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CASE REPORT - BRAIN TUMORS

Plurihormonal pituitary adenoma with concomitant adrenocorticotropic hormone (ACTH) and growth hormone (GH) secretion: a report of two cases and review of the literature

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Abstract Plurihormonal pituitary adenomas are tumours that show immunoreactivity for more than one hormone that cannot be explained by normal adenohypophysial cytodifferentiation. The most common combinations in these adenomas include growth hormone (GH), prolactin (PRL) and one or more glycoprotein hormone sub-units (β-TSH, β-FSH, β-LH and αSU). The authors report two cases of a plurihormonal pituitary adenoma expressing the rare combination of ACTH and GH. They both underwent successful transphenoidal hypophysectomy (TSH). Long-term post-operative follow-up revealed no evidence of tumour recurrence. Due to the multiple secretions and plurihormonal characteristics clinical diagnosis of composite pituitary adenomas can be difficult. The authors discuss the diagnosis and management of composite pituitary adenomas and review the literature regarding this rare phenomenon.

Keywords Plurihormonal pituitary adenoma · Composite pituitary adenoma · Dual differentiation · Adrenocorticotropic hormone (ACTH) · Growth hormone (GH) · Transphenoidal hypophysectomy (TSH)

Introduction

The diagnosis of plurihormonal adenomas relies on the application of immunohistochemistry to demonstrate specific reactivity to unrelated pituitary hormones, and the prevalence varies in different series depending on the diagnostic methods used. Nevertheless, it is apparent that plurihormonal adenomas constitute a significant proportion of pituitary adenomas. Some studies suggest a prevalence of approximately 31-36 % in surgically removed tumours [7, 17]. In keeping with the transcriptional regulation of adenohypophysial cells synthesising individual hormones, pituitary adenomas often co-express GH and PRL or FSH and LH; however, the 2004 WHO classification of pituitary adenomas does not include combinations of GH, PRL and TSH or FSH and LH as plurihormonal pituitary adenomas [1, 9]. True plurihormonal pituitary adenomas exhibit immunoreactivities that cross the expected lines of cytodifferentiation. Such tumours are rare, and currently, there is lack of definitive evidence regarding the incidence and clinical relevance of such plurihormonality.

GH-producing adenomas often co-secrete other hormones. Approximately 25 % concurrently secrete prolactin (PRL) [13]. Single adenomas producing ACTH and GH are rare although they have previously been described. Diagnosis may be especially difficult due to the potential range of signs and symptoms. Excessive ACTH secretion may lead to Cushing's syndrome with characteristic features such as moon face, buffalo hump, truncal obesity and abdominal striae. Oversecretion of GH may cause acromegaly and a distinctive appearance with enlargement of the hands, feet, nose, lips, ears and thickening of the skin in addition to multiple other clinical features.

Case presentation

Case 1: A 61-year-old female under the care of the otolaryngology team underwent a MRI brain for left-sided otalgia as an investigation for possible retrocochlear pathology. An incidental pituitary mass and anterior clinoid/planum sphenoidale

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meningioma were diagnosed. She was referred to the multidisciplinary endocrine clinic. On discussion, it became apparent that she had been suffering from hyperhidrosis for several years and her physical appearance was acromegalic. Serum analysis revealed elevated GH serum level of 18.3 mU/L, random cortisol of 356 nmol/l (reference range 171–536) and IGF-1 108 nmol/l (reference range 9.8–27.6). Formal visual field assessment revealed no abnormalities. Initially she underwent a subfrontal craniotomy for removal of an anterior clinoid meningioma. Three months later she underwent TSH for removal of the pituitary mass. Gross microscopic resection of the pituitary tumour was achieved. No peri-operative complications occurred and the patient was discharged with replacement hydrocortisone.

The resected tumour fragments were fixed in 10 % buffered formalin and after processing in graded ethanols, embedded in paraffin blocks. From these blocks, 4-μm-thin sections were cut and stained with routine histochemical methods for haematoxyin-–eosin (H&E), Periodic acid Schiff–Orange G (PAS-OG) and reticulin. The sections were also immunostained for anterior pituitary hormones: GH (Novocastra, polyclonal antibody (ab), 1:200), prolactin (DAKO, polyclonal ab, 1:800), ACTH (LabVision-Thermo monoclonal ab, clone AH26 1:800), Thyroid Stimulating Hormone (Novocastra, monoclonal ab, clone QB2/6, 1:100), Luteinising Hormone (LabVision-Thermo, monoclonal ab, clone SPM103, 1:8000), Follicle-stimulating hormone (Novocastra, monoclonal antibody, clone INN-hFSH-60, 1:500) using automated immunostainer (Leica BondMax) as per manufacturer's guidelines and using horseradish peroxidase-conjugated streptavidin complex and diaminobenzidine as a chromogen. The proliferative activity was assessed by Ki67 (DAKO, monoclonal ab, clone MIB-1). Histology showed features of a pituitary adenoma with an unusual biphasic appearance in both the tinctorial properties (PAS-OG method) and the immunohistochemical expression profile. The tumour contained large confluent areas of acidophilic cells expressing GH (and very focal FSH and LH), which were sharply demarcated from equally prominent geographic areas of cells that strongly expressed ACTH. Proliferative activity overall remained low (<3 %). No atypical features were found (Figs. 1 and 2).

At two-year follow-up under joint neurosurgical and endocrine care, she was clinically well with no clinical or radiological evidence of pituitary tumour recurrence. She remained on replacement hydrocortisone and her pre-operative signs/symptoms of endocrine dysfunction had fully resolved.

Case 2: A 78-year-old female was referred to the chest physicians with symptoms of daytime somnolence and sleep disturbance. On clinical review, she was found to have classic acromegalic facies. A glucose tolerance test confirmed acromegaly with a nadir growth hormone of 16.8 mU/L. Fasting serum cortisol was 388 nmol/l (reference range 171–536) and IGF-1 level was 35.6 nmol/l (reference range 7.7–23.0). No

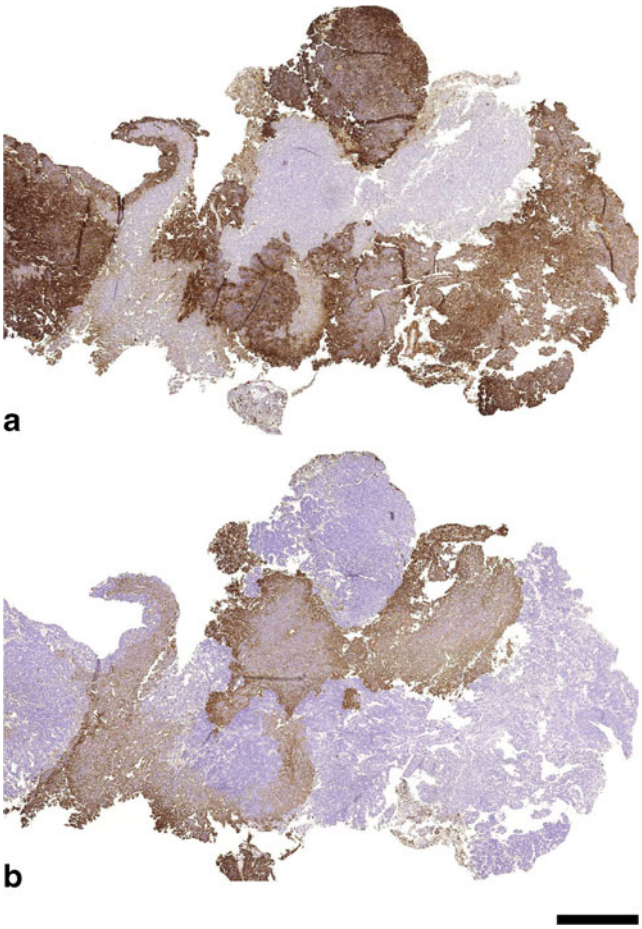

Fig. 1 Low-power view of the tumour showing the distribution and proportions of ACTH (**a**) and GH (**b**) expressing areas. Scale bar: 1,000 μm (**a**-**b**)

obvious pituitary tumour was visible on MRI scan and the patient was managed with cabergoline 500 μg twice weekly. Unfortunately, she developed presumed Raynaud's syndrome, a recognised side effect of cabergoline. Cabergoline was discontinued and she was referred to the pituitary surgeon. It was difficult to ascertain whether the new symptoms were reflective of true Raynaud's phenomenon or carpal tunnel syndrome due to acromegaly.

She underwent TSH and gross microscopic resection was achieved. Post-operatively, she developed the syndrome of inappropriate antidiuretic hormone release (SIADH). This was successfully managed with conservative medical therapy. Her GH level reduced to 1.2 on glucose tolerance test and she was discharged on no further treatment.

The resected tumour fragments were processed and analysed histologically in an identical manner as in Case 1. Histology showed a pituitary adenoma composed of two different cell types, the majority of the fragments comprised Orange G+ acidophilic cells predominantly expressing GH with only very focal prolactin expression. A single smaller fragment was mainly comprised of PAS+basophilic cells expressing ACTH

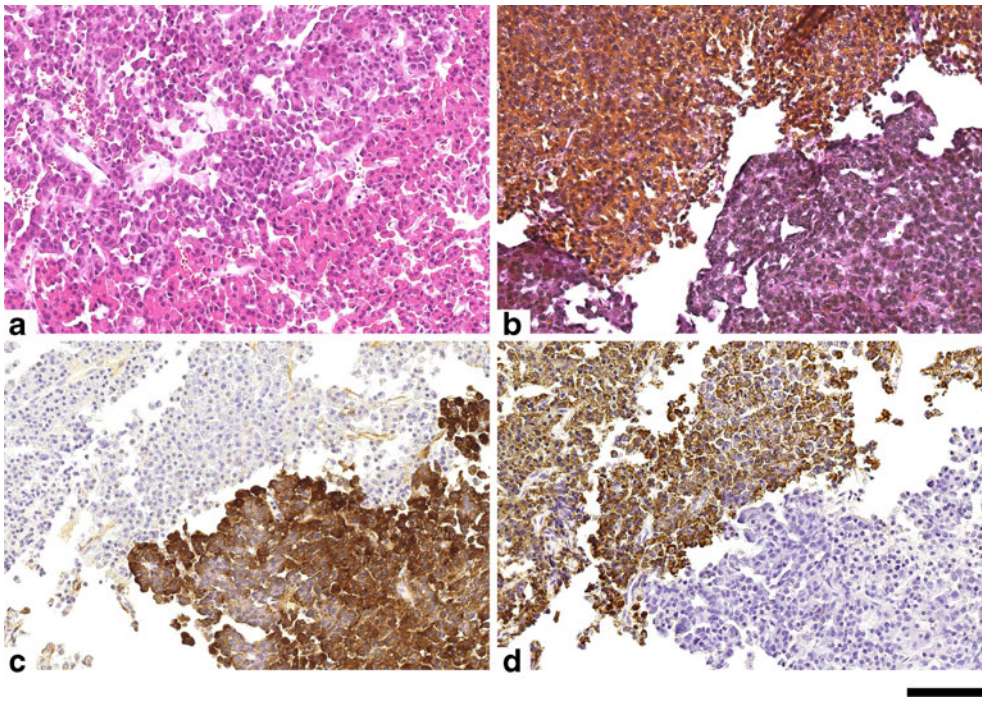

Fig. 2 On higher power, two separate populations of neuroendocrine cells can be distinguished on H&E (**a**) and PAS-OG stain (**b**). Immunostaining for ACTH (**c**) shows positive labelling in PAS positive basophilic cells; while orange G-positive acidophilic cells show positive labelling for GH (**d**). Scale bar: 100 μm (**a**-**d**)

with a few GH expressing acidophilic cells intermingled at the periphery of this fragment. The effaced reticulin fibre pattern in the tumour was consistent with an adenomatous proliferation rather than normal or hyperplastic pituitary tissue. The tumour showed no atypical features (Fig. 3).

At one-year follow-up, she showed clinical improvement with shrinking of her hands and feet, normal post-operative GH levels, and there was no radiological evidence of tumour recurrence.

Discussion

The routine application of immunohistochemistry and its ancillary techniques in the diagnosis of pituitary pathology has challenged and negated the long held "one-cell-one-hormone" concept, as it has been demonstrated beyond doubt that plurihormonality is common in adenomas and non-neoplastic pituitary tissue [18]. Pituitary precursor cells are capable of differentiating towards the spectrum of cell types that populate the adult adenohypophysis. Current evidence points to a distinct lineage for corticotrophs from that of other adenohypophysial cell types. Expression of pituitary hormones in adenohypophysial cells is regulated by several transcription factors. PIT-1 regulates expression of GH, PRL and TSH, whereas STF-1 and GATA-2 regulate expression of FSH and LH. ACTH expression is regulated by T-PIT. It is therefore not unexpected that pituitary adenomas often co-express GH and PRL or FSH and LH [15]. However, true plurihormonal pituitary adenomas that exhibit immunoreactivities that cross expected lines of cytodifferentiation are rare entities; one such example being the clinically aggressive silent subtype 3 adenoma. Plurihormonal adenomas may be further sub-classified as monomorphous (different hormones expressed from a single morphological cell type) or plurimorphous (different hormones expressed by morphologically divergent cells). Only ten cases of plurihormonal single pituitary adenomas secreting both GH and ACTH have previously been described [2–4, 10–12, 14, 16, 19, 20]. Table 1 shows the characteristics from previous reports. Both of our cases showed histologically distinct populations of cells expressing GH and ACTH with some intermingling only at the boundaries. These findings would be compatible with a plurimorphous (composite) plurihormonal pituitary adenoma. The presence of very focal FSH and LH expressing cells within the dominant GH expressing component in the first case is interesting; in the absence of any normal anterior pituitary in the vicinity, this could be interpreted as focal evidence of further divergent hormonal differentiation within this GH predominant component.

It is common for GH and ACTH-secreting pituitary tumours to show no clinical signs of Cushing's syndrome. This phenomenon has been termed subclinical Cushing's disease (CD). A few such cases have been reported [5, 6, 8]. Oki et al. proposed that subclinical CD may be due to insufficiency of autonomic ACTH production or that the ACTH produced was inactive. They reported the presence of high molecular weight (HMW) ACTH, a biologically inactive form in a patient with acromegalic features but no cushingoid features. Our two cases exhibited subclinical CD. The association between

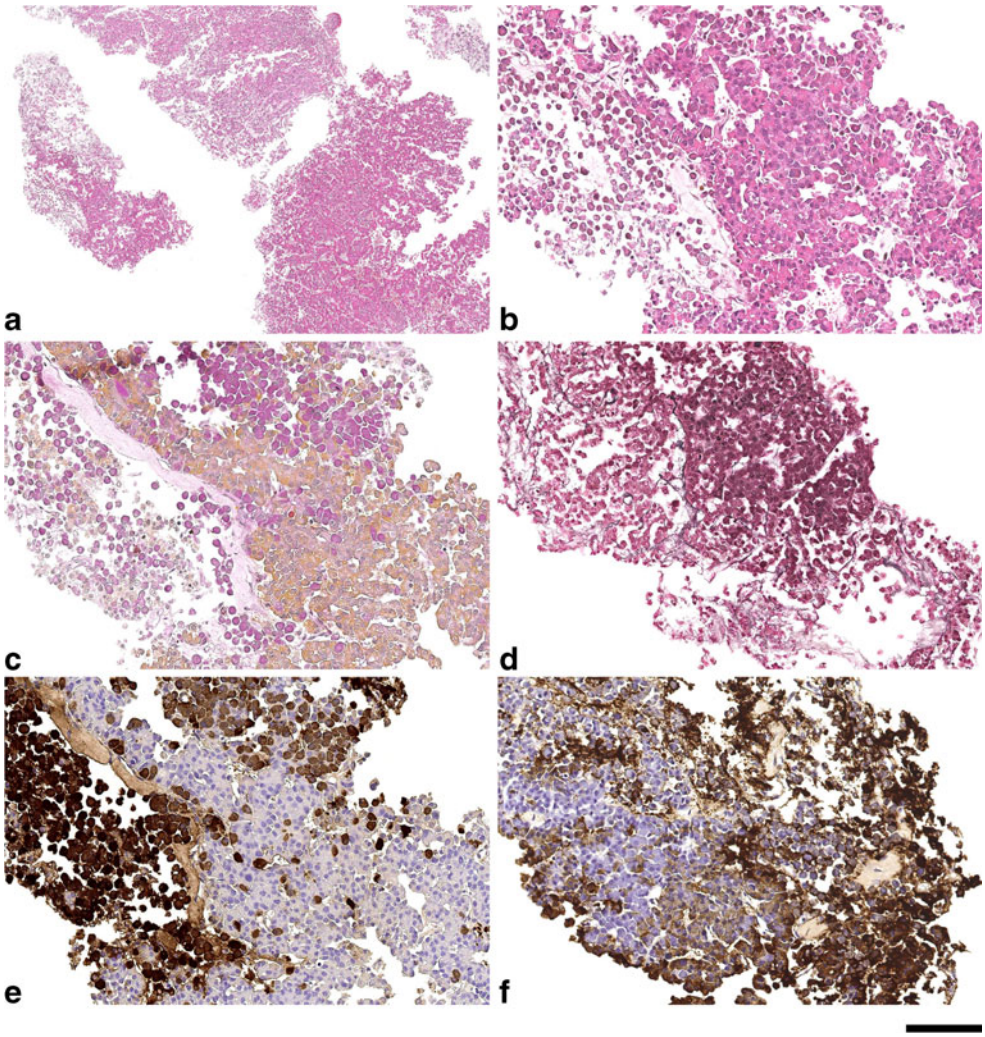

Fig. 3 H&E stained sections (**a** and **b**) reveal several moderately cellular tissue fragments comprising mildly pleomorphic cells. PAS-OG stain (**c**) reveals two populations of cells, PAS positive and orange G positive. In both components the cells are arranged in patternless sheets with effaced reticulin fibre pattern (**d**). Immunostaining for ACTH (**e**) shows positive labelling in PAS positive basophilic cells; while the remaining acidophilic cells are positive for GH (**f**). Scale bar: 500 μm (**a**), 100 μm (**b**-**f**)

subclinical CD and plurihormonal pituitary adenomas has not yet been confirmed although our cases may suggest a similar mechanism. We did not explicitly test this. The clinical relevance of the subclinical CD phenomenon has not previously

Table 1 Characteristics of reported plurihormonal ACTH and GH secreting pituitary adenomas

Citation	Age/Sex	Clinical features	Staining pattern
Kannan et al. 2012 [11]	52 M	Acromegaly and Cushing's	Rare GH positivity and complete PRL positivity in large adenoma with ACTH staining in the small pieces of adenoma
Zada et al. 2011 [20]	31 F	Acromegaly and Cushing's	Not explicitly documented
Oki et al. 2009 [16]	36 M	Acromegaly	Diffuse strong GH staining and focal ACTH and TSH staining
Tsuchiya et al. 2006 [19]	54 M	Acromegaly	Diffuse GH and PRL staining with focal ACTH staining
Kageyama et al. 2002 [10]	45 F	Acromegaly	Distinct groups of cells with weak GH staining diffusely located and sparse but distinct ACTH stained cells
Mazarakis et al. 2001 [14]	53 M	Acromegaly	Focal GH immunoreactivity mixed with ACTH immunoreactive cells
Kovacs et al. 1998 [12]	62 M	Acromegaly	Mainly variable GH+cells with few ACTH+cells
Apel et al. 1994 [2]	76 F	Acromegaly	Distinct areas of ACTH and GH staining
Blevins et al. 1992 [4]	40 F	Acromegaly and Cushing's	Distinct groups of cells staining for ACTH and GH
Arita et al. 1991 [3]	29 F	Acromegaly and Cushing's	Diffuse GH staining with focal ACTH in tumour

been discussed. It is possible that performing gel chromatography to detect subclinical CD may affect pituitary surgeons decision-making regarding the need and timing of operative intervention. Although subclinical CD suggests that ACTH is produced in a low biologically active form so as to not cause unwanted signs or symptoms it is possible that with tumour progression there may be greater and/or altered production of HMW ACTH which may have the potential to manifest as Cushing's syndrome. The natural history of this phenomenon is unknown, however, a better understanding may encourage earlier tumour removal.

In all reported cases of plurihormonal single pituitary adenomas producing both GH and ACTH the dominant clinical characteristics have been of GH overproduction. Here we have also presented two cases with clinical features of acromegaly only. Kovacs et al. described a plurihormonal tumour with ACTH hypersecretion that was not evident clinically or biochemically postulating that the somatotrophs with GH hypersecretion were associated with silent subtype I cells of adenomatous corticotrophs.

The post-operative MRI scans for both patients shows successful resection of the tumours (Fig. 4) leading to resolution of endocrine dysfunction.

It is difficult to draw strong conclusions regarding the nature of pituitary adenomas secreting both ACTH and GH. Our two cases support previous reports that GH secretion dominates the clinical picture of such tumours. The origin of such tumours remains elusive. Several hypotheses have been put forward, including neoplastic transformation of two different cell lines, transdifferentiation of one neoplastic clone and divergent differentiation from non-committed stem cells. In the absence of ultrastructural and transcriptional studies, any conclusions for our two cases will remain speculative. Plurihormonal tumours secreting ACTH and GH are uncommon. Our two cases add a significant body of knowledge to what is currently known about these rare tumours. Further work is needed to elucidate several features of these tumours including their cellular origin and natural history.

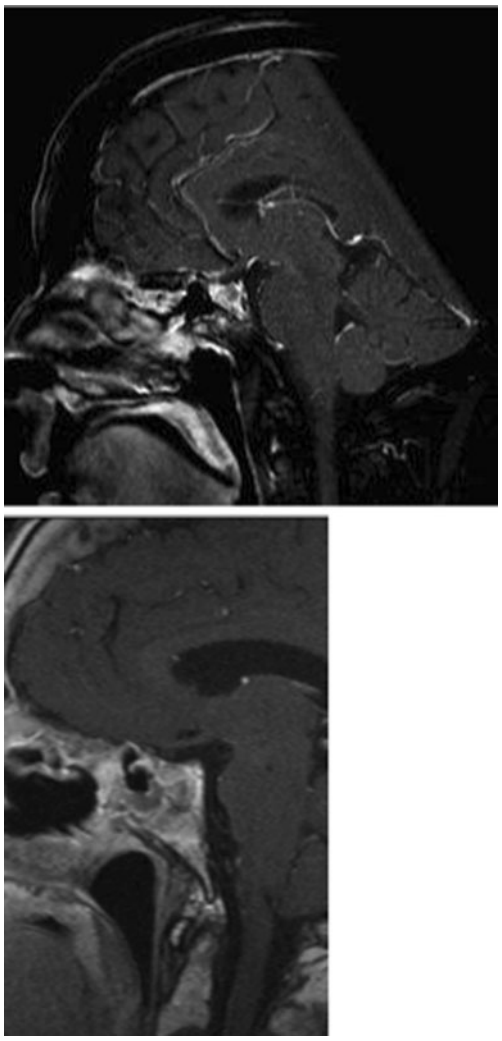

Fig. 4 Patient 1 (above) and patient 2 (below) post-operative sagittal MRI scans (T1 with gadolinium)

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Conflict of interest None.

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