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Therapeutic outcomes in patients undergoing surgery after diagnosis of Cushing's disease: A single-center study

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Abstract. This study aimed to investigate early and late outcomes of patients who underwent neurosurgical procedures for the preoperative diagnosis of Cushing's disease (CD). Clinical, endocrine, imaging, and histologic data from 252 patients undergoing pituitary surgery at Toranomon Hospital through the end of 2012 were entered into a database and statistically analyzed. In 22 of these patients (8.7%; positive venous sampling in 15 and negative venous sampling in 7 patients), tumors were invisible on magnetic resonance imaging (MRI) and 42.9% of them achieved remission. In the remaining 230 patients, 93.5% of those with microadenomas (n=154) and 71.1% of those with macroadenomas (n=76) achieved early postoperative remission, with recurrence rates of 2.7% and 14.8%, respectively, during a 72.5-month median follow-up. In multivariate analyses, cavernous sinus invasion (CSI; odds ratio [OR], 13.0), type of surgery (OR, 4.0), and tumor size (OR, 2.7) were significant preoperative factors affecting early postoperative results, whereas peak cortisol levels ≥9.4 μg/dL in response to corticotropin-releasing hormone (CRH) and CSI were significant factors predicting recurrence. Tumor recurrence was more common in patients with non-densely granulated adenomas than in patients with densely granulated adenomas. We propose that the higher remission and lower recurrence rates in this series are due to our surgical strategies, including extracapsular tumor removal, aggressive resection of tumors with CSI, extended transsphenoidal surgery (TSS), or a combined approach for large/giant adenomas. Appropriate multimodal treatments, including radiotherapy, medication, and repeated surgery in patients with persistent or recurrent CD, could result in better overall outcomes than previously achieved.

Key words: Cushing's disease, Transsphenoidal surgery, Long-term results, Temozolomide, Radiosurgery

CUSHING'S DISEASE (CD), which is caused by an ACTH-secreting pituitary adenoma (ACTH adenoma), is considered an aggressive pituitary endocrinopathy due to devastating effects that result from untreated hypercortisolemia [1, 2]. Although they are histologically benign, these ACTH adenomas are associated with significant morbidity and premature death [1, 2]. Currently, CD remains primarily a surgical disease, with remission rates ranging from 65% to 95% following microscopic or endoscopic transsphenoidal surgery (TSS) [2-4]. Some patients, however, will not achieve sustained remission after TSS and can exhibit persistent or recurrent CD, which is more challenging for neurosurgeons and endocrinologists and requires multimodal treatment to achieve remission. In these patients, treatment options include irradiation therapy, medical therapy, or repeat surgery [3, 5]. However, some patients may ultimately require bilateral adrenalectomy to control unresponsive severe hypercortisolism. Others may develop deadly pituitary carcinoma despite undergoing multidisciplinary treatments. We herein report our surgical results and treatment outcomes for patients with persistent or recurrent CD after unsuccessful surgery. Our single center series consisted of 252 consecutive patients who underwent surgery after the diagnosis of CD.

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Materials and Methods

Patient population

A total of 307 patients were operated on by the same

surgeon (SY) after CD diagnosis at Toranomon Hospital between 1988 and 2014. Among them, 252 consecutive patients, including 18 patients <20 years of age, underwent pituitary surgery until the end of 2012. All patients were followed up at least two years [median follow-up after surgery was 72.5 months and interquartile range (IQR) was 41-106 months]. The median age at surgery was 42.7 years (range: 7-81 years), with a male to female ratio of 1: 5.8. Of these 252 patients, tumors were not visible on extensive preoperative MRI studies (including 3T MRI) in 22 patients (8.7%), whereas microadenoma or macroadenoma was suspected in the remaining 230 patients based on MRI (154 microadenoma patients and 76 macroadenoma patients). These 230 cases included two ectopic microadenomas located within the cavernous sinus (CS) [6], three double adenomas associated with nonfunctioning macroadenoma, GH and PRL microadenoma, and PRL microadenoma [7], two invasive macroadenomas that progressed to pituitary carcinoma associated with liver metastases or dissemination to the central nervous system [8]. However, there were no tumors with Nelson syndrome in this series. The ethics committee at Toranomon Hospital, Tokyo, approved this study (Acceptance No. 706).

MRI analyses and tumor size

MRI was performed using a 1.5 T scanner (Magnetom Symphony Syngo MR, Siemens). Conventional T1-weighted spin echo and T2-weighted turbospin echo sequences were obtained in the coronal, sagittal, and axial planes with 2-mm-thick interleaved sections without a pre-gadolinium gap. After contrast enhancement, spoiled gradient-recalled acquisition (SPGR) with contiguous 1.5-mm-thick sections and/or dynamic study with contiguous 2-mm-thick sections, as well as T1-weighted coronal, sagittal, and axial MRI, were obtained. In addition, MRI examinations using a 3.0-T scanner (Achieva, Philips Medical Systems, Best, The Netherlands) have been performed since 2007. The maximum size of each tumor was measured, and classified as a microadenoma (maximum diameter <10 mm) or a macroadenoma (maximum diameter ≥10 mm). In this study, the residual or recurrent tumor was also defined as a microadenoma or a macroadenoma based on the maximum diameter before a repeat surgery.

Simultaneous bilateral IPSS and criteria of central origin of hypercortisolemia

Each of the 22 patients with MRI-invisible tumors underwent inferior petrosal sinus sampling (IPSS) to confirm the source of ACTH overproduction according to our previously described protocol [9]. Inferior petrosal sinus/peripheral (IPS/P) ratio ≥2.0 in the basal state, or a peak ratio ≥3.0 after corticotrophin releasing hormone (CRH) administration, was considered CD [10]. CD was also diagnosed if the normalized ACTH/PRL IPS:P ratio was ≥1.3, suggesting central origin of hypercortisolemia [11], despite the patient did not meet the other two criteria. In addition, positive lateralization was defined when the side-to-side ACTH ratio was ≥1.4 [10].

Surgical procedures

Endoscopy was used as an adjuvant to microscopic surgery after 2000, and pure endoscopic surgery has been advocated since 2012. The same transsphenoidal surgical (TSS) procedure was used in all cases regardless of microscopic or endoscopic surgery. The pituitary gland was exposed horizontally to both cavernous sinuses and vertically to both intercavernous sinuses. When a tumor was identified in surface view after dural opening, selective adenomectomy was performed with additional removal of normal pituitary around the adenoma. Tumors were aggressively attacked and removed as much as possible even when they showed CS invasion (CSI) by wide opening of the sella to expose the sinus floor. CSI was strictly assessed by direct observation of the entire medial wall when the tumor was dissected and excised from the medial wall of the cavernous sinus. In addition, we performed extended TSS or used a simultaneous combined transsphenoidal and transcranial approach to remove large/giant multilobulated invasive adenomas [12]. In the current series, extended TSS was performed in three patients, the simultaneous combined approach was performed in two, and TSS followed by craniotomy was performed in one patient. When no adenoma was observed in the surface view after dural opening, a microsurgical approach was adopted as described in our previous report [9]. When an adenoma was still not detected after careful search of adenoma, hemihypophysectomy of the side suspected by IPSS was typically performed. Total hypophysectomy leaving the posterior gland was performed in only one of the earliest cases in this series.

Remission and recurrence criteria

No perioperative hydrocortisone was administered. Serum cortisol levels were assayed the morning after

surgery to determine the necessity for steroid replacement within days. Biochemical investigations were performed 2 weeks after surgery. Postoperative corticotropic function was assessed 3 months after surgery. Patients were considered in complete remission (CR) when following conditions were fulfilled: 1) the tumor was completely removed during surgery, which was confirmed by postoperative MRI, and 2) hypocortisolism (nadir level obtained after surgery <3 μg/dL) requiring steroid replacement, or normal suppression to 1 mg low-dose dexamethasone tests (nadir cortisol <3 μg/dL) and normal midnight cortisol level <5 μg/dL regardless of serum cortisol levels. Persistent normal cortisol levels with normal suppression to low-dose dexamethasone tests without recurrence of CD symptoms was considered remission in long-term assessments. In contrast, CD was considered persistent or recurrent in patients who did not meet the above-mentioned criteria. Early postoperative endocrine conditions were assessed by our endocrinologists. Serum ACTH and cortisol levels were measured using Elecsys ACTH (Roche Diagnostics K.K., Tokyo, Japan) and ST AIA-PACK CORT (Tosoh Corporation, Tokyo, Japan), respectively. Moreover, postoperative endocrine functions were also assessed using TRH, GnRH, and GRH or GHRP administration 2 ~ 3 weeks after surgery.

Pathological studies

Specimens were obtained during surgery, fixed in 10% buffered formaldehyde, dehydrated in graded ethanol, embedded in paraffin, and studied using routine histological methods, including immunohistochemistry. Microsections were stained with hematoxylin-eosin. Immunocytochemical studies were performed as previously described [13]. Ki-67 labeling index (LI) immunostaining was performed using antibodies [MIB-1; clone 30-9 (prod. no 790-4286, Ventana)] to assess tumor aggressiveness. We counted a mean of 1000 tumor cells per case. Results are expressed as a percentage of tumor cells with positive nuclei. Only nuclei with a strong positive label were counted. For electron microscopy, samples were fixed in 2.5% glutaraldehyde for a few hours, osmicated, and embedded in an epoxy mixture. All obtained adenomas were histologically investigated or reviewed by the same pathologist (NI).

Statistical analyses

Data are expressed as the mean ± standard deviation (SD) in normally distributed samples and the median ± interquartile range (IQR) in non-normally distributed samples. Statistical analyses were performed using JMP software (version 9.0.2; SAS Institute Inc. Cary, NC, USA). Logistic regression analyses were performed for all continuous data. Categorical variables were analyzed using Chi-square tests. Multivariate stepwise logistic regression analyses were performed to detect significant independent factors using factors that had significant associations in univariate analyses. P <0.05 was considered statistically significant.

Results

MRI-invisible tumors

No definitive adenomas were demonstrated despite extensive MRI examinations in 22 (8.7%) of 252 patients. These 22 patients were diagnosed with CD by their local referral endocrinologists based on clinical findings and were also examined by our endocrinologists to confirm the CD diagnosis. An ectopic source of ACTH hypersecretion was rigorously excluded. Therefore, IPSS with CRH administration was performed in these 22 patients to confirm excess ACTH secretion. Fifteen patients met the CD criteria (positive sampling), whereas seven patients did not meet the criteria (negative sampling). Although no definitive pituitary tumor was detected and the following sampling was negative in seven patients, TSS was finally selected by the patients after adequate informed consent because their endocrine data corresponded to CD and no definite tumors responsible for ectopic ACTH hypersecretion were observed in these patients. Of these seven patients, a tiny adenoma (maximum diameter of 2 mm) was found in only one patient. Complete endocrine remission was obtained in two patients, including one with the apparent tiny tumor found during surgery. In contrast, no responsible tumors were noted in the remaining 5 patients during a mean follow-up period of 6 years. Regarding the other 15 patients with positive IPSS sampling, Cushing's syndrome was cured in one patient after removal of the pulmonary endocrine tumor found 1.5 years after negative exploration of the pituitary. Of the remaining 14 patients, complete remission was achieved in seven patients (adenomas ranging from 2-4 mm in diameter were found in six patients during surgery, whereas no adenoma was detected in one patient). Interestingly, in one patient, a tumor was found in the inner wall of the left CS that was completely separated from the pituitary gland, thus

suggesting an ectopic pituitary adenoma [6]. In contrast, there was no postoperative improvement in disease in the remaining seven patients with no adenomas during surgery. All tissues obtained during surgery were submitted to pathologists and examined using histology and immunohistochemistry. No tumors or hyperplasia were observed in the eight patients that had no tumors found during surgery. There were no major perioperative complications and no postoperative hormonal replacement except in one patient with negative sampling who showed transient cerebrospinal fluid leakage with meningitis and required thyroid hormone replacement after total anterior hypophysectomy. Complete remission was accomplished in nine of the 22 patients and there was no recurrence of CD during a median follow-up of 6.6 years (range, 2-12.5 years). Hypercortisolism was either well controlled (seven patients) or partially controlled (three patients) using metyrapone, mitotane, trilostane, or cabergoline in ten patients with no tumors. However, the remaining two patients needed bilateral adrenalectomy to control CS. One of these patients died in a car accident three years after surgery. The surgical outcome of these 22 patients is summarized in Fig. 1 (some of these data have been previously published [9]).

Surgical outcomes and follow-up results in microadenomas

Of the 230 patients (except the 22 patients described above), 154 were diagnosed with microadenomas (67.0%) and 76 were diagnosed with macroadenomas (33.0%). Of 154 patients with microadenomas, primary surgery or repeat surgery was performed in 134 and 20 patients, respectively. Moreover, CSI was observed in 36 patients (23.4%). Postoperative CR was achieved in 144 of 154 patients (93.5%) whereas CD persisted in the remaining 10 patients. Of these 10 patients, all showed CSI (Knosp grade 2 in four patients, grade 3 in three patients, and grade 4 in three patients) and six underwent repeat surgery at our hospital. In nine patients, CD became well controlled after postoperative treatment (local radiation in one patient, cyber-knife in two patients, cyber-knife plus metyrapone in two patients, cyber-knife plus octreotide LAR in one patient, cabergoline in one patient, repeated surgery in one patient, and follow-up without any treatment in one patient). In the final patient, CD has not been controlled even after postoperative cyber-knife followed by cabergoline.

Of 144 patients judged as CR after surgery, recurrence occurred in four patients 2 years, 3 years, 7 years, and 13 years after surgery, respectively. Repeated surgery was performed in all four patients and CR was achieved again in three patients and has been maintained for 2.5 and 7 years in two patients, whereas one patient again showed CD recurrence two years after remission obtained by repeated surgery followed by cyber-knife. In contrast, the disease persisted in the remaining one patient because no tumor was found during repeated surgery.

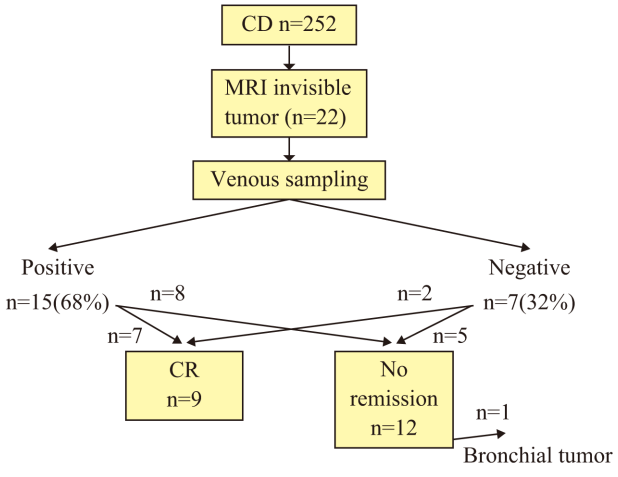

Fig. 1 Flow diagram summarizing surgical outcomes and inferior petrosal sampling (IPSS) results in 22 patients with invisible adenoma on preoperative MRI out of 252 patients undergoing pituitary surgery for a CD diagnosis. n, number; CR, complete remission.

Surgical outcomes and follow-up results in Macroadenoma

Of 76 patients with macroadenoma, extended TSS was performed in three patients, a combined approach was used in two patients, and TSS followed by craniotomy was employed in one patient. CSI was found in 47 (61.8%) of these 76 patients.

CR was accomplished in 54 (71.1%) patients, whereas CD persisted in 22 patients after surgery. All patients showed tumor invasion into the CS to some extent, with Knosp grade 4 in 17 patients, grade 3 in three patients, and grade 2 in two patients. Moreover, surgery performed at our institute was repeated surgery in 9 of these 22 patients due to persistent or recurrent tumors after prior surgery performed at other hospitals. Of these 22 patients, three died of pneumonia, systemic fungal infection, or renal failure within one year after surgery. One patient underwent surgery four times

with local radiation, including γ-knife. This tumor progressed to carcinoma that resulted in death 9 years after the first operation. Of the remaining 18 patients, CD was well controlled in 16 patients. Radiotherapy was used in 10 patients (local radiation in three patients and radiosurgery in seven patients), radiation followed by temozolomide (TMZ) was used in four patients, radiation followed by cabergoline was used in one patient, and pasireotide was used in one patient. CD persisted in one patient after radiosurgery and metyrapone due to poor compliance. In addition, in one patient where TSS was performed three times at another hospital and twice at our hospital, serum basal cortisol levels decreased to within a normal range. This patient was followed up without any adjuvant treatment. However, CD recently recurred (two years after surgery) and the patient is on a waiting list for surgery. In four patients treated with TMZ, all had invasive macroadenomas: one had carcinoma with liver metastases, two were densely granulated ACTH adenomas (Ki67 LI of 3.6 and 3.5, respectively), and one was Crooke's cell adenoma (Ki67 LI of 0.6).

Of 54 patients that had CR after surgery, eight patients experienced recurrence: one patient had recurrence after 1 year, two patients after 3 years, one patient after 4 years, three patients after 6 years, and one patient after 9 years. Repeat surgery followed by radiosurgery was performed in three of these patients and CR was again achieved. Radiosurgery only was used in three patients and resulted in CR. In the remaining two patients, repeat surgery followed by pasireotide and radiosurgery was performed in one patient; however, control of CD was not achieved. However, CR was finally obtained after metyrapone administration in this patient. In contrast, the final patient showed CD recurrence one year after surgery and was treated with cabergoline. At present, CD is not well controlled in this patient.

Short and long-term postoperative outcomes of these 230 patients with MRI-visible tumors are summarized in Fig. 2.

Analyses of preoperative factors that influence postoperative results

Various preoperative variables, including age, sex,

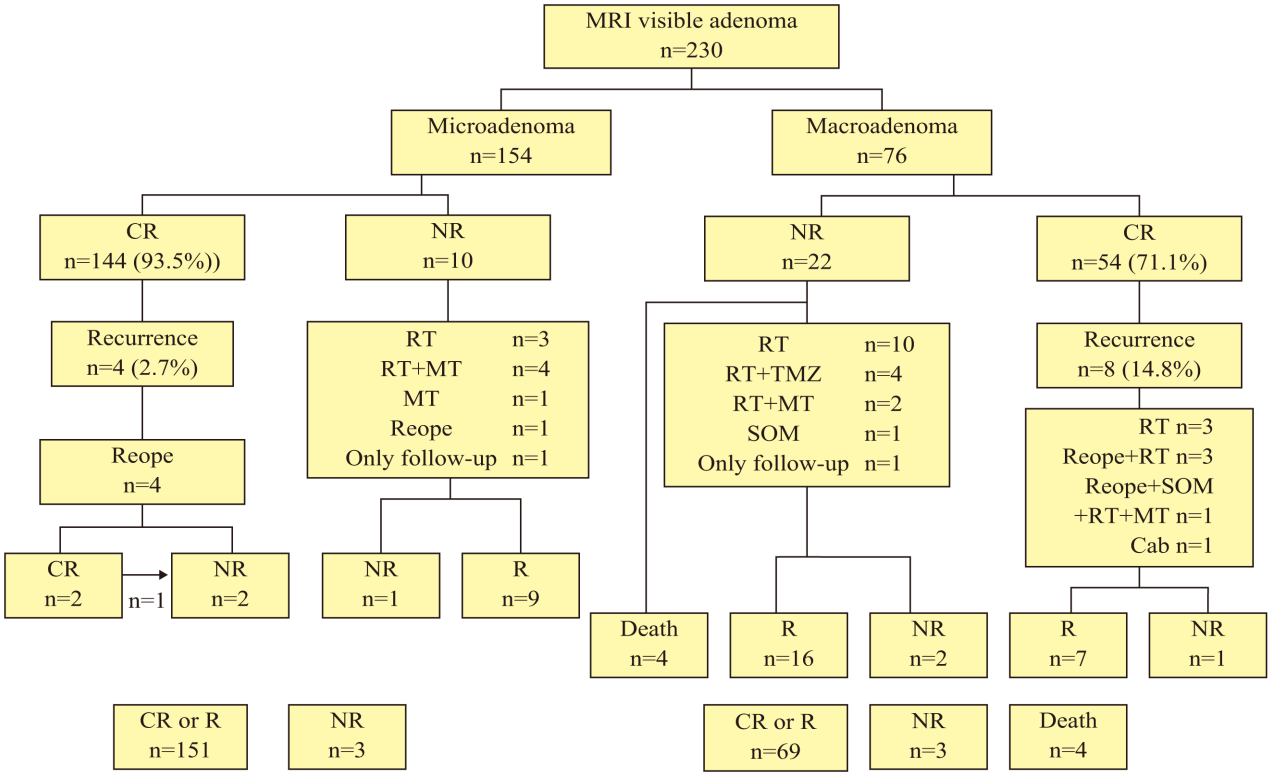

Fig. 2 Flow diagram summarizing short and long-term postoperative outcomes of 230 patients with MRI-visible adenoma. CR, complete remission; R, remission; NR, no remission (persistence of CD); RT, radiation; MT, medication; Reope, reoperation; TMZ, temozolomide; SOM, SOM230; Cab, cabergoline; n=number of patients.

tumor size, cavernous sinus invasion, and type of surgery, were analyzed to determine preoperative factors that influence postoperative surgical outcome (Tables 1 and 2). These patients were classified into two groups based on surgical results: the successful group fulfilled the criteria for remission (198 patients) and the unsuccessful group had persistent CD (32 patients). Univariate analyses identified the four factors that significantly correlated with surgical outcome: age, tumor size, cavernous sinus invasion, and type of surgery (primary *vs*. repeat surgery) (Table 1). According to multivariate analyses, cavernous sinus invasion was the most unfavorable preoperative factor affecting the surgical result. However, the type of surgery and tumor size were also significant independent predictors of early postoperative outcome (Table 2).

Analyses of postoperative factors that influence long-term surgical results

Among 198 patients who achieved early postoperative remission, 12 patients showed recurrence of CD after an average of 5.3 years after surgery (range, 1-13 years) during a median follow-up period of 6 years Various factors affecting long-term surgical results were also analyzed, including age, sex, type of surgery, tumor size, cavernous sinus invasion, early postoperative lowest ACTH and cortisol levels, and peak ACTH and cortisol levels in CRH loading tests (Tables 1 and 2). Univariate analyses indicated the following factors significantly correlated with long-term surgical outcome: tumor size, cavernous sinus invasion, postoperative lowest ACTH levels, postoperative lowest cortisol levels, peak ACTH levels after CRH loading, peak cortisol levels after CRH loading, and histological type (Table 1). According to multivariate analyses, peak cortisol levels ≥9.4 μg/dL after CRH loading (cut off value was determined by receiver operating characteristic analyses) were the best predictor of tumor recurrence (sensitivity 64%, specificity 88%). Cavernous sinus invasion was also a significant independent predictor of tumor recurrence (Table 2).

Histological findings

Of 230 patients, histological data, including immunohistochemistry and electron microscopy, were available for 222 patients. ACTH producing adenomas were confirmed in all cases examined and no patient showed ACTH hyperplasia in this series. Densely granulated ACTH cell adenoma (DG adenoma) was diagnosed in 200 patients, sparsely granulated ACTH cell adenoma (SG adenoma) in two patients, Crooke's cell adenoma (CCA) in 15 patients, and oncocytic ade-

Table 1 Factors influencing surgical results (univariate analyses)

Factor	Successful surgery	Unsuccessful surgery	
Number of cases	n=198	n=32	*P* value
Age median (IQR, years)	42 (33-54)	52 (38-60)	0.004
Sex (female/male)	170/28	28/4	0.803
Type of surgery (primary/repeat)	175/23	17/15	<0.001
Tumor size (micro/macro)	144/54	10/22	<0.001
Cavernous sinus invasion (+/-)	50/148	29/3	<0.001
	Persistence of remission	Tumor recurrence	*P* value
Number of cases	186	12	0.382
Age (mean ± SD, years)	42±13.6	42±18.5	0.585
Sex (female/male)	161/25	9/3	0.265
Type of surgery (primary/repeat)	166/20	9/3	0.135
Tumor size (micro/macro)	140/46	4/8	0.002
Cavernous sinus invasion (+/-)	42/144	8/4	0.001
Postoperative factors			
Lowest ACTH level*	5.0 (2.0-5.6)	11.1 (5.3-22.1)	<0.001
Lowest cortisol level*	1.0 (0.5-1.7)	1.4 (1.1-2.9)	0.011
Peak ACTH level (CRH test)*	15.2 (6.7-35.5)	51.4 (39.4-83.1)	0.001
Peak cortisol level (CRH test)*	2.5 (1.1-5.9)	12.0 (4.7-17.5)	0.001
Histological type (densely/others)	163/9	8/4	<0.001
Mib-1 index*	0.9 (0.3-2.5)	1.4 (0.3-8.1)	0.410

IQR, interquartile range; micro, microadenoma; macro, macroadenoma; SD, standard deviation. * values are expressed as median (IQR), a unit of ACTH (pg/mL), a unit of cortisol (μg/dL), densely: densely granulated ACTH adenoma. Others include sparsely granulated ACTH adenoma, Crooke's cell adenoma, and oncocytic ACTH adenoma.

noma (OCA) in five patients. Tumor recurrence was significantly more common in non-densely granulated adenomas compared to densely granulated adenomas (p <0.001). However, Ki67 LI was not significantly different between patients with successful and unsuccessful surgical outcomes (p=0.257) or noninvasive and invasive tumors (p=0.460). Details of the relationship between each histological type and clinical characteristics are summarized in Table 3.

Pituitary function

In our series, the frequencies of early postoperative hypopituitarism of any degree other than ACTH was 27% (41/154) in microadenomas and 47% (36/76) in macroadenomas, indicating a significantly lower rate in microadenomas compared to macroadenomas (P=0.002). Hypopituitarism included GH [18% (28/154) in microadenomas, 36% (27/76) in macroadenomas], LH/FSH [9% (14/154) in microadenomas, 24% (18/762) in macroadenomas] and TSH deficiencies [8% (13/154) in microadenomas, 21% (16/76) in macroadenomas]. These anterior hormone deficiencies were assessed in response to each provocation test. However, the number of patients requiring hormonal replacement was small.

Surgical complications other than endocrine disturbance

There was no mortality associated with surgery. However, internal carotid artery (IC) injury was occurred in two patients with Knosp grade 4. High flow bypass was performed immediately after repeated surgery in one patient who had been operated on twice at another hospital. Blindness in the right eye occurred after bypass surgery. Fortunately, the other patient could tolerate IC occlusion without any neurological deficit. Permanent oculomotor nerve palsy occurred in one patient when the invasive macroadenoma was removed using the simultaneous combined approach. In addition, transient III rd and VI nerve palsy or VI nerve palsy were complications in one patient each. Moreover, one patient experienced a transient postoperative cerebrospinal fluid leak that was resolved by lumber drainage. One patient showed permanent severe visual disturbances due to candida retinitis that occurred one week after surgery.

Discussion

According to the present treatment algorithm, TSS remains the first choice of treatment for CD [14]. It has been reported that surgical results depend on tumor size and extension, adenoma visibility on preoperative MRI, and neurosurgical expertise [15-17].

MRI-invisible tumors

The frequency of MRI-invisible adenomas (8.3%) in this series was lower than what has previously been reported (17-63%) [15-17]. This is likely due to our extensive MRI examinations using high-field-

Table 2 Factors influencing surgical results (multivariate analyses)

Early postoperative results	Category	Odds ratio (95% CI)	*P* value
Type of surgery	Repeat/primary	4.0 (1.5-10.9)	0.006
Size of tumor	Macro/micro	2.7 (1.0-7.4)	0.046
Cavernous sinus invasion	+/−	13.0 (3.5-47.5)	<0.001
Long-term results	Category	Odds ratio (95% CI)	*P* value
Peak cortisol level (post operative CRH test)	≥9.4/<9.4 μg/dL	10.3 (2.0-53.0)	0.005
Cavernous sinus invasion	+/−	6.6 (1.0-18.4)	0.020

CI, confidence interval; micro, microadenoma; macro, macroadenoma

Table 3 The relationship between histological subtypes and clinical characteristics

Subtype	No. of cases	Age Mean±SD	Sex Female/Male	Micro/macro	CS invasion No. (%)	Persistent No. (%)	Recurrence No. (%)	Mib-1 index Median (IQR)
DG adenoma	200	42±14.3	175/25	140/60	63 (32)	22 (11)	8 (4)	0.9 (0.2-2.7)
SG adenoma	2	62,64	2/0	1/1	2 (100)	1 (50)	1 (50)	0.3,2.9
CC adenoma	15	51±13.6	11/4	4/11	10 (67)	6 (40)	1 (7)	1.4 (0.7-4.75)
O adenoma	5	51±23.5	4/1	1/4	2 (40)	1 (20)	2 (40)	3.2 (1-5.6)

CS, cavernous sinus; Micro, microadenoma; Macro, macroadenoma; IQR, interquartile range; DG, densely granulated; SD, sparsely granulated; CC, Crooke's cell; O, oncocytic

strength MRI with various methods including SPGR and/or dynamic study with 1.5-2.0 mm thick sections. It remains controversial whether negative MRI affects surgical results [16, 18-20]. The remission rate of our 21 MRI-invisible patients (45%) was significantly lower than that of MRI-visible microadenomas (93.5%), which is in agreement with previous studies [18-20]. In our previous report, we concluded that TSS should be considered the first-line treatment in MRI-invisible patients if IPSS suggests pituitary adenoma, whereas other therapeutic options must be considered in patients with negative IPSS [9]. However, remission was obtained in two of seven patients (28.6%) with both MRI and IPSS negative findings in our current study. Therefore, in accordance with another report [21], transsphenoidal exploration may be considered even in MRI-invisible and IPSS-negative adenomas if they meet the following requirements: if no ectopic source can be identified after further body imaging and the patients agree with surgery after informed consent. In our institute, IPSS has been performed only in patients whose pituitary adenomas are invisible even after extensive MRI studies.

Remission and recurrence criteria

The wide range of remission and recurrence rates previously reported may be due to different remission and recurrence criteria used in the studies. In addition, there is no agreement on the definitions of these outcomes [22]. In this series, the following conditions were also judged as early postoperative CR: no remaining tumor mass with normal suppression to 1 mg low-dose dexamethasone tests (nadir cortisol <3 μg/dL) and normal midnight cortisol levels <5 μg/dL irrespective of morning cortisol basal levels. This condition occurred in some cases with long-term preoperative metyrapone treatment. It is important to be careful in assessing remission using early postoperative cortisol and ACTH levels when the patient has received long-term pretreatment with cortisol-lowering drugs such as metyrapone or ketoconazole because these medications could result in higher postoperative basal and CRH-stimulated cortisol levels due to early recovery of suppressed non-tumor corticotropic cells and subsequent restoration of pituitary-adrenal function [23].

Postoperative remission of microadenomas and macroadenomas

The early postoperative remission rate was 93.5% in microadenomas and 71.1% in macroadenomas in this series. These remission rates were higher than those of other previous studies [15, 16, 19, 20, 24]. Moreover, our overall remission of 86.1% is also higher than those recently reported [15, 16, 19, 20, 25, 26], particularly considering the high prevalence of macroadenomas (31%) in this series. The higher incidence of macroadenomas in this series is primarily because our department serves as a referral center for difficult and complicated cases. The higher remission rates achieved might be attributed to the following our surgical strategies: adenomectomy with pseudocapsule, removal of the normal pituitary surrounding the tumor when it does not have definite pseudocapsule, aggressive attack of the tumor with CSI, and adequate extensive approaches for large or giant lobulated adenomas. Additionally, all procedures were performed by an experienced surgical team. Petersenn *et al.* reported in their meta-analysis that the highest remission rate was achieved when 36–40 patients were operated on per year of study duration with a slight upward trend in remission rates observed with increasing surgical experience [22].

Our current univariate analyses also highlighted factors affecting surgical results that were previously reported [9, 14-18, 20]. In addition, our current multivariate analyses indicated that CSI, type of surgery, and tumor size were independent unfavorable factors. Patil *et al.* also reported that the odds ratio of failure to achieve remission for patients with repeat TSS for recurrent CD were 3.7 times that of patients undergoing first-time TSS, which is similar to the odds ratio to our series [27].

Tumor recurrence during follow-up

A recent meta-analysis reported recurrence rates of 15.7% (11.5–47.4%) using biochemical assays only and 14.4% (5.0–20.8%) using a combination of clinical and biochemical endpoints with a mean time to recurrence of 50.8 months (range 3–158 months) [22]. These authors also reported a slightly higher, but not statistically significant, recurrence rate of 17.6% in patients with macroadenomas compared with those (13.4%) with microadenomas [22]. In our study, 2.7% of patients with microadenomas and 14.8% of patients with macroadenomas with early postoperative remission showed recurrence after an average of 63.6 months (range, 12-156 months) during a median follow-up period of 6 years. This lower recurrence rate, particularly in microadenomas, is likely due to our

aggressive surgical strategies, including extracapsular removal with additional removal of adjacent normal pituitary tissue and removal of the inner medial wall of the cavernous sinus when CSI was found. There have been many reports studying predictive factors for CD recurrence after TSS [16, 28-33]. Our current multivariate analyses confirmed CRH-stimulated peak serum cortisol levels ≥9.4 μg/mL and CSI were significant independent unfavorable predictors of recurrence. However, others have claimed that long-term recurrence may still occur and thus lifetime clinical and biochemical follow-up is necessary, even when strict cure criteria are applied [16, 30, 32].

Postoperative treatment after unsuccessful TSS or CD recurrence

Persistent or recurrent CD after unsuccessful TSS requires multimodal treatment to achieve remission. In these patients, options for treatment include repeat TSS, radiation therapy, and medical therapy [34]. Despite undergoing multiple treatment modalities, some patients may ultimately require bilateral adrenalectomy for definitive treatment to eliminate hypercortisolemia associated with CD [34]. In this series, only two patients, whose CD persisted after negative pituitary exploration, underwent bilateral adrenalectomy without the development of Nelson syndrome. Our principle for the treatment of persistent or recurrent CD after surgery is as follows: 1) reoperation for persistent or recurrent CD in which a distinct tumor is located in a surgically accessible area [35], 2) radiosurgery with or without subsequent medication for the tumor in which surgical removal looks impossible, and 3) various appropriate medications for persistent or recurrent CD without any MRI-visible tumors. In our current series, higher control rates have been achieved in persistent or recurrent CD after unsuccessful surgery. It should be noted that both CD and tumor mass have been controlled well in four patients (one carcinoma and three invasive atypical macroadenoma) treated with TMZ [8].

Postoperative pituitary functions and surgical complications

In our series, the frequencies of early postoperative hypopituitarism of any degree other than ACTH was 27% in microadenomas and 47% in macroadenomas, indicating a significantly lower rate in microadenomas compared to macroadenomas. Hypopituitarism included GH, LH/FSH, and TSH deficiencies compared favorably with those reported in previous studies [15, 36].

There was no mortality associated with surgery. However, internal carotid artery injury occurred in two patients with Knosp grade 4, one of whom needed high flow bypass. This suggests the importance of preoperative angiography perfusion analysis by balloon occlusion in patients with Knosp grade 4 to predict whether carotid artery occlusion can be tolerated [37]. The severe complication rate in this series was slightly less than a previous report [38]. In general, patients with CD may have higher post-TSS complication risks than patients with other pituitary tumors [39].

Pathological findings

CD pathology can be divided into several types, including densely granulated ACTH adenoma, sparsely granulated ACTH adenoma, and Crooke's cell adenoma [40, 41]. In this study, tumor recurrence was significantly more common in non-densely granulated adenomas than in densely granulated adenomas, although multivariate analyses could not indicate that histological type was a significant independent predictor of tumor recurrence. In contrast, Ki67 LI was not significantly different between non-recurrent and recurrent tumors or noninvasive and invasive tumors, although there was a tendency toward higher Ki67 LI in recurrent tumors or invasive tumors.

We propose that our surgical strategies, including extracapsular tumor removal, aggressive attack of the tumors with CSI, extended TSS, and a simultaneous combined approach for large/giant adenomas, enable us to achieve higher remission and lower recurrence rates in this series. Moreover, appropriate multimodal treatments, including radiotherapy, medication, and repeat surgery for patients with persistent or recurrent CD, could result in better overall outcomes compared to those previously reported. Interestingly, both CD and tumor mass have been controlled in four patients treated with TMZ. However, long-term recurrence may still occur and lifetime clinical and biochemical follow-up is necessary, even when the strict cure criteria are used.

Disclosure

The authors have no potential conflicts of interest associated with this study.

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