ORIGINAL ARTICLE/*ARTYKUŁ ORYGINALNY*

The relationship between efficacy of surgical treatment of Cushing disease and pathological – immunohistochemical and ultrastructural – confirmation of corticotroph tumour presence

Związek skuteczności operacyjnego leczenia choroby Cushinga z patomorfologicznym – immunohistochemicznym i ultrastrukturalnym – potwierdzeniem obecności gruczolaka kortykotropowego przysadki

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Abstract

Background and purpose: The most common cause of Cushing disease (CD) is ACTH-secreting pituitary adenoma. Transsphenoidal selective adenomectomy is the treatment of choice. Frequency of remission varies from 60% to 90%, depending on the site and the surgeon's experience. This study aims to answer the question whether confirmation of corticotroph adenoma in pathological examinations increases the probability of surgical cure for CD.

Material and methods: The prospective study involved 36 patients with CD operated on with the transsphenoidal approach and followed up for at least 18 months. Following the surgical procedure, the specimen was examined by a pathologist. The study evaluated the significance of positive histological (immunohistochemical and ultrastructural) examination results for achieving surgical cure for CD.

Results: Twenty-three of 36 patients (63.9%) were regarded as being surgically cured of CD. Persistent CD was confirmed in 13 patients (36.1%). Pituitary insufficiency was

Streszczenie

Wstęp i cel pracy: Najczęstszą przyczyną choroby Cushinga jest gruczolak przysadki wydzielający kortykotropinę (ACTH), a leczeniem z wyboru – przezklinowa selektywna adenomektomia. Częstość remisji waha się od 60% do 90% w zależności od ośrodka i doświadczenia operatora. Celem pracy była odpowiedź na pytanie, czy stwierdzenie gruczolaka kortykotropowego w badaniu histopatologicznym, immunohistochemicznym i ultrastrukturalnym wiąże się z większym prawdopodobieństwem operacyjnego wyleczenia choroby Cushinga.

Materiał i metody: Prospektywne badanie obserwacyjne, do którego włączono 36 kolejnych pacjentów z chorobą Cushinga operowanych z dostępu przezklinowego, a następnie obserwowanych przez co najmniej 18 miesięcy. Doświadczony patolog ocenił materiał operacyjny. Przeanalizowano związek dodatniego wyniku badania histologicznego, immunohistochemicznego i ultrastrukturalnego z klinicznymi wskaźnikami operacyjnego wyleczenia choroby Cushinga.

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found in 5 patients (13.9%), whereas 3 patients (8.3%) were diagnosed with diabetes insipidus. A significant difference was demonstrated between the cured and the non-cured group with reference to the results of pathological examination of surgical specimens. Pathomorphological confirmation of corticotroph adenoma was significantly more frequently observed in the cured group in comparison with the non-cured group ($p = 0.028$).

Conclusions: Pathological confirmation of corticotroph pituitary adenoma may be regarded as an important predictor of the surgical cure of CD.

Key words: Cushing disease, transsphenoidal surgery, corticotrophic adenoma, immunohistochemistry, electron microscopy.

Wyniki: Za wyleczonych operacyjnie z choroby Cushinga uznano 23 z 36 badanych pacjentów (63,9%). U 13 osób (36,1%) potwierdzono przetrwałą chorobę Cushinga. Pooperacyjną niedoczynność przysadki stwierdzono w 5 (13,9%), a trwałą moczówkę prostą w 3 przypadkach (8,3%). Wykazano różnicę pomiędzy grupą wyleczoną i niewyleczoną w odniesieniu do patomorfologicznej oceny materiału pobranego śródoperacyjnie. W grupie wyleczonej istotnie częściej obserwowano obecność histologicznych, immunohistochemicznych i ultrastrukturalnych cech gruczolaka kortykotropowego w porównaniu z grupą niewyleczoną ($p = 0,028$).

Wnioski: Potwierdzenie obecności gruczolaka kortykotropowego przysadki w badaniu patomorfologicznym może być uważane za jeden z czynników rokowniczych operacyjnego wyleczenia choroby Cushinga.

Słowa kluczowe: choroba Cushinga, operacja przezklinowa, gruczolak kortykotropowy, badanie immunohistochemiczne, mikroskopia elektronowa.

Introduction

Cushing disease is a relatively rare endocrine disorder with incidence estimated at 1-3 new cases per million persons per year. In the majority of patients, the cause of the disease is an ACTH-secreting pituitary microadenoma. A macroadenoma is responsible for about 10% of all cases, whereas ACTH hyperplasia without evidence of a pituitary tumour is a particularly rare finding [1-4]. The disease is associated with a significant increase in mortality due to chronic hypercortisolaemia complications such as arterial hypertension, secondary diabetes, osteoporosis, thromboembolic episodes and higher risk of infections. They lead to impaired quality of life and reduced life expectancy. It is estimated that about half of all untreated patients die within five years [1,4].

Currently, transsphenoidal selective adenomectomy is the treatment of choice. The success rate of pituitary surgery in reference centres is high, reaching 60% to over 90% [2,3,5]. It should be expected that a prospective assessment of all potential factors influencing the surgical treatment efficacy will contribute to optimizing the management of Cushing disease with real help towards improving patients' prognosis.

Once the diagnosis of Cushing disease is established, the key to successful treatment is total tumour resection confirmed by a pathologist according to the 2004 WHO classification [6]. Therefore, the aim of the present study was to evaluate the significance of the positive results of histopathological, immunohistochemical and ultrastructural examination of the specimens collected during the transsphenoidal surgery in terms of predicting cure of Cushing disease.

Material and methods

Patient population

The study population comprised 36 consecutive patients with Cushing disease (30 women and 6 men; F : M ratio 5 : 1) hospitalized at the Department of Endocrinology, the Medical Centre of Postgraduate Education from 2005 to 2009. The mean age was 36.3 ± 12.9 years (range 17-57 years). After confirmed diagnosis of Cushing disease, the patients were referred to the Department of Neurosurgery, Military Institute of Medicine in Warsaw. They were all operated on by the same neurosurgeon using the same surgical protocol.

All patients were informed about the aims and methods of the study and they signed the informed consent form. The study protocol was approved by the Bioethics Committee at the Medical Centre of Postgraduate Education in Warsaw.

Clinical course of the disease and preoperative endocrine evaluation

All patients underwent a standard clinical evaluation. Particular attention was devoted to the symptoms

of Cushing syndrome, the previous surgical treatment and complications of chronic hypercortisolaemia.

The diagnosis of ACTH-dependent Cushing syndrome was based on the clinical signs and standard hormonal criteria: increased urinary excretion of urinary free cortisol (UFC) or 17-hydroxysteroids (17-OHCS), increased serum cortisol level at 8.00, the loss of cortisol circadian rhythm (serum cortisol level above 7.5 μg/dL in the late-night hours: 22.00-24.00) and increased or detectable level of plasma ACTH at 8.00, and serum cortisol more than or equal to 1.8 μg/dL following the low-dose dexamethasone suppression test (LDDST; 0.5 mg q.i.d. for 48 hours). The pituitary aetiology of Cushing syndrome was confirmed based on serum cortisol and UFC or 17-OHCS suppression greater than 50% in the high-dose dexamethasone suppression test (HDDST; 2 mg q.i.d. for 48 hours) and a positive result of magnetic resonance imaging (MRI). In case of equivocal results of hormonal assessment and pituitary imaging, the diagnosis of Cushing disease was confirmed by a positive result of the stimulation test with intravenous CRH injection (100 μg).

Preoperative MRI

Prior to neurosurgical treatment, all patients underwent high resolution MRI of the pituitary-hypothalamic region (SIEMENS Symphony 2004; 1.5 Tesla). The MRI scans were performed before and after intravenous injection of gadolinium (Gd-DTPA). A radiologist, neurosurgeon and endocrinologist recorded the presence, size and position of any focal lesion. It was determined that the presence of the hypodense lesion after contrast injection indicates the pituitary adenoma. Microadenoma was defined as a pituitary tumour with a diameter of less than 10 mm in any dimension, whereas macroadenoma was defined as a pituitary tumour having at least one diameter more than 10 mm. The MRI was qualified as equivocal if the pituitary tumour was not precisely visualized or only indirect traits of the tumour were present such as deviation of the pituitary stalk or a convex upper surface of the pituitary gland.

Surgical procedure

In all cases, a microsurgical transseptal transsphenoidal approach was used for resection of an ACTH-secreting pituitary adenoma. The sella was exposed and an H-shaped incision of the dura was made. The dura mater was opened and separated from the pituitary capsule to expose the entire anterior surface of the pituitary gland. Then, the pituitary gland was carefully explored, irrespective of the MRI findings. Selective adenomectomy was performed in all cases of pituitary adenoma visualized on MRI. When the MRI results were either equivocal or no tumour was evident, a series of vertical and horizontal incisions of the pituitary gland was carried out and all tissue that seemed to be abnormal was removed and submitted for pathological examination. In patients with no identifiable abnormal tissue, intraoperative hemihypophysectomy (compared to the corresponding site showing the MRI abnormality), subtotal hypophysectomy, or total hypophysectomy was performed.

Histopathological, immunohistochemical and ultrastructural assessment

The pathological analysis was carried out at the Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology in Warsaw.

The specimens were collected for histopathological analysis and routinely stained with haematoxylin and eosin (H&E). Immunohistochemical staining was performed on paraffin-embedded specimens according to the labelled EnVision Flex Visualisation System (Dako, K8000) with DAB as chromogen using antibodies against all anterior pituitary hormones (GH, PRL, ACTH, β-TSH, β-FSH, β-LH; all antibodies from LabVision) and the glycoprotein α-subunit (Novocastra).

For electron microscopy, small blocks of tissue were fixed in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide, dehydrated in graded alcohols and embedded in Epon 812. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined using a CM120 BioTween Philips electron microscope.

The result of the histopathological assessment was considered 'positive' if the presence of adenoma in the histological specimens as well as the immunopositivity for ACTH was confirmed. The result was treated as 'negative' if there was neither histopathological evidence of a corticotrophic adenoma nor ACTH staining.

Postoperative hormonal evaluation and criteria of cure

Blood samples for serum cortisol measurements were collected from all patients at 6.00 on the first postoperative day. Afterwards, the standard dose of hydrocortisone (20 mg in the morning and 10 mg at 15.00) was started and continued until the next hormonal evaluation.

Following the surgical procedure, all patients were subjected to further postoperative evaluation lasting at least 18 months (median 30 months; range 18-36 months). The first biochemical evaluation of corticotroph function took place at the Department of Endocrinology, Medical Centre of Postgraduate Education, within 7 days after the transsphenoidal surgery. Subsequent reassessments were performed at 6 weeks and at 3, 6, 12, 18, 24 and 36 months after surgery. Patients on hydrocortisone replacement therapy had their cortisol measurements taken 48 hours after the last administered dose. If the morning serum cortisol reached the lower limit of the referral range (5 μg/dL), it was assumed that normal function of the pituitary-adrenal axis was being restored. This enabled a reduction or even withdrawal of the hydrocortisone replacement therapy.

The patients were regarded as cured postoperatively if they fulfilled the following criteria for remission: subnormal serum cortisol level on the first postoperative day and (at the end of follow-up) clinical and biochemical evidence of eucortisolaemia or adrenal insufficiency. Eucortisolaemia was defined as: serum cortisol concentration and UFC within referral range, normal circadian rhythm and serum cortisol less than or equal to 1.8 μg/dL following the overnight 1 mg dexamethasone suppression test.

Hormone assay

Chemiluminescent immunometric assays (IMMULITE 2000; Siemens, UK) were used to measure serum cortisol and UFC. Method sensitivity was 0.2 μg/dL (5.5 nmol/L) for serum cortisol and for UFC. The normal range for serum cortisol was 5-25 μg/dL (138-690 nmol/L) and for UFC 20-90 μg/24 hours. Plasma ACTH was measured using a specific two-step radioimmunometric assay (IRMA; coated tube technique; Brahms, Germany). Method sensitivity was 1.2 pg/mL and the referral range was 10-60 pg/mL. The daily urinary excretion of 17-OHCS was determined by the method based on Silber-Porter reaction. The normal range was 2.2-7 mg/24 hours.

Table 1. Study group characteristics – basic data

Variable	Value
Number of patients	36
Age [years]; mean ± SD	36.3 ± 12.9
Sex	
females; *n* (%)	30 (83%)
males; *n* (%)	6 (17%)
BMI [kg/m^2]; mean ± SD	29.7 ± 6.04
Duration of symptoms [months]; mean ± SD	43.9 ± 37.9
Surgical treatment; *n* (%)	
first surgery	28 (77.8%)
second or third surgery	8 (22.2%)
Follow-up period [months]; mean ± SD	28.5 ± 7.9

SD – standard deviation, BMI – body mass index

Statistical analysis

Methods of descriptive statistics (mean, median, standard deviations, proportion) were employed in the statistical analysis. Verification of hypotheses concerning the relationship between two categorical variables were expressed as frequencies and compared using the exact chi-square test (Fisher's exact test). The level of significance was set at $p < 0.05$. The calculations were performed using the commercially available statistical software package SPSS v.18.0.

Results

Clinical and epidemiological evaluation

Demographic data and basic preoperative characteristics of the study group are given in Table 1.

Detailed results of clinical evaluation covering age, sex, number of operations, results of preoperative MRI of the pituitary and duration of postoperative follow-up are shown in Table 2.

Criteria of surgical cure

Based on hormonal evaluation on the 1st postoperative day and at the end of follow-up, 23 patients (63.9%) fulfilled the criteria of cure, whereas 13 patients (36.1%) were regarded as not cured. We considered the patient to be surgically cured if serum cortisol concentration was lower than or equal to 2.5 μg/dl on the first postoperative day (the cut-off point was based on our unpublished data) and either the results of hormonal evaluation of the pituitary-adrenal axis were normal or adrenal insufficiency was confirmed. Detailed results of hormonal evaluation performed in the immediate postoperative period and at the end of follow-up are presented in Table 3.

Simultaneously, correct thyro- and gonadotropic function of the anterior pituitary was confirmed in all cured patients. There was no case of diabetes insipidus in this group. In the non-cured group, thyro- and gonadotropic insufficiency was found in 5 cases (13.9% of all operated patients and 38.5% of non-cured patients). Permanent diabetes insipidus was diagnosed in 3 uncured patients.

Histopathological and immunohistochemical evaluation considering ultrastructure of the surgical specimens

The histopathological and immunohistochemical examination of the surgically removed specimens revealed the presence of corticotrophic adenoma in 27 cases (75%). In the other 9 cases (25%) no corticotrophic adenoma was found by the routine histopathological or by immunohistochemical examination of the obtained specimens. In a group of 27 patients with definitely positive results of histopathological examination obtained in light microscopy, at electron microscopy level a densely granulated ACTH adenoma was revealed in 20 patients (74.1%) whereas a sparsely granulated corticotrophic tumour was detected only in 2 patients (7.4%). In the 5 remaining cases (18.5%), tissue specimens either were not evaluated using electron microscopy, or revealed normal acinar architecture of the pituitary. Detailed results of histopathological evaluation are shown in Table 4. A typical result of histopathological assessment of corticotroph adenoma is shown in Fig. 1, whereas strong ACTH positivity is presented in Fig. 2. Examples of densely granulated ACTH and sparsely granulated ACTH adenomas in electron microscopy are presented in Figs. 3 and 4.

To assess the relationship between the efficacy of the surgical treatment of Cushing disease and results of the histological and immunohistochemical and electron microscopy examination, we compared the cured and non-cured groups in terms of frequencies of positive and negative histopathology. The difference was statistically significant ($\chi^2 = 4.86; p = 0.028$), demonstrating a relationship between pathological confirmation of the presence of corticotrophic adenoma and higher efficacy of transsphenoidal surgery for Cushing disease (Table 5).

Additionally, in order to verify the relationship between history of previous surgery and the efficacy of surgical treatment of Cushing disease we compared

Table 2. Detailed results of the demographic data, magnetic resonance imaging (MRI) and duration of postoperative follow-up period

No.	Age	Sex	MRI findings	Number of operations	Follow-up period (months)
1	52.6	F	microadenoma	1	36
2	24.8	F	macroadenoma	1	36
3	26.3	F	microadenoma	1	36
4	19.9	M	microadenoma	1	36
5	52.4	F	microadenoma	1	18
6	26.2	F	microadenoma	1	36
7	57.2	F	microadenoma	1	36
8	43.7	F	macroadenoma	1	36
9	28.0	F	equivocal	1	18
10	29.9	F	microadenoma	1	18
11	34.4	F	macroadenoma	2	18
12	51.0	F	microadenoma	1	24
13	41.0	F	microadenoma	1	24
14	29.1	F	microadenoma	1	18
15	29.7	F	microadenoma	1	24
16	20.9	M	microadenoma	1	18
17	18.4	F	microadenoma	1	24
18	40.8	F	microadenoma	1	24
19	24.3	F	equivocal	1	24
20	26.3	F	microadenoma	1	36
21	25.1	F	equivocal	1	36
22	56.3	F	microadenoma	1	36
23	52.1	F	microadenoma	1	18
24	57.6	F	equivocal	2	36
25	34.1	F	equivocal	2	18
26	28.4	F	microadenoma	2	36
27	34.8	F	microadenoma	1	24
28	49.3	M	macroadenoma	1	24
29	55.8	F	macroadenoma	1	36
30	16.8	M	microadenoma	1	24
31	32.1	F	microadenoma	1	36
32	32.4	F	equivocal	3	36
33	39.8	M	macroadenoma	1	36
34	18.8	M	equivocal	2	18
35	53.8	F	equivocal	2	36
36	42.5	F	microadenoma	2	36

F – female, M – male

Table 3. Detailed results of hormonal evaluation of pituitary-adrenal axis and summary of postoperative pituitary function

Demographic data			Serum cortisol on 1st day after surgery 2.5 μg/dL	Final evaluation of pituitary-adrenal axis at the end of follow-up				Postoperative pituitary function		
No.	Sex	Age (years)		Cortisol (μg/dL) (8.00)	Circadian rhythm	Cortisol after LDDST (μg/dL)	Remission	Gon.	Tyr.	Diabetes insipidus
1	F	52.6	yes	10.4	N	1.0	yes	C	C	no
2	F	24.8	yes	10.5	N	1.0	yes	C	C	no
3	F	26.3	yes	1.8	*	**	yes	C	C	no
4	M	19.9	yes	16.4	N	1.0	yes	C	C	no
5	F	52.4	yes	4.6	N	1.0	yes	C	C	no
6	F	26.2	yes	10.9	N	1.0	yes	C	C	no
7	F	57.2	yes	10.2	N	1.4	yes	C	C	no
8	F	43.7	yes	5.3	N	1.6	yes	C	C	no
9	F	28.0	yes	1.0	*	**	yes	C	C	no
10	F	29.9	yes	10.9	N	1.0	yes	C	C	no
11	F	34.4	yes	9.0	N	1.0	yes	C	C	no
12	F	51.0	yes	2.8	N	1.5	yes	C	C	no
13	F	41.0	yes	11.9	N	1.0	yes	C	C	no
14	F	29.1	yes	9.7	N	1.0	yes	C	C	no
15	F	29.7	yes	14.1	N	1.1	yes	C	C	no
16	M	20.9	yes	14.3	N	1.0	yes	C	C	no
17	F	18.4	yes	8.7	N	1.0	yes	C	C	no
18	F	40.8	yes	7.0	N	1.8	yes	C	C	no
19	F	24.3	yes	13.9	N	1.0	yes	C	C	no
20	F	26.3	yes	10.2	N	1.1	yes	C	C	no
21	F	25.1	yes	6.5	N	1.2	yes	C	C	no
22	F	56.3	yes	13.6	N	1.0	yes	C	C	no
23	F	52.1	yes	8.4	N	1.7	yes	C	C	no
24	F	57.6	no	15.5	A	3.7	no	C	C	no
25	F	34.1	no	16.8	A	9.0	no	C	C	no
26	F	28.4	no	14.6	A	3.5	no	C	C	no
27	F	34.8	no	13.5	N	3.67	no	C	C	no
28	M	49.3	no	23.7	A	4.3	no	I	I	yes
29	F	55.8	no	11.6	N	3.2	no	C	C	no
30	M	16.8	no	14.8	A	8.79	no	I	I	yes
31	F	32.1	no	14.4	A	8.47	no	I	I	yes
32	F	32.4	no	32.4	A	17.8	no	I	I	no
33	M	39.8	no	33.1	A	7.72	no	C	C	no
34	M	18.8	no	28.5	A	6.38	no	C	C	no
35	F	53.8	no	18.3	A	11.7	no	I	I	no
36	F	42.5	no	27.3	A	8.7	no	C	C	no

M – male, F – female, LDDST – low-dose dexamethasone suppression test (1 mg of dexamethasone), Gon. – gonadotropic, Tyr. – thyrotropic, I – incorrect, C – correct, N – normal, A – abnormal

** Due to subnormal cortisol levels a proper assessment of circadian rhythm was impossible (adrenal insufficiency)*

*** Due to subnormal cortisol levels, the LDDST was not performed*

Table 4. Detailed results of pathological evaluation of the surgical specimens in 36 patients with Cushing disease

No.	Age	Sex	Histopathology	
			LM	EM
1	52.6	F	positive	DG-ACTH
2	24.8	F	positive	SG-ACTH
3	26.3	F	positive	no data
4	19.9	M	positive	no data
5	52.4	F	positive	DG-ACTH
6	26.2	F	positive	DG-ACTH
7	57.2	F	positive	DG-ACTH
8	43.7	F	positive	DG-ACTH
9	28.0	F	negative	
10	29.9	F	positive	DG-ACTH
11	34.4	F	positive	DG-ACTH
12	51.0	F	negative	
13	41.0	F	positive	DG-ACTH
14	29.1	F	positive	DG-ACTH
15	29.7	F	positive	DG-ACTH
16	20.9	M	positive	no data
17	18.4	F	positive	DG-ACTH
18	40.8	F	positive	no data
19	24.3	F	positive	SG-ACTH
20	26.3	F	positive	DG-ACTH
21	25.1	F	negative	
22	56.3	F	positive	DG-ACTH
23	52.1	F	positive	DG-ACTH
24	57.6	F	negative	
25	34.1	F	negative	
26	28.4	F	positive	no data
27	34.8	F	positive	DG-ACTH
28	49.3	M	positive	DG-ACTH
29	55.8	F	positive	DG-ACTH
30	16.8	M	positive	DG-ACTH
31	32.1	F	negative	
32	32.4	F	negative	
33	39.8	M	positive	DG-ACTH
34	18.8	M	negative	
35	53.8	F	negative	
36	42.5	F	positive	DG-ACTH

F – female, M – male, LM – light microscopy, EM – electron microscopy, DG – densely granulated adenoma, SG – sparsely granulated adenoma

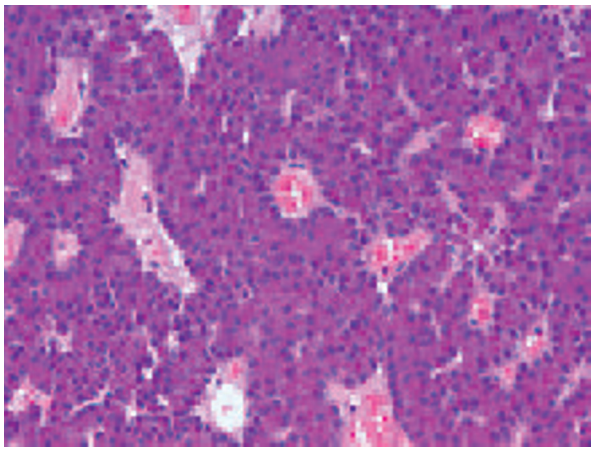

Fig. 1. Histopathology of corticotrophic pituitary adenoma (H&E; original magnification × 20)

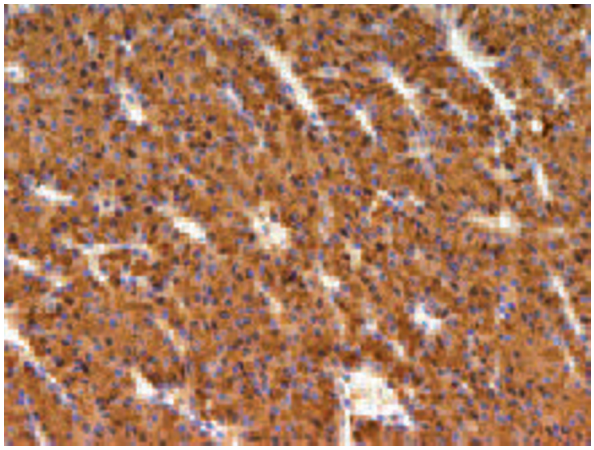

Fig. 2. Strong immunostaining for ACTH in corticotrophic pituitary adenoma (original magnification × 20)

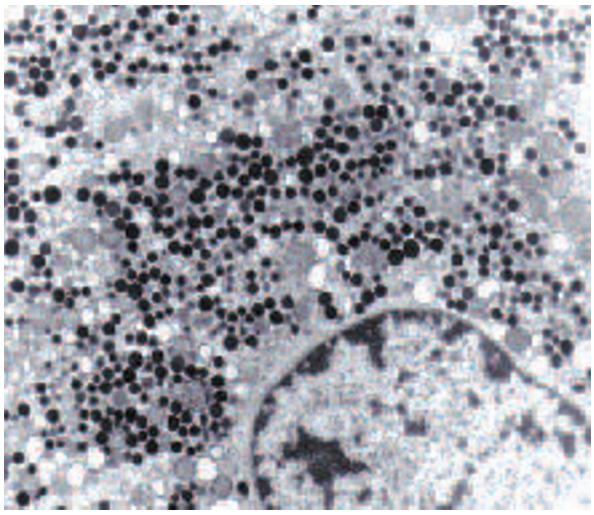

Fig. 3. Ultrastructural features of densely granulated corticotrophic pituitary adenoma (original magnification × 4800)

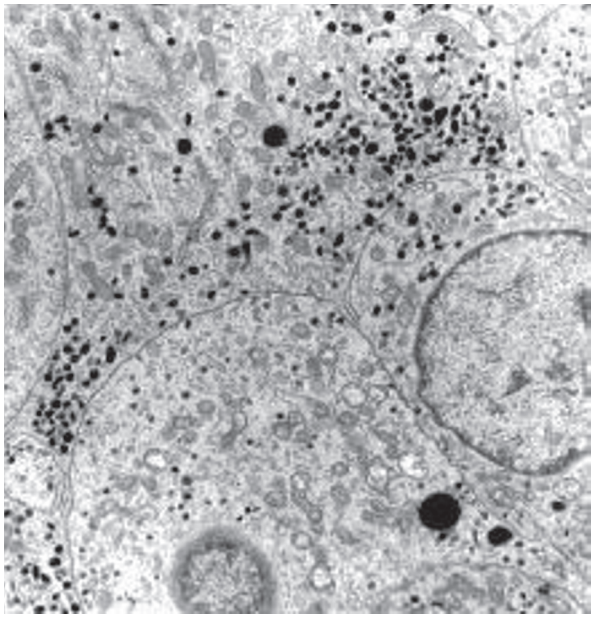

Fig. 4. Ultrastructural features of sparsely granulated corticotrophic pituitary adenoma (original magnification × 4800)

Table 5. Relationship between histopathological confirmation of corticotrophic adenoma and efficacy of transsphenoidal surgery for Cushing disease – comparison of cured and non-cured subgroups (Fisher's exact test)

Histopathological confirmation of ACTH-staining adenoma	Number of patients	Cured patients (n = 23)	Non-cured patients (n = 13)	p-value
Yes	27 (75%)	20 (87%)	7 (53.8%)	0.028
No	9 (25%)	3 (13%)	6 (46.2%)	

Table 6. History of previous surgical treatment for Cushing disease: comparison of cured and non-cured subgroups – Fisher's exact test

Previous surgical treatment	Number of patients	Cured patients (n = 23)	Non-cured patients (n = 13)	p-value
No	28 (77.8%)	22 (95.7%)	6 (46.2%)	0.001
Yes	8 (22.2%)	1 (4.3%)	7 (53.8%)	

cured and non-cured patients with regards to the history of previous surgical treatment for Cushing disease. There was a significant difference between the cured and non-cured groups with reference to previous surgical treatment of corticotrophic pituitary adenoma ($\chi^2 = 11.77; p = 0.001$). The results are given in Table 6.

Discussion

The importance of histopathological and immunohistochemical assessment in predicting efficacy of transsphenoidal surgery for Cushing disease is discussed in the literature [7-10]. Interestingly, in some patients it is impossible to detect the corticotrophic adenoma in surgically removed tissues but they meet the criteria for cure in Cushing disease, even after many years of follow-up [10-13]. Also in our study, no ACTH-staining adenomas were found in 3 of 23 surgically cured patients (13%). Thus, it seems that the reasons for this situation should be considered.

Retrospective analysis from the University of Virginia demonstrated remission of Cushing disease in as many as 50% of patients with a negative result of histopathological examination. At the same time, the study showed that remission of Cushing disease occurred in 88% (significantly more frequent) of patients where the presence of ACTH-staining adenoma was definitely confirmed [13].

When explaining high efficacy of surgical treatment despite lack of a immunohistochemical confirmation, some authors indicate a possibility of losing 'small' microadenomas intraoperatively, e.g. lost in suction or at the stage of processing the submitted specimen in a histopathological laboratory [10,12]. It also seems possible that a small focal lesion (frequently not visualized in MRI) is not removed, yet during surgical manipulation conducted in an adjacent region, the tumour becomes compromised in terms of blood supply (ischaemia) or irreversibly damaged, thus resulting in cure of Cushing disease [10,13].

In our own material such a situation could have occurred in two surgically cured patients (patients no. 9 and 19), where it was decided to perform sellar exploration, despite the fact that the tumour was not precisely visualized by MRI. In both cases this exploration resulted in a surgical cure, despite the lack of histopathological confirmation of tumour removal.

Therefore significant importance should be attached to results of histopathological examinations conducted in patients with pituitary corticotrophic microadenomas and – as described above – in a situation when sellar exploration is performed following equivocal MRI results. In such cases, confirmation of ACTH-staining adenoma definitely suggests complete removal of a small pituitary tumour. In turn, patients with invasive pituitary macroadenomas (in particular those with suprasellar extension and cavernous sinus invasion) more fre-

quently are not cured, despite a positive histology. Even removing a part of a 'large' corticotrophic adenoma obviously translated into a positive result of a histopathological assessment and, coupled with simultaneous lack of complete efficacy of the surgical procedure, results in continued persistence of Cushing disease. In the presented material, such a situation probably occurred in three patients who, despite evidence of immunostaining for ACTH in removed surgical specimens (patients no. 28, 29, and 33), were not cured.

The efficacy of surgical treatment in our series reached 63.9%. In larger retrospective studies, the efficacy of treatment varies from 60 to over 90%. The lower efficacy of surgical treatment in the material from our site is probably linked to the fact that the more difficult cases from all over the country are brought precisely there, as our site has the tradition of being the first centre diagnosing and treating patients with Cushing disease. In the examined group, there were 8 subjects (22.2%) operated on for the second time and 4 of them were previously operated on either in another neurosurgical centre or by another neurosurgeon. In only 3 out of 8 subjects operated on for the second time, the presence of a pituitary tumour was confirmed in the preoperative MR imaging.

In terms of postoperative complications, diabetes insipidus was seen in three patients in our group (8.5 %); in one patient the ADH insufficiency was partial and in two subjects (5.5%) the condition was complete and permanent. In two cases with diabetes insipidus the MRI showed a microadenoma, and in one other case a pituitary macroadenoma. This frequency is higher compared to the series of Hammer *et al*., Höybye *et al*., Semple *et al*. and Black *et al*. with dominating microadenomas (3.1%, 3%, 0.95% and 0.4%, respectively) [14-17]. On the other hand, the frequency of diabetes insipidus reported by Tommasi was 21.6% for the group of macroadenomas [18].

Conclusions

1. The results of our prospective study indicate that the histopathologically confirmed presence of corticotrophic adenoma is an important predictor of surgical cure for Cushing disease. Our observations are consistent with previous, retrospective studies.
2. Positive histopathology and ACTH immunostaining seem to be the most important in cases of sellar exploration performed in patients with Cushing disease without precise visualisation of the tumour in preoperative imaging studies.
3. Additionally, our data support the view that the more favourable cure rate after the transsphenoidal procedure is attributed to the first attempt of surgical treatment for Cushing disease. However, one should remember that the 5-year risk of recurrent hypercortisolism in Cushing disease is high and in larger studies reaches even 25.5% [12]. Therefore, every patient after transsphenoidal surgery for Cushing disease requires prolonged and careful endocrine follow-up.

Disclosure

The authors report no conflict of interest.

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