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ARAŞTIRMA / RESEARCH

Clinical, hormonal, radiological and morphological comparison of patients with clinically evident Cushing's disease and patients with silent corticotroph cell adenoma

Klinik olarak belirgin Cushing hastalığı olan hastalar ile sessiz kortikotrof hücreli adenomu olan hastaların klinik, hormonal, radyolojik ve morfolojik olarak karşılaştırılması

Gamze Akkuş[1], Barış Karagün[2], Nuri Eralp Çetinalp[3], Arbil Açıkalın[4], Fulya Odabaş[2], Mehtap Evran Olgun[2], Murat Sert[2], Suzan Zorludemir[4], Bekir Tamer Tetiker[2]

[1]Cukurova University Faculty of Medicine, Department of Internal Medicine, [2]Department of Endocrin and Metabolism, [3]Department of Brain and Nerve Surgery, [4]Department of Pathology, Adana, Turkey

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Abstract

Purpose: The aim of the study was to review our clinical 7 years experiences with silent corticotroph adenoma (SCA) and Cushing disease (CD) with regard to clinical, radiological features, immunohistochemical and surgical outcomes and compare the results between two groups.
Material and Methods: We retrospectively reviewed a series of patients (n=17; SCA 10, CD 7) with corticotroph adenomas and collected biochemical, neuroradiological and pathological data of those during follow-up time.
Results: Mean pre-operative ACTH values of the patients with CD and SCA were 60.4±29.5, 45.5±34.6 pg/mL, respectively. Mean preoperative cortisol of the patient with CD (21.2±4.4 vs 11.5±4.0 mcg/dL) was increased than the patients with SCA measurements'. Patients with SCA had larger pituitary mass (20.4±4.5 vs 8.8±4.0 mm) than the patients with CD. And cavernous sinus invasion also was determined in all patients with SCA. Sparsely granulation staining was more common in all patients with SCA (7/10) or CD (4/7).
Conclusion: SCAs are totally different from functional CD adenomas related with clinical characteristics and postoperative outcomes. SCA can be thought of as a diverse group of pituitary adenomas.

Keywords:. Pituitary adenomas, silent corticotroph adenomas, clinical comparison

Öz

Amaç: Bu çalışmada retrospektif olarak kendi kliniğimizde takip edilen sessiz kortikotrof adenomu (SKA) olan hastalar ile belirgin kortizol sekrete eden adenomu (Cushing Hastalığı (CH)) olan hastaları klinik, radyolojik, immunohistokimyasal ve cerrahi sonuçlarına göre karşılaştırılması amaçlanmıştır.
Gereç ve Yöntem: Retrospektif olarak takip edilmiş 17 hastanın 10 tanesi SKA; diğer 7 tanesi aşikar CH olan bireyleri klinik, biyokimyasal, nöroradyolojik ve patolojik verilerine göre toplayıp karşılaştırdık.
Bulgular: CH olan hastalar ile SKA'sı olan hastaların preoperatif ACTH değerleri (60.4±29.5, 45.5±34.6 pg/mL) birbirine benzer olup, preoperatif kortizol değerleri (21.2±4.4 vs 11.5±4.0 mcg/dL) arasında anlamlı farklılık vardı. SKA'sı olan hastaların ortalama hipofiz adenom boyutları (20.4±4.5 mm), CH olan bireylerin ortalama adenom boyutlarından (8.8±4.0 mm) daha fazla idi. Aynı şekilde kavernöz sinüs invazyonu SKA'sı olan hastalarda daha fazla görülmekte idi. Her iki grupta immunohistokimyasal olarak seyrek granüllü boyanma paterni (SKA 7/10, CH 4/7) daha fazla görülmekte idi.
Sonuç: Sessiz kortikotrof adenomlar klinik ve postoperatif sonuçları itibari ile aşikar CH olan bireylerden tamamı ile farklıdır. Bu yönü ile sessiz kortikotrof adenomları pituiter adenomların farklı bir sub-grubu gibi değerlendirmek daha doğru olacaktır.

Anahtar kelimeler: Sessiz kortikotrof adenomlar, pituiter adenomlar, immunohistokimyasal boyanma

Yazışma Adresi/Address for Correspondence: Dr. Gamze Akkuş, Cukurova University Faculty of Medicine, Department of Internal Medicine, E-mail: tugrulgamze@hotmail.com
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INTRODUCTION

Corticotroph adenomas, also known as ACTH pituitary adenomas, are usually associated with the occurrence of Cushing's disease (CD), which is characterized by clinical manifestations of hypercortisolism, including central obesity, moon face, diabetes mellitus, hypertension, and osteoporosis[1,2]. A majority of cases with CD (80%) have pituitary microadenomas in contrast to silent corticotroph pituitary adenomas (SCA) that show strong immunopositivity for adrenocorticotropic hormone and do not manifest either biochemical or hormonal evidence of hypercortisolism[3,4]. SCAs were first reported in the 1970s; they are a distinct subtype of pituitary adenomas that comprise 20% of all corticotroph adenomas, and approximately 5% of all non-functional surgically treated pituitary adenomas[5,6]. The definitive diagnosis of SCA is determined after pathological examination of resected tumour tissue[7]. Although the preoperative clinical findings are similar to those of other non-functional pituitary adenomas, SCAs are potentially more aggressive and show resistance to therapy[8,9]. According to the WHO 2017 classification criteria, histologically, SCAs can be further divided into type I (densely granulated) and type 2 (sparsely granulated). It has been shown that both types display aggressive clinical behaviour compared with their functional counterparts[10,11]. However, regardless of its aetiology, the natural history of SCAs, and the factors influencing clinical outcome remain controversial; the pathophysiology of SCAs is still poorly understood. Although there are several studies that advocate the inability of SCAs to manifest clinical features of hypercortisolism, SCAs have consistently been shown to present clinically aggressive behaviour in contrast to non-functioning pituitary adenomas associated with CD[12,13]. Nevertheless, there is a limited availability of clinical, molecular, and immunohistochemical data about functional corticotroph adenomas and non-functioning corticotroph pituitary adenomas.

In view of the above, in this study, we aim to demonstrate the clinical and radiological manifestations of SCAs and CD based on our 5-year direct clinical experience and the literature data. Further, we analysed the immunohistochemical characteristics and pre- and post-surgical outcomes in patients with SCAs and CD and compared the results between the two groups. Additionally, we highlight their distinctive characteristics.

MATERIALS AND METHODS

In this retrospective-prospective cohort study, clinical, radiological, immunohistochemical, and biological data 2012 and 2019 from patients with SCA and those with CD were considered (n=17). The study was approved by the Local Ethical Committee of the University (Cukurova University, Number:99, on May 2020).

Sample

All patients who have been admitted to our hospital clinic were evaluated by multidisciplinary team including neurosurgery, endocrinology and radiology. We recorded data regarding age, sex, clinical features, ACTH and cortisol levels at diagnosis and various time points, urine cortisol levels, adenoma size (pre and post-treatment) and detailed finding of magnetic resonance imaging (MRI), immunohistochemical data and follow-up periods. Pre- and post-operative magnetic resonance imaging (MRI) findings with characteristics of corticotroph adenomas (SCAs and CD) were available for all patients.

We included patients with positive ACTH immunohistochemical staining results but without clinical diagnosis of CD. We also verified the diagnosis of all patients with central ACTH-dependent CD. CD was diagnosed on the basis of the clinical signs and symptoms of hypercortisolism, including localised adiposity, buffalo hump, abdominal striae, facial plethora, hirsutism, abnormal weight gain, hypokalaemia, and osteoporosis. The patients with CD showed increased plasma ACTH levels, elevated serum and urinary cortisol levels, loss of the cortisol diurnal rhythm, impaired pituitary function and the presence of adenomas in pituitary MRI. Dexamethasone suppression tests using low (1–2 mg) and high (8 mg) doses of dexamethasone were performed in all patients with CD[14]. Plasma cortisol and ACTH levels were measured at 08.00 h on day. Further, urinary cortisol levels were determined in all patients with CD.

Clinical SCA was diagnosed on the basis of the following characteristics: lack of clinical signs or symptoms associated with hypercortisolism at initial presentation, normal cortisol levels, normal or only slight elevations of serum ACTH levels and

immunohistochemical positivity of ACTH in pathological specimens. Patients with SCA were further divided into two subtypes based on the immunohistochemical staining patterns, densely granulated (subtype 1) and sparsely granulated (subtype 2).

Patients were excluded if they had clinically and biochemically other functional (i.e., hormone-secreting) pituitary adenomas associated with conditions such as acromegaly, prolactinomas. And patients with hormone-negative (non-functional) adenomas were also excluded for the current study.

Procedure

Radiological assessment

Tumours were classified based on the largest diameter of the tumour seen on the MRI scan—macroadenomas (>10 mm) or microadenomas (<10 mm), and cavernous sinus invasion and tumour extension were classified according to the Knosp criteria[15]. The Knosp grading system has five grades (0–4) based on a series of lines drawn through the supra- and intracavernous segments of the internal carotid arteries as seen on midsellar coronal MR. Grades 0, I and II were considered non-invasive, while grades III/ IV were considered invasive. Magnetic resonance imaging were evaluated by the same two expert neurodiologists.

Histopathological assessment

Immunohistochemical (IHC) analyses were performed on all tumour samples. To achieve this, 3-μM sections were prepared from formaldehyde-fixed paraffin-embedded tissues. Subsequently, they were stained with the haematoxylin and eosin (H & E) stain and immunohistochemical staining. Immunohistochemical staining was performed using markers for pituitary hormones ACTH (BioSB, clone 02A3) and keratin 8 (Thermo Scientific, mouse) according to the manufacturer's protocols on an automated IHC staining platform (Ventana Benchmark XT, Roche Tissue Diagnostics).

Hormonal evaluation

The plasma ACTH levels were measured using an immunoassay kit (UniCel DxI 800, Beckman Coulter; ng/mL). The plasma cortisol measurements were performed by human ELISA methods. Urine cortisol levels were measured by RIA.

Statistical analysis

Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation and as median and minimum-maximum where appropriate. Chi-square test was used to compare categorical variables between the staining groups. The normality of distribution for continuous variables was confirmed with the ShapiroWilk test. For comparison of continuous variables between two groups (SCA vs CD), the Student's t-test was used depending on whether the statistical hypotheses were fulfilled or not. For comparison of preop-postop continuous variables (cortisol-ACTH values), paired samples t-test or Wilcoxon Signed Rank test was used depending on whether the statistical hypotheses were fulfilled or not. All analyses were performed using IBM SPSS Statistics Version 20.0 statistical software package. The statistical level of significance for all tests was considered to be 0.05.

RESULTS

We conducted a retrospective-prospective cohort study in a total of 17 patients (n = 17) with SCAs and CD. Based on our diagnostic inclusion criteria, out of these 17 patients, 7 displayed clinical and biochemical manifestations of hypercortisolism suggestive of CD, while 10 patients presented with SCA (Table 1).

The mean age of the patients with CD (5 women and 2 men) and that of patients with SCA (8 women and 2 men) were 35.28 ± 7.4 and 40.8±13.5 years, respectively (P=0.69). Additionally, two patients (patients 2 and 4) presented with pathological fractures due to severe osteoporosis. Further, the patients who had been diagnosed with SCA presented the following symptoms suggestive of the presence of a pituitary tumour: headaches (n = 4), visual field defects (n = 4), and amenorrhoea/galactorrhoea (n = 2).

Immunohistochemical analyses revealed that approximately 70% of the adenomas from patients with SCA (n = 7) showed a sparsely granulated pattern.

Also, 4 adenomas from patients with CD (n = 4, 57.1%) were found to be sparsely granulated. Notably, only 1 patient with CD (patient 7) exhibited Crooke's hyaline changes in the pituitary gland (Table 1).

Table 1. Clinical and demographic characteristics of sample

	Type	Sex	Age (years)	Tumour Size (mm)	İnvasion Knosp	Mass Signs*	Hypercortisolism**	08.00 cortisol (6.7-22.5)	08.00 ACTH (10-40)	IHC ACTH positivity
1	CD 1	F	45	10	No Grade 1	-	+	27.18	41	+ Densely granulation/Ki 67 1%
2	CD 2	M	41	7	No Grade 0	-	+	25	67	+ Sparsely granulation / Ki 67 2%
3	CD 3	F	24	18	Yes Grade 2	-	+	22	80	+ Sparsely granulation / Ki 67 2-3%
4	CD 4	M	35	10	No Grade 1	-	+	24.39	75	+ Sparsely granulation /Ki 67 2%
5	CD 5	F	40	8	No Grade 0	-	+	23.31	75	+ Densely granulation/Ki 67 1%
6	CD 6	F	39	5	No Grade 0	-	+	16.71	55	+ Densely granulation/ Ki 67 1%
7	CD 7	F	46	4	No Grade 0	-	+	18.59	60	+ Sparsely/ Crooke cell adenoma/ Ki 67 1%
			38.5±7.4	8.8±4.6				21.2±4.6	60.4±29.5	
8	SCA 1	F	63	30	Yes Grade 4	headache	-	10	28.9	+ Sparsely granulation/Ki 67 1%
9	SCA 2	M	47	15	Yes Grade 3	hypogonadism	-	5.3	23	+ Sparsely granulation/ Ki 67 1%
10	SCA 3	F	48	21	Yes Grade 4 Cystic adenoma	headache	-	8.8	43	+ Densely granulation/Ki 67 1%
11	SCA 4	F	45	24	Yes Grade 4 Cystic adenoma	Visual acuity	-	9.5	56	+ Sparsely granulation/Ki 67 4%
12	SCA 5	F	18	20	Yes Grade 4 Cystic adenoma	headache	-	12.2	32	+ Densely granulation/Ki 67 3-4%
13	SCA 6	F	28	30	Yes Grade 4	hypogonadism	-	11.8	13.8	+ Sparsely granulation/Ki 67 1%
14	SCA 7	F	31	35	Yes Grade 4	Visual acuity	-	14	24	+ Densely granulation/Ki 67 2%
15	SCA 8	M	50	15	Yes Grade 3	Visual acuity	-	7.6	17	+ Sparsely granulation/ Ki 67 1%
16	SCA 9	F	38	25	Yes Grade 4	headache	-	8.01	26	+ Sparsely granulation/Ki 67 1%

					Cystic adenoma					
17	SCA 10	F	43	20	Yes Grade 4	Visual acuity	-	6.4	29	+Sparsely granulation/ Ki 67 1-2%
			40.8±13.5	20.5±4.4				11.7±4.0	45.5±34	

SCA: Silent Corticotroph Adenoma, CD: Cushing Disease; Mass Signs*: headache, visuel acuity, hypogonadism or pituitary apoplexy Hypercortisolism**: Weight gain, centripedal fat deposition, muscle wasting, hyperpigmentation, acne, hypertension, cognitif disfunction, osteoporosis etc.

However, there were no statistically significant differences in the hormonal parameter values of cortisol and ACTH, radiological data, postoperative biochemical remission with adrenal insufficiency, and in the granulation patterns between patients with CD and SCA.

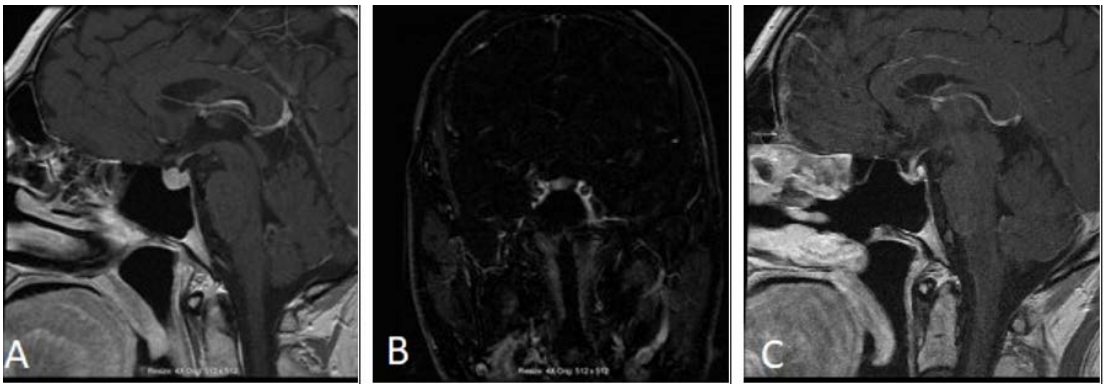
The normal morning ACTH range is 10–50 pg/mL. The plasma cortisol was measured by a specific in-house. The normal range of cortisol is 6.7–22.6 mcg/dL. Urinary cortisol was measured by RIA, and the normal range was 28.5–213.7 mcg/24 h

The observed mean preoperative ACTH levels in patients with CD and SCA were 60.4 ± 29.5 pg/mL and 45.5 ± 34.6 pg/mL, respectively (P = 0.3). Expectedly, the mean preoperative cortisol levels were found to be significantly elevated in patients with CD when compared with those of patients with SCA (21.2 ± 4.4 mcg/dL and 11.5 ± 4.0 mcg/dL, respectively; P = 0.00).

Besides, we analysed the serum cortisol responses during the low-dose (1 and 2 mg) and high-dose (8 mg) dexamethasone suppression tests in patients with ACTH-dependent CD (Table 3). The observed mean urine cortisol level in patients with CD was found to be 1465 mcg/24 h (Table 3). Among the patients with CD, 42.8% (n = 3) presented with macroadenomas, and 57.2% (n = 4) had microadenomas. On the other hand, all the 10 SCA patients presented with macroadenomas (100%). Notably, patients with SCA had significantly larger pituitary mass (20.4 ± 4.5 and 8.8 ± 4.0 mm, respectively; P = 0.00) than the patients with CD. Furthermore, cavernous sinus invasion was also identified in all patients with SCA (P = 0.00; Table 1)

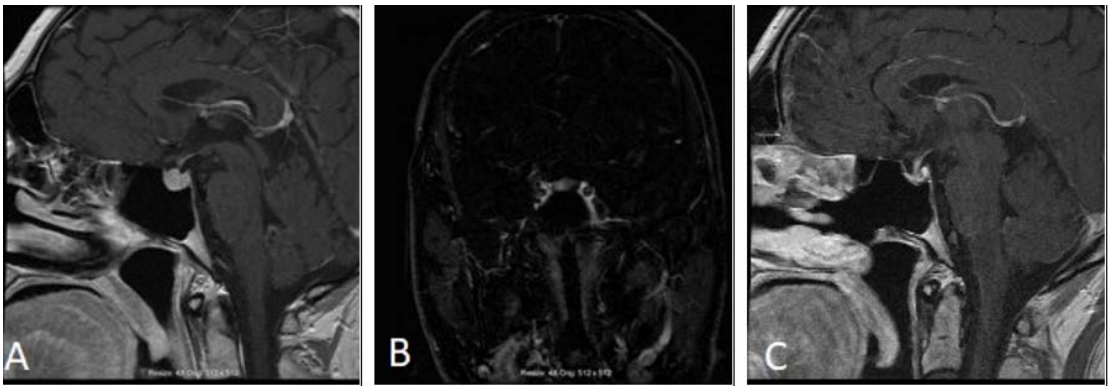
Pre-operative and post-operative imaging of adenomas (patient 2-3-8-10-11) were presented in figure 1. In contrast to all patients with CD (n = 7, 100%), only 70% of the patients with SCA (n = 7) underwent endoscopic transsphenoidal surgery. Gross total resection was achieved in 85.6% of patients with CD. Notably, the gross total resection was significantly lower in SCA patients than in the patients with CD (n = 2, 20%). Gross total resection was defined as the absence of any abnormal enhancement on the postoperative CT scan.

Adenomas from patients from both the CD and SCA groups displayed comparable postoperative ACTH values (P = 0.29) (table 2). Similarly, there was no significant correlation between the postoperative cortisol values of patients with CD and that of those with SCA (16.9 ± 20.9 and 13.8 ± 11.0 mcg/dL, respectively; P = 0.8) (Table 2).

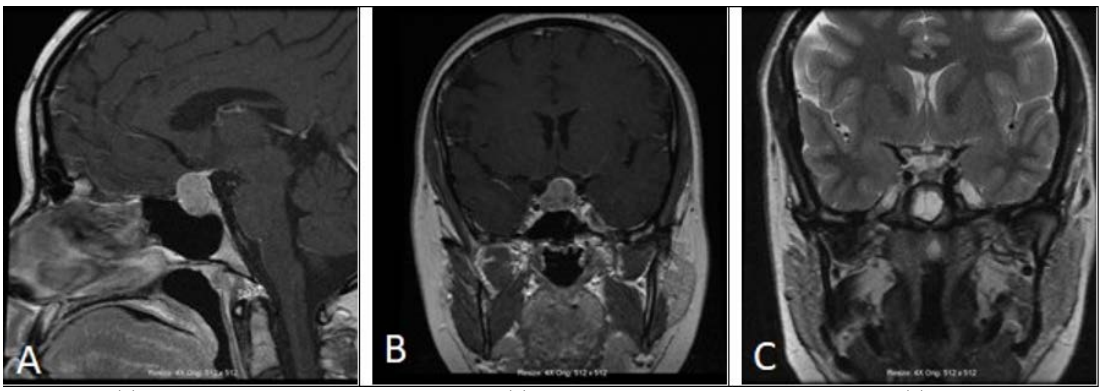


Patient 2 - 41 year old- with a 7 mm pituitary adenoma was diagnosed as Cushing Disease

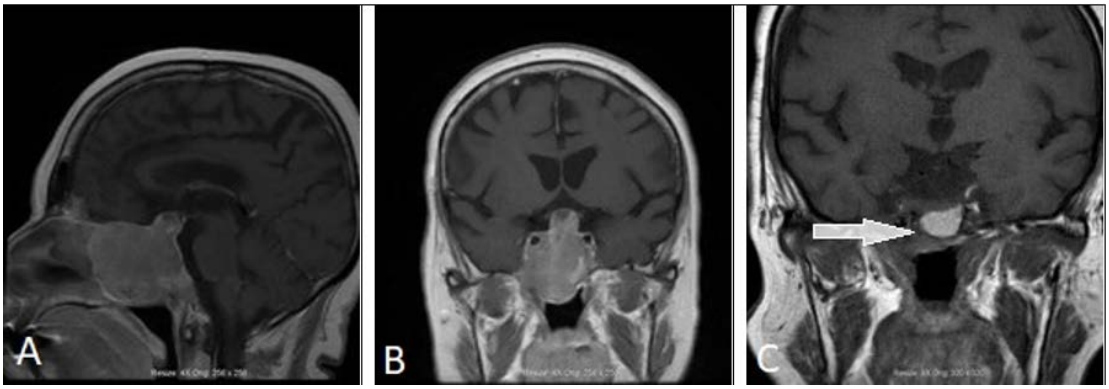
Sagittal T1-weighted preoperative (B) Coronal T1-weighted subtraction preoperative (C) Sagittal T1-weighted post operative MR images



Patient 3-24 year old-with a 18 mm pituitary adenoma was diagnosed as Cushing Disease

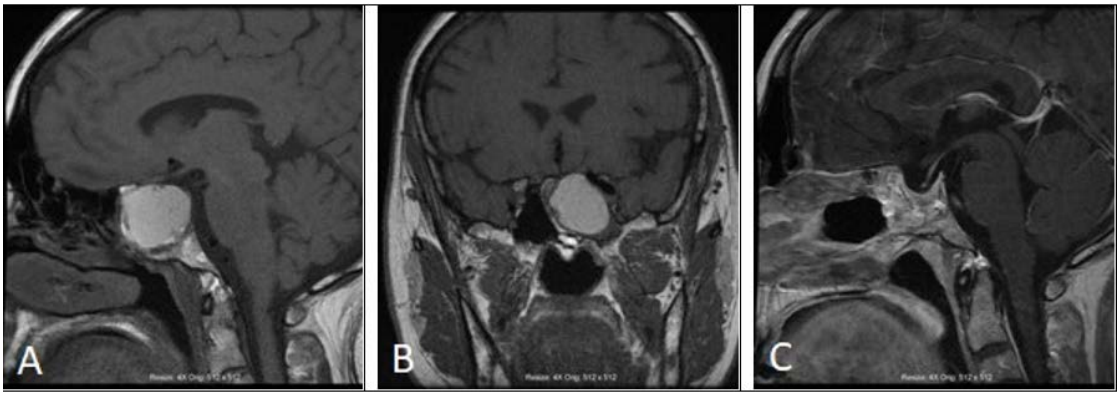


(A)Sagittal T1-weighted preoperative (B) Coronal T1-weighted preoperative (C) Coronal T2-weighted post operative MR images



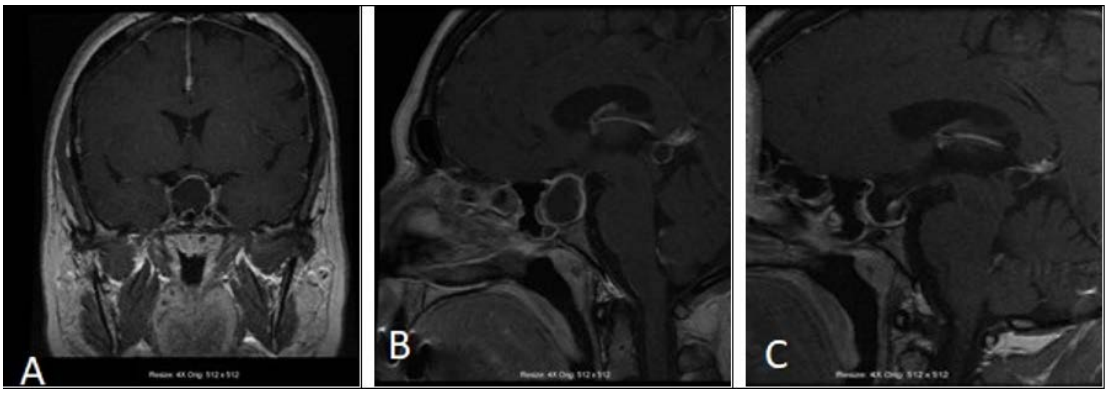
Patient 8-63 year old-with a 30 mm pituitary macroadenoma was diagnosed as silent corticotroph adenoma

(A) Sagittal T1-weighted preoperative MR image (B) Coronal T1-weighted preoperative MR image (C) Coronal T1-weighted post operative MR image shows residual adenoma (arrow)



Patient 10-48 year old-with a 21 mm cystic macroadenoma was diagnosed as silent corticotroph adenoma

(A) Sagittal T1-weighted preoperative (B) Coronal T1-weighted preoperative (C)Sagittal T1-weighted post operative MR images



Patient 11-45 year old-with a 24 mm cystic macroadenoma was diagnosed as silent corticotroph adenoma

(A) Coronal T1-weighted preoperative (B) Sagittal T1-weighted preoperative (C) Sagittal T1-weighted post operative MR images

Table 2. Post-operative hormon measurements and surveillance findings of all patients with CD and SCA

	Type	Sex	Age (years)	Residive Mass size	Post-op cortisol (6.7-22.5)	Post-op ACTH (10-40)	Post-op hypopituitarsim/ adrenal Failure	Additional treatment	Remission	IHC ACTH positivity
1	CD 1	F	45	-	5	9.45	-	-	+	+ Densely granulation/ Ki 67 1%
2	CD 2	M	41	-	10.19	26	-	-	+	+ Sparsely granulation / Ki 67: 2%
3	CD 3	F	24	5	13	29.7	-	-	+	+ Sparsely granulation / Ki 67: 2-3%

4	CD 4	M	35	-	15	43	-	-	+	+ Sparsely granulation / Ki 67: 2%
5	CD 5	F	40	-	8.02	50	-	-	+	+ Densely granulation/ Ki 67: 1%
6	CD 6	F	39	-	7.24	17	-	-	+	+ Densely granulation/ Ki 67: 1%
7	CD 7	F	46	- Empty sella	28	34	-	+ medical treatment	-	+ Sparsely/ Crooke cell adenoma / Ki 67: 1%
				13.8±11.5	46.7±29.2					
8	SCA 1	F	63	15	6.2	22	Hypopit.	+ Re-surgery		+ Sparsely granulation/ Ki 67: 1%
9	SCA 2	M	47	10	5.5	19	Hypopit.	-		+ Sparsely granulation/ Ki 67: 1%
10	SCA 3	F	48	15	4.3	23	Hypopit.	+		+ Densely granulation/ Ki 67: 1%
11	SCA 4	F	45	Empty sella	9.5	28	-	-		+ Sparsely granulation / Ki 67: 4 %
12	SCA 5	F	18	10	12.5	39	-	-		+ Densely granulation/Ki 67: 3-4%
13	SCA 6	F	28	15	7.8	13.8	-	-		+ Sparsely granulation/ Ki 67: 3-4
14	SCA 7	F	31	Empty sella	7.2	24	-	-		+ Densely granulation/Ki 67: 2%
15	SCA 8	M	50	10	0.31	5.7	Hypopit.	-		+ Sparsely granulation/ Ki 67: 1%
16	SCA 9	F	38	20	7.2	25	-	+ Re-surgery		+ Sparsely granulation/ Ki 67: 1%

17	SCA 10	F	43	10	6.5	27	-	-		+Sparsely granulation/ Ki 67:1-2%
						13.8±11.5	35.7±30.3			

SCA: Silent Corticotroph Adenoma, CD: Cushing Disease, Hypopit: Hypopituitarism

Postoperatively, patients with CD experienced no signs or symptoms of adrenal insufficiency, while 4 patients with SCA presented with new-onset hypopituitarism within 3 months of pituitary surgery. Hormone replacement therapy (corticosteroids, L-thyroxine and gonadal steroids) was recommended to all patients with postoperative pituitary deficiency (Table 3).

Table 3. Basal, after low dose and high dose dexamethasone hormone measurements of the of the patients with CD were indicated in this table.

	Basal Cortisol	**After 1 mg dxm test**	**After 2 mg dxm test**	**After 8 mg dxm test**	**Preop. ACTH (pg/ml)**	**Urinary cortisol (mcg/24 h)**
CD 1	27.18	19.23	6.56	4	41	500
CD 2	25	16	8.98	8.98	67	1080
CD 3	24	21	16	16	80	579
CD 4	22.39	23.31	22.14	22.14	75	1010
CD 5	23.31	8.32	20.74	20.74	75	569
CD 6	16.71	13.47	14.47	14.47	55	990
CD 7	18.59	35.78	44.42	44.42	60	5533

In patients with SCA, the mean follow-up period was 6.89 ± 5.011 years, and 2 patients (patients 10 and 16) were surgically treated again. On the other hand, the mean follow-up period of patients with CD was 3.86 ± 4.70 years, and only 1 patient diagnosed with Crooke's cell adenoma of the pituitary was followed up for subsequent medical therapy with pasireotide. There was no significant correlation in the follow-up time periods between the two groups (P = 0.07).In addition, during the follow-up period for SCA (6.89 ± 5.011 years), we did not observe SCA transforming into hormonally active disease.

Table 4. Demographical parameters and hormonal parameters (pre and post-operative) of two groups were demonstrated in this table.

	Gender(F/M)	**Age(year)**	**Adenoma size (mm)**	**Preop. Cortisol (mcg/dL)**	**Post-op. Cortisol (mcg/dL)**	**Pre-op. ACTH (pg/mL)**	**Post-op. ACTH (pg/mL)**
CD	5/2	35.28±7.4	8.8±4.6	21.2±4.6	13.8±11.5	60.4±29.5	46.7±29.2
SCA	8/2	40.8±13.5	20.5±4.4	11.7±4.0	13.8±11.5	45.5±35	35.7±30.3

DISCUSSION

In this study, we conducted a retrospective analyses of clinical and biochemical data, and assessed MRI sequences in 17 patients with corticotroph adenoma prior to immunohistochemical evaluation. Among these, 7 patients presented with CD, characterised by clinical manifestations associated with hypercortisolism, while the other patients (n = 10) were diagnosed with clinically silent corticotroph adenomas as they did not exhibit because they did not exhibit Cushingoid features. Our findings are

consistent with those of several previous studies that have demonstrated that SCAs do not manifest clinical or biochemical hypercortisolism[16,17,18].

Ever since the first description of SCAs, published almost 40 years ago by Kovacs et al.[5], molecular mechanisms underlying clinical presentation, SCA tumorigenesis and its progression, its associated clinical manifestations, and its characteristically distinct immunohistological subtypes have not been elucidated till date. A previous study by Kim et al.[26] compared the clinical parameters of patients with SCA with that of patients having non-functional pituitary tumours, and showed that female gender, cavernous sinus invasion, intratumoural haemorrhage on sella MRI, and decreased ACTH response in the combined pituitary function test were independent indicators of SCA.

There are limited data related to the comparison of clinical characteristics, including radiological features, immunohistochemical findings and postoperative findings in patients with SCA and CD. In view of this, our study assessed the clinical outcomes, including radiological features of SCAs and CD, and further analysed the immunohistochemical characteristics and pre- and post-surgical outcomes in patients with SCAs and CD and compared the results between the two groups.

Thus, this mechanism may cause dysfunctional processing and biological inactivity of pituitary hormones. We have shown that the preoperative ACTH levels of patients with SCA were similar to those with CD. In contrast, a previous study reported that plasma ACTH levels correlate with abundance of ACTH-immunopositive cells in CD but not in SCAs[20]. Another hypothesis is that differences occur in post-translational processing of pituitary hormone by prohormone convertase (PC) 1/3[21]. This enzyme may determine whether or not corticotroph adenomas will secrete. Some studies[22,23] have shown that this enzyme is decreased in silent corticotroph adenomas than in functional corticotroph adenomas. All these hypotheses are still insufficient to explain the mechanism underlying silent corticotroph adenomas. Traditionally, SCAs have been more aggressive lesions based on the 2017 World Health Organization Classification of pituitary tumours and they are also recognised as 'high-risk pituitary adenomas'. This classification has mostly relied on studies reporting more resistance to surgical treatment or higher recurrence rates compared with other non-functional pituitary tumours[24,25].

In this study, basic demographic parameters, such as age, female sex ratio were similar between the two groups. All patients with SCA presented with symptoms associated with pituitary mass effect (headache, visual acuity, or hypogonadism). However, none of the patients developed clinical or biochemical evidence of cortisol excess. As expected, patients with CD presented with typical features associated with hypercortisolism, such as buffalo hump, abdominal striae, facial plethora, hirsutism, abnormal weight gain, hypokalaemia, and osteoporosis. These was starkly evident in patients #2 and #4 who additionally showed pathological fractures due to severe osteoporosis.

In our series, all SCAs were macroadenomas that displayed invasion of adjacent anatomical structures (cavernous sinus or sphenoid sinus). Notably, 4 of them had a prominent microcystic component, a unique distinguishing feature that differentiates them from corticotroph adenomas linked to CD. These findings are further strengthened by a previous study by Cazabat et al.[27], which highlighted the presence of multiple microcysts to be a good diagnostic marker that helps distinguish SCAs from other pituitary tumours.

Transsphenoidal surgery has been shown to be safe and an effective treatment method for patients with SCA and CD[28,29]. The gross total resection rate is based on the diminutive size of adenomas and the pattern of invasiveness of adjacent anatomical structures. Generally, SCAs are more likely to be macroadenomas with invasive characteristics rather than resembling other non-functioning pituitary tumours[30]. As functional corticotroph adenomas are more commonly microadenomas, it is not surprising to find that the gross total resection rates have increased. This interesting finding is further supported by another study by Smith et al.[31], which reported that the gross total resection rate after transsphenoidal surgery was 94.1% in a cohort of patients with CD (n= 68), where 60 patients harboured microadenomas (n=60); while the gross total resection rate was only 78.6% among patients with SCA (n=14) where 8 of them had macroadenomas (n=8). These results are consistent with those of ours, where the gross total resection rate was 85.6% in patients with CD who had macroadenomas (42.8%), while it was only 20% in patients with SCAs who harboured macroadenomas.

Several previous studies have shown that clinically silent corticotroph adenomas were clinically more

aggressive when compared with their functional counterparts. In this study, SCAs were immunopositive for ACTH and were associated with elevated plasma ACTH levels, and they exhibited unique characteristics, including clinical and biochemical features, radiologic features, and postoperative surveillance findings.

In this study, we grouped the patients with SCA into two subtypes based on the immunohistochemical staining pattern, densely granulated pattern (subtype 1) and sparsely granulated pattern (subtype 2). It has been shown that Type 1 SCAs cannot be differentiated from microadenomas associated with CD and show strong ACTH immunoreactivity[8]. Both the subtypes of SCA are associated with clinically more aggressive behaviour in contrast to functional CD adenomas[32]. Functional adenomas associated with CD featuring Crooke's hyalinisation have been termed as high-risk pituitary adenomas due to their aggressive clinical behaviour according to the recent 2017 WHO classification[10].

In our study, the immunohistochemical analyses revealed that sparsely granulated staining pattern was more predominant than the densely granulated pattern in tissue samples of patients with SCA and CD. Further, when we compared the clinical characteristics of the corresponding adenomas with sparsely and densely granulated staining patterns within each group of patients with SCA and CD, it was found that immunohistological characteristics had no effect on clinical outcomes.

Further, it was observed that patients with SCA (n = 7, 70%) and those with functional adenomas associated with CD (n = 4, 57.1%) exhibited sparsely granulated pattern of staining. Contrary to the findings of the literature, in this study, the resulting granulation pattern (sparsely granulated) was similar in tissue samples of patients with SCA and that of those with CD. This discrepancy could be attributed to the small sample size. Notably, the patient with CD (patient 7) who harboured Crookes' hyalinisation did not opt for surgical treatment and so we could not achieve biochemical remission in this case yet.

While evaluating the postoperative outcomes, we had observed that four patients with SCA presented with new-onset pituitary deficiency related to surgical complications within 3 months of surgery.

In addition, during the follow-up period of patients with SCA (mean ± SD, 6.89 ± 5.011 year) we did not encounter any case of SCA that transformed into hormonally active corticotroph adenoma. One of the most important clinical implications after surgery is the higher risk of recurrence that might be associated with the tumour's aggressive clinical behaviour, thus transforming into hormonally active CD. Zoli et al.[33] reported that 5 patients, including 2 men and 3 women, had corticotroph tumours that displayed transformation from an SCA variant to manifest CD and vice versa after a mean follow-up period of 3.5 years. This transformation could be attributed to the phenomenon of cyclic cortisol secretion. However, in this study neither recurrence nor tumour transformation was observed.

One strength of our study was the use of consistent evaluation criteria in all patients with SCA and CD with regard to clinical outcomes, biochemical features, immunohistochemical analyses, and pre-and postoperative surveillance assessments.

The limitations of this study include its retrospective nature, small sample size, and design, as it is a single-centre cohort study performed at a tertiary-care institution with possible referral bias. Further, the pathological evaluations of the specimens changed over time, although the immunostaining of ACTH was systematically quantified repeatedly by an expert neuropathologist. Since the pituitary-specific transcription factor especially TPIT, SF1 were not studied earlier, clear distinction criteria for classifying adenomas as clinically or totally silent adenomas, or null cell adenomas could not be set. Besides, follow-up periods have influenced the observed outcome in our study.

In conclusion, our study demonstrated that corticotroph adenomas exhibit much variation in terms of clinical features, histology, and immunohistochemistry. To the best of our knowledge, our study is the first of its kind to compare the clinical outcomes, including radiological features, immunohistochemical findings, hormonal measures, and pre- and postoperative surveillance findings in patients with SCA and CD. Even though our sample size is too low, we have shown that SCAs are distinctly different from functional adenomas associated with CD with regard to clinical characteristics and postoperative outcomes. Therefore, despite the minor variations in the immunohistochemical staining pattern of ACTH in SCA specimens, SCAs could be categorised as a diverse group of pituitary adenomas. Taken together, our study warrants the need for larger multicentre research studies in order to elucidate the

epidemiological and distinct biological characteristics of SCAs.

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