→5.09), GH(0.46→9.82), PRL(12.05→43.63), LH(4.98→69.17), FSH(2.24→11.41), ACTH(24.52→27.87), Cortisol(21.66→20.44)	fT4 1.22, TSH(1.31→12.42), GH(<0.01→1.79), PRL(25.03→44.69), LH(2.61→12.50), FSH(2.01→3.72), ACTH(2.13→7.05), Cortisol(8.75→12.31)	3
5	fT4 0.80, TSH(1.71→6.19), GH(2.76→11.25), PRL(15.11→62.28), LH(8.11→27.75), FSH(4.02→12.92), ACTH(28.20→47.63), Cortisol(16.18→14.68)	fT4 1.23, TSH(3.40→12.04), GH(1.54→3.64), PRL(13.46→49.12), LH(5.28→14.24), FSH(3.76→5.41), ACTH(6.03→36.41), Cortisol(7.87→17.32)	2.5
6	fT4 0.98, TSH(0.32→2.63), GH(0.45→0.57), PRL(12.12→69.01), LH(2.58→17.84), FSH(2.58→18.60), ACTH(83.70→60.93), Cortisol(24.94→24.12)	fT4 1.24, TSH(1.39→12.65), GH(0.36→1.00), PRL(5.33→10.70), LH(6.50→14.75), FSH(12.90→17.03), ACTH(4.90→10.80), Cortisol(2.47→11.21)	2.2
*7	fT4 0.40, TSH(0.07→0.64), GH(0.06→0.04), PRL(1.13→2.22), LH(2.04→2.60), FSH(1.60→1.80), ACTH(40.58→46.75), Cortisol(32.58→31.48)	fT4 0.38, TSH(0.02→0.03), GH(<0.04→0.04), PRL(<0.3→<0.3),LH(1.25→3.43), FSH(2.80→3.70), ACTH(23.28→33.18), Cortisol(9.6→11.5)	2
*8	fT4 0.99, TSH(2.68→9.40), GH(0.93→0.69), PRL(10.85→80.36), LH(8.40→63.10), FSH(25.70→72.08), ACTH(35.22→37.46), Cortisol(20.30→22.23)	fT4 0.90, TSH(1.41→8.81), GH(0.06→0.12), PRL(6.53→18.89), LH(3.45→20.46), FSH(2.54→5.12), ACTH(56.69→64.42), Cortisol(16.37→17.37)	1.5
9	fT4 0.73, TSH(0.94→11.05), GH(<0.01→1.05), PRL(6.65→68.52), LH(5.53→96.51), FSH(1.83→10.73), ACTH(9.10→27.18), Cortisol(10.32→15.31)	fT4 1.04, TSH(0.72→6.11), GH(0.47→2.89), PRL(14.14→101.20), LH(17.96→146.42), FSH(3.77→12.43), ACTH(16.46→20.90), Cortisol(17.06→24.39)	1.3
10	fT4 1.47, TSH(0.62→1.16), GH(0.08→0.42), PRL(13.69→34.55), LH(0.32→1.57), FSH(4.07→4.03)ACTH(149.50→153.10), Cortisol(45.68→51.36)	fT4 1.20, TSH(0.66→8.61), GH(0.04→1.47), PRL(8.31→65.68), LH(1.55→4.32), FSH(6.60→2.92), ACTH(2.84→7.90), Cortisol(0.90→3.06)	1.2
11	fT4 1.02, TSH(2.13→11.81), GH(0.38→1.57), PRL(88.97→>186.0), LH(1.71→9.27), FSH(3.37→10.67), ACTH(56.21→63.19), Cortisol(14.83→20.87)	NA	1.4
12	fT4 1.24, TSH(2.59→10.60), GH(0.06→1.84), PRL(2.52→37.14), LH(7.34→27.46), FSH(22.19→32.01), ACTH(16.76→361.13), Cortisol(3.56→18.25)	fT4 1.20, TSH(0.99→5.69), GH(0.10→1.53), PRL(1.96→48.46), LH(7.49→28.40), FSH(22.47→31.77), ACTH(8.42→115.3), Cortisol(6.72→22.32)	6.7
13	NA	fT4 1.23, TSH(2.89→14.98), GH(0.35→5.62), PRL(9.15→61.04), LH(7.40→49.68), FSH(5.46→11.12), ACTH(11.27→40.15), Cortisol(14.14→21.72)	3.3
14	fT4 0.88, TSH(1.30→4.85), GH(0.32→0.31), PRL(8.91→20.24), LH(1.89→8.56), FSH(5.85→10.18), ACTH(39.84→18.50), Cortisol(23.60→17.53)	fT4 1.00, TSH(3.04→12.39), GH(0.12→2.55), PRL(11.85→22.05), LH(2.31→9.83), FSH(4.25→6.13), ACTH(4.91→15.62), Cortisol(3.61→7.44)	2
15	fT4 0.96, TSH(0.58→4.63), GH(0.57→0.36), PRL(9.13→53.80), LH(27.49→73.55), FSH(77.52→77.12)ACTH(20.60→24.95), Cortisol(20.32→33.56)	NA	1.3
16	fT4 1.13, TSH(0.45→2.69), GH(0.53→4.53), PRL(5.1→61.1), LH(0.94→13.60), FSH(3.0→31.4), ACTH(154.0→62.2), Cortisol(26.8→24.1)	fT4 0.87, TSH(0.66→6.23), GH(0.22→4.39), PRL(8.72→48.43), LH(3.16→25.60), FSH(5.23→11.09), ACTH(5.61→23.56), Cortisol(0.43→5.49)	1.1
17	NA	NA	1.1
18	fT4 1.08, TSH(1.08→10.97), GH(0.97→1.08), PRL(21.39→95.47), LH(1.05→10.57), FSH(2.78→7.34),ACTH(23.52→28.30), Cortisol(14.24→17.38)	fT4 0.88, TSH(1.87→11.48), GH(0.40→0.57), PRL(7.20→14.85), LH(2.16→7.16), FSH(2.51→2.49), ACTH(1.97→2.92), Cortisol(2.74→7.69)	1

CPFT combined pituitary function test; *NA* not available

fT4 free T4 (ng/dl); *TSH* thyrotropin stimulating hormone (μIU/ml); *GH* growth hormone (ng/ml); PRL prolactin (ng/ml)

LH luteinizing hormone (mIU/ml); *FSH* follicle stimulating hormone (mIU/ml); *ACTH* adrenocorticotrophic hormone (pg/ml); Cortisol (μg/dl)

* The patient who underwent reoperation

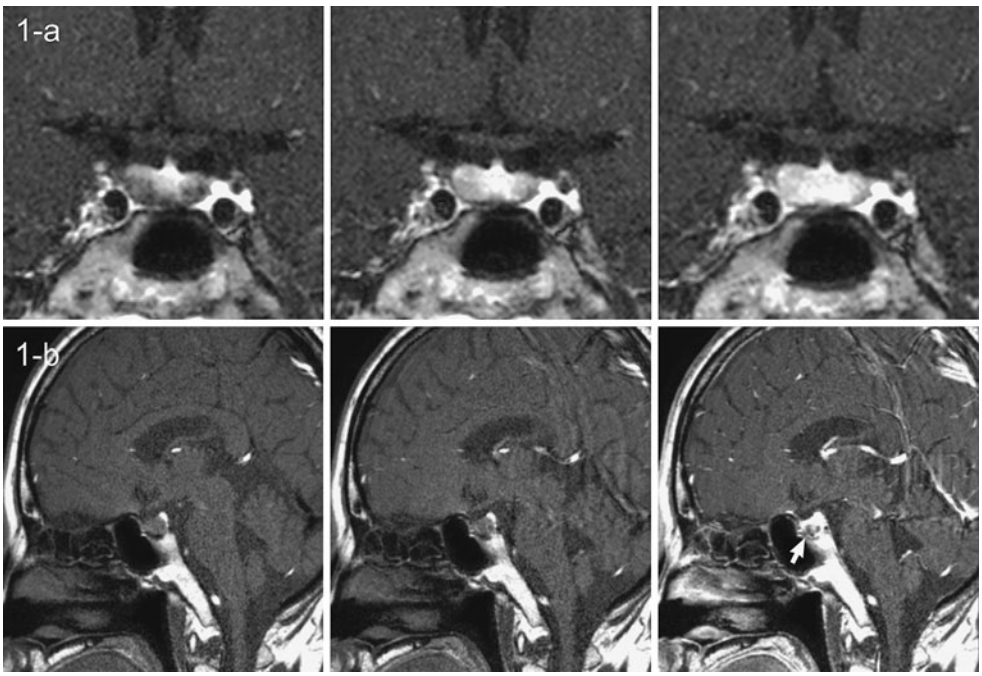

Fig. 1 The example of intraoperative surgical and histological identification (SHI) in patient #15. **a** and **b** is T1-weighted coronal and sagittal dynamic views which were obtained after intravenous gadolinium injection, respectively. The initial coronal dynamic image showed no pituitary adenomas within pituitary gland; however, the second sagittal dynamic image detected the tumor in the anterior portion of pituitary gland (*white arrow* indicates the tumor). The specimens were frozen and sectioned serially, and the surgeon made a drawing of the location of the tissue obtained intraoperatively using a surgical marking pen. The resection margin of the tumor was determined according to the results of frozen sections

et al., surgical removal is crucial for identifying the site of the ACTH-producing tumor [4]. These studies indicate that multiple-staged resection and tumor tissue identification (i.e., SHI) is useful for localizing corticotroph adenomas and for diagnosing CD. The usefulness of SHI for localizing tumors has received little attention in previous studies.

Some studies reported that histologic evidence of an ACTH-secreting adenoma occurs less frequently in MRI-negative patients [38–40]; these results differ from ours showing no significant difference between the MRI-positive and MRI-negative groups. We verified the presence of adenomas using SHI in all patients, although the tumors were confirmed in 16 patients (88.9%) in the final pathology reports. In case of patient #2, left-sided tumor was identified and removed during TSS. SHI using frozen biopsy confirmed tumor tissues, however, the corticotroph adenoma was not confirmed in permanent pathological specimen. It was probably because the resected tumor tissues were insufficient or lost during the procedure. The error of pathological tissue preparation can also lead to this result due to minute tumor tissues of CD. This patient achieved remission of clinical signs and laboratory findings. That means SHI is particularly useful for localization and management of corticotroph adenomas with discordant findings.

In addition, Testa et al. [39] reported that pathologic findings may not predict outcome after surgery in some patients with CD. However, regardless of whether microadenomas can be identified using MRI, the remission rate (100%) in our patients was higher than the rates of 59–93.7% reported previously [29]. The authors have been used SHI technique in other endocrine active pituitary tumors. In our previous report [15], we found tumor tissue infiltration frequently on the pseudocapsule with SHI technique and it was very helpful to achieve high rate of surgical remission especially in growth hormone secreting tumor. Moreover, because most of the ACTH-secreting tumors are microadenoma and it is very difficult to identify from the surrounding normal pituitary gland, it should be investigated more aggressively. Considering these aspects, SHI would be clearly useful and effective in achieving surgical remission in patients with CD.

In conclusion, even if MRI and IPSS are of limited value, a multidisciplinary approach is still required for localizing pituitary adenomas [29]. In this context, high-resolution MRI may be better than IPSS for localizing ACTH-secreting tumors. In patients with no definite

finding of a lesion on the initial MRI, performing a second MRI using a different protocol may be helpful. SHI may be a better method for localizing tumors accurately, especially in patients with microadenomas that are not visible on high-resolution MRI.

Disclosure statement The authors have nothing to disclose.

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