Clinicopathological study of pituitary adenomas in the region of northeast India

Mrinal Bhuyan[a], Dipti Sarma[b], Deep Dutta[a], Yogesh Yadav[b], Sarat Das[c]

[a]Departments of Neurosurgery, [b]Endocrinology, [c]Pathology, Gauhati Medical College, Guwahati, Assam, India

Correspondence to Deep Dutta, MBBS, MS, MCh, Department of Neurosurgery, Gauhati Medical College, Guwahati 781032, Assam, India; Mobile no: 8822049217; e-mail: duttadeep2844@yahoo.co.in

Received 7 June 2016
Accepted 21 September 2016

Journal of The Arab Society for Medical Research 2016, 11:43–49

Background/aim
Pituitary adenomas (PA) are the most common tumours of the pituitary gland. Clinicopathological characteristics of these tumours may vary from population to population. The present study aimed to understand the detailed clinical and pathological characteristics of surgically resected PA in the region of Northeast India.

Patients and methods
Thirty-two consecutive cases of PA diagnosed and operated at Gauhati Medical College were studied for clinical, pathological, and treatment characteristics in this study between January 2014 and December 2015. The patient group included 20 female and 12 male patients with a median age of 37 years (range: 22–58 years).

Results
A total of 13/32 (40.63%) cases were nonfunctioning adenomas. Among the hormonally active PA, prolactin-producing adenomas were most common (25%), followed by growth hormone-producing adenomas (18.8%) and adrenocorticotrophic hormone-producing adenomas (15.6%). The most common symptom was headache, followed by visual disturbances, galactorrhoea, acromegaloid and cushingoid features. Menstrual disturbances were present in 50% of the female patients. 23.1% of the cases of nonfunctioning PA were negative for all anterior pituitary hormones on immunohistochemistry.

Conclusion
PA can have varied presentations. Detailed clinical, hormonal and pathological evaluation is essential for better understanding the nature of the tumour growth and its progression. This study is the first of its kind in the region of Northeast India, showing the relationship of the functioning and nonfunctioning PA with their hormonal profile and immunohistochemical analysis.

Keywords:
immunohistochemistry, pituitary adenoma, pituitary surgery, trans-sphenoidal

J Arab Soc Med Res 11:43–49

1687-4293

Introduction

Pituitary adenomas (PA) are the most common tumours of the pituitary gland. From a neurosurgical perspective, it is the third most common intracranial primary tumour after gliomas and meningiomas [1]. PA is a familiar neoplasm of the sella turcica [2] and reports also suggest increasing incidence of PA in different populations [3]. However, there is scarcity of data on PA from India and especially from the North-Eastern region.

Each subtype of PAs has its own clinical presentation, hormone secretion profile, including different histopathological characteristics, tendency towards invasiveness, patient prognosis, and alternate treatment protocols [4]. The estimated prevalence of PA varies from 14.4% in autopsy studies to 22.5% in radiologic studies [2].

Primarily, PA is classified on the basis of the following: (a) histological criteria; (b) immunohistochemical criteria, which is the gold standard for diagnosis and for analysing the main pituitary hormones [prolactin (PRL), growth hormone (GH), adrenocorticotrophic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH)]; (c) ultrastructural criteria, through electron microscopy [5]; (d) clinical and biochemical criteria, such as clinical presentation and function of pituitary to confirm whether or not the tumour is functioning; (e) imaging criteria to determine tumour size and sellar/extrasellar extension; and (f) surgical findings.

On the basis of size, PAs are divided traditionally into microadenomas (dimension$<$1 cm) and macroadenomas (dimension $\geq$1 cm). Currently, this

classification has been improved by classifying on the basis of immunohistochemistry (IHC) and electron microscopic findings. PA are classified on the basis of size as determined by means of MRI as macroadenoma and microadenoma, on the basis of cytoplasmic staining affinity as acidophilic, chromophobic, or basophilic, or on the basis of measurement of hormone production of GH, PRL, TSH, FSH, ACTH, and LH, and ultrastructural characteristics [6]. There is a remarkable variation in the biological behaviour of PA, which has formed the basis for their classification as follows: clinically functioning and nonfunctioning adenomas.

According to Hardy [7], PA are classified into four grades on the basis of the size and degree of local invasion as follows:

Grade I: Microadenomas (<10 mm in diameter), cause focal bulging of the sellar floor.
Grade II: Macroadenomas (>10 mm in diameter), have suprasellar expansion or enlargement of sella.
Grade III: Locally invasive adenomas with partial destruction of the sella.
Grade IV: Strongly invasive adenomas invade adjacent bony structures. Moreover, they show suprasellar outgrowth that includes the cavernous sinus, hypothalamus and bone.

Patients present with a variety of signs and symptoms related to either excess or impaired pituitary function and/or mass effects. Tumours that produce hormones such as PRL, GH and ACTH are the most common PAs. Moreover, no clinical or biochemical evidence of hormone excess can be found in about one-third of PA. The majority of clinically nonfunctioning pituitary tumours are gonadotroph cell adenomas.

In this study, we analysed surgically resected and immunohistochemically evaluated pituitary macroadenomas in our institute over 2 years to determine the clinicopathological characteristics and proportion of hormonal subtypes of PA in the Northeast Indian population.

Patients and methods

Ethics statement

Ethical clearance was obtained from the Ethics Committee of Gauhati Medical College and Hospital. Study methodology was approved by institutional ethics committee of Gauhati Medical College and Hospital, Assam (No.233/2014/229).

Study population

All consecutive, nonduplicate 32 cases of PA between January 2014 and December 2015 diagnosed at Gauhati Medical College, India, were included in the study. Patients were admitted both in the Department of Endocrinology and Neurosurgery. They were operated in the Department of Neurosurgery, Gauhati Medical College, Guwahati. According to the Hardy classification [7], they were classified by their degree of local invasion, endocrine symptoms (clinically functioning and clinically nonfunctioning PA) and by their hormonal secretion, computed by means of IHC. Disease evolution time, tumour regrowth, bromocriptine treatment, and time to outcome of the patients were evaluated at the time of diagnosis to analyse the biological behaviour of PA.

Clinical data

Adenomas were classified into functional and nonfunctional on the basis of preoperative clinical evaluation. Information documented included age, sex, clinical features, and duration of symptoms, ophthalmologic evaluation, hormonal evaluation, and preoperative size of the PA. Hormone measurements were performed using commercially available immunoassays. Central hypothyroidism was diagnosed based on the finding of a low T4 in the presence of a low or inappropriately normal TSH. A morning serum cortisol less than 5 μg/dl was used as a cutoff to define hypocortisolism. A diagnosis of hypogonadotropic hypogonadism can be made when there is a normal or low level of LH and FSH with less than 20 pg/ml of serum estradiol or less than 300 ng/dl of testosterone level.

Magnetic resonance imaging study and tumour size

MRI studies were carried out before and after surgery using a 1.5 or 3.0-T scanner with injection of gadolinium. The maximum diameter of each tumour was measured, and the tumours were grouped as microadenomas (maximum diameter <10 mm) or macroadenomas (maximum diameter ≥10 mm). Tumour volume was calculated according to the Di Chiro and Nelson formula: volume=height×length×width×0.5233 [8]. Lateral tumour growth was assessed using the Knosp classification [9]. A thyroid ultrasound study was also carried out in some patients to assess the condition of the thyroid gland.

We used the following approaches for tumour excision.

(1) Trans-sphenoidal microsurgery.
(2) Endoscopic endonasal approach to the sella using the paraseptal, middle meatal, or middle turbinectomy approach.

Immunohistochemistry

The samples were applied on each slide [10], and deparaffinized, rehydrated, and rinsed in PBS. Later, endogenous peroxidase was blocked with 0.25% H_2O_2/ distilled water for 15 min and blocked with 3% BSA in PBS. After 1 h of incubation, the slides were treated with monoclonal antibodies of pituitary hormones: PRL, GH, LH, FSH, TSH and ACTH. Positive control consisted of normal postmortem pituitaries for pituitary hormones. After washing, the sections were incubated for 30 min and treated with the secondary antibody, biotinylated anti-Ig. After washing in PBS, the sections were allowed to incubate for 30 min along with the peroxidase-conjugated streptavidin complex. The sections were counterstained with hematoxylin. The immunoreactivity of the tumour cells to different hormones were observed as positive or negative.

Results

The number of PAs studied was 32, which were organized according to their characteristics. Tumours mainly affected young adult population with a mean age of 38.12 years. Twelve (37.3%) were male, with a mean age of 40.17 years and SD of 9.8 (range: 23–58 years), and 20 (62.5%) were female patients, with a mean age of 36.8 years and SD of 8.92 (range: 22–55 years). However, the mean age in the nonfunctional group was higher than that in the other groups consisting of Cushing, prolactinoma, and acromegaly (P=0.035). Although statistically nonsignificant (P=0.577), there were more female than male patients in the study cohort (female-to-male ratio was 1.67 : 1).

Nonfunctioning adenomas constituted 40.6% of PA. Among the hormonally active PA, PRL -producing adenomas were most common (25%), followed by GH-producing adenomas (18.8%) and ACTH-producing adenomas (15.6%).

Headache was the most common symptom, present in 24 (75%) cases, whereas visual symptoms were reported by 16 (50%) patients. Visual field defects were noted in 56.2% of the cases on evaluation. Menstrual disturbances (amenorrhoea and oligomenorrhoea) were present in 10 (50%) female patients and galactorrhoea was reported by seven (35%) female patients, and an additional four (20%) patients were diagnosed as having galactorrhoea on clinical examination. All of the patients with Cushing's disease and acromegaly had symptoms suggestive of respective disease.

Patients with nonfunctioning PA presented with symptoms of headache (84.6%), visual disturbances (61.5%), amenorrhoea (30.8%) and hypogonadism (30.8%). Almost half (46.2%) of the patients had visual field deficits (hemianopia and quadrantanopia) on clinical evaluation.

Three (9.4%) cases were incidentally detected as adenomas, whereas the rest presented with symptoms as mentioned above. The mean duration of symptoms was 7 years and 8 months in patients with acromegaly, whereas it was only 1 year and 5 months in cases of Cushing's disease.

Operative results

All operations were performed with the patient positioned supine, the torso raised about 15–20°, and the head placed with the forehead–chin line set horizontally. The head was rotated towards the surgeon at 10–20°.

The tumour was approached through either of the two techniques:

(1) Trans-sphenoidal microsurgery.
(2) Endoscopic endonasal approach to the sella using the paraseptal, middle meatal, or middle turbinectomy approach.

Sublabial approach was used for patients with large tumours with small nostril. An incision is made horizontally in the gingival mucosa by retracting the upper lips. Thereafter, the maxilla and nasal cavity floor are accessed. A vertical incision is made to separate the nasal mucosa from the septum, and the anterior septum is subluxed and deviated. A speculum is inserted. The microscope or endoscope is brought into the field at this point. An osteotome and Kerrison rongeur are used to open the sphenoid sinus and enlarge it until the surgeon can visualize the lateral portion of the sella. To confirm the position of the sella we use the fluroscopy. The sellar floor is opened with an osteotome and enlarged with an up-biting Kerrison rongeur, revealing the dura. A midline vertical or cruciate incision is made in the dura with a number 11 blade, and is enlarged using upangled scissors. Tumour is exposed and intracapsular decompression and piecemeal excision is performed using pituitary rongours, curettes, gentle suction and irrigation. Most of the tumours we encountered were soft and suckable. In three of the cases, the cavernous sinus was encroached by the tumour, and during its resection there was bleeding from the cavernous sinus. We achieved haemostasis using gelfoam and surgicel (oxidized cellulose polymer).

We prefer not to use any packing material. While dissecting we try not to breach the arachnoid, and in case the arachnoid gets ruptured we pack the sella using the fascia lata supported by fat and muscle. We usually repair the floor with pieces of bone harvested from the septum. We also use fibrin glue in most of our cases.

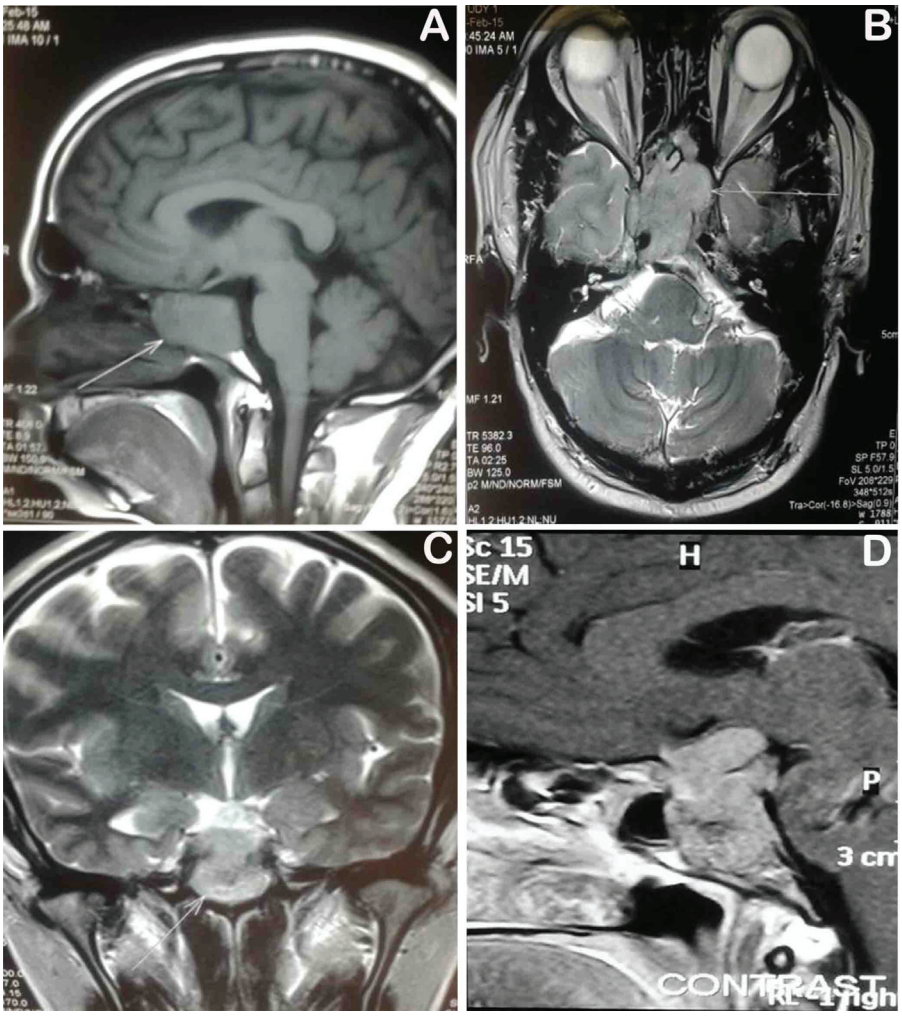
Tumours were predominantly sellar and 60–100% excision could be achieved in all cases. Postoperative transient diabetes insipidus was observed in two (6.25%) cases, which were managed with fluid replacement only (without vasopressin supplementation). Postoperative cerebrospinal fluid rhinorrhoea occurred in three (9.3%) cases, which resolved with conservative treatment. MRI images of few cases in our study population are shown in Fig. 1a–d.

In follow-up MRI, significant tumour recurrence was seen in two (6.25%) cases, both of which were cases of acromegaly with tumour size more than 2 cm (preoperative) and with tumour extension to the cavernous sinus and with carotid encasement. Reoperation was performed in both cases to reduce the tumour bulk and subsequently sent for radiotherapy.

Immunohistochemistry

Overall, PRL was the most frequently detected hormone by means of IHC (13 cases, 40.63%), followed by FSH (9/32, 28.13%), ACTH (7/32, 21.88%) and GH (7/32, 21.88%), as shown in Fig. 2. However, among the nonfunctional adenomas, PRL was present in 2/13 as compared with 11/19 in the others. FSH was present in 8/13 among nonfunctional adenomas as compared with 1/19 in others. ACTH was present in 1/12

Figure 1



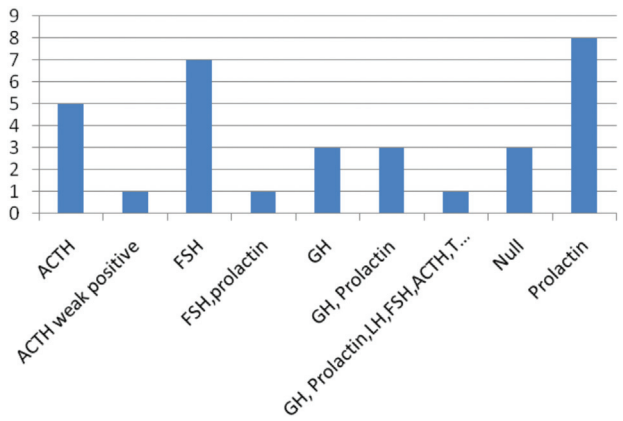
MRI of selected cases from the study: (a) Noncontrast T1WI sagittal MR: Large, relatively well-defined mass filling the entire sella with some suprasellar extension paralleling grey matter signal intensity. Pituitary is not seen separately – large noncomplicated Macroadenoma; (b) Precontrast T2WI Axial MR: Large lobulated pituitary macroadenoma invading the skull base and the cavernous sinus on right showing an ill-defined border between it and adjacent brain. There is encasement of the right ICA, which is otherwise not narrowed. (c) Precontrast T2WI Coronal MR showing a large 'figure of 8' appearing. Pituitary macroadenoma due to suprasellar extension encasing bilateral ICA with normal arterial lumen, and (d) postcontrast T1WI sagittal MR revealing inhomogeneously enhancing mass with intrasellar and suprasellar component compressing the floor of the third ventricle.

nonfunctional cases as opposed to 6/19 in the others. GH was not present in the nonfunctional group. Figure 3 a–f shows photomicrographs of PA in our study population showing immunopositivity for various anterior pituitary hormones.

IHC correlated well in Cushing's disease cases, with the tumour positive for ACTH in all cases. In patients with acromegaly, tumour cells were positive for GH in all cases and half of the cases were additionally positive for PRL. IHC was positive for PRL in all cases of prolactinoma, with one case showing positivity for all hormones. More than half (53.8%) of the cases of nonfunctioning PA were positive for FSH only, and one (7.7%) case each was positive for ACTH (weakly positive) only, FSH and PRL, and PRL only. Approximately one-fourth (23.1%) cases of nonfunctioning PA were negative for all anterior pituitary hormones on IHC.

In relation to raised serum hormone levels, 85.7% of cases with adenoma that were positive for GH on IHC had nonsuppressible serum GH levels, 71.4% of cases with ACTH positivity had raised serum cortisol levels and 92.3% of the PRL positive cases had raised serum PRL levels. None of the cases with IHC positive for FSH, LH or TSH had raised corresponding hormones.

Figure 2



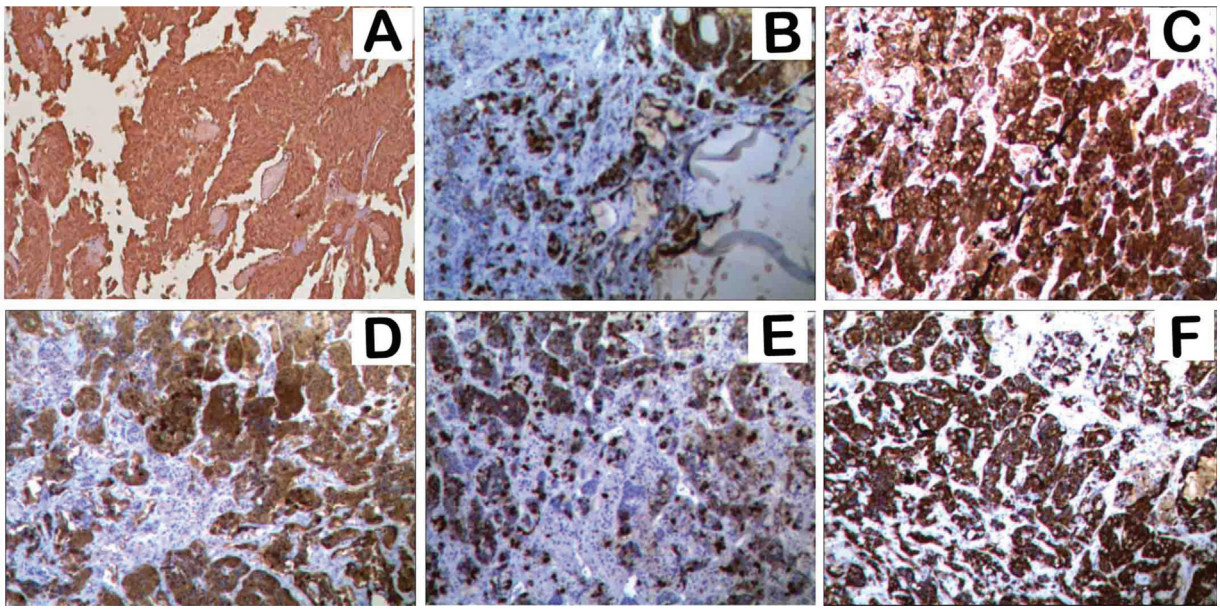
Frequency of pituitary adenomas based on immunohistochemical positivity of different hormones. Note that adenomas showing immunohistochemistry positivity for prolactin and follicle-stimulating hormone are the most frequent ones.

Discussion

Pituitary adenomas are tumours with heterogeneous nature, which constitute 10–25% of intracranial neoplasms and are the most common lesions in the sellar region [11]. In this study, we are presenting the reports of 32 cases of PA, which occurred between 22 and 58 years of age. There was a slightly higher incidence in women compared with men.

In a large series by Mindermann and Wilson [12] it was observed that pituitary adenomas are usually not seen in people younger than 20 years of age and are mostly prolactinomas (39%), followed by endocrine-inactive adenomas, GH-releasing adenomas, and ACTH-releasing adenomas. In the same study, they also observed that PA were more common in female than in male population (1.7 : 1). In the present study, we also found a similar female

Figure 3



A photomicrograph of pituitary adenoma showing immunopositivity for (a) prolactin (×20), (b) follicle-stimulating hormone (×10), (c) thyroid-stimulating hormone (×10), (d) adrenocorticotrophic hormone (×10×), (e) luteinizing hormone (×10), and (f) growth hormone (×10).

preponderance (female-to-male ratio 1.67 : 1), and also none of the participants in our study were younger than 20 years of age. Similarly, we also found clinically nonfunctioning adenomas and prolactinomas to be the most common type of adenoma in our study. In line with our study results, Gruppetta et al. [13] had reported that the most common subtypes of PA were the prolactinomas and nonfunctioning adenomas. Prolactinomas have a much higher incidence in this study, which is attributed to the fact that most cases of prolactinomas are managed medically. As our study investigated only those cases of adenomas that underwent surgery, the prevalence of prolactinomas was relatively lower.The results of our study indicate that the most common mass effect symptoms among patients with PA undergoing surgical resection were headache and visual field defects. Consistent with our study results, Gruppetta et al. [13] reported that the most common presenting features in patients with PA were headache, followed by menstrual irregularities and visual impairment. Similar to our results, Hennessey and Jackson [14] demonstrated that the most common clinical symptoms due to intracranial mass effects of PA are visual defects and headache.

Nonfunctioning pituitary adenomas are a heterogeneous group with different types of cells [15,16]. They are usually diagnosed as macroadenomas due to the absence of clinical manifestations and cause tumour growth for long time. Between 15 and 45% of pituitary adenomas are nonfunctioning [17,18]. Report shows that 95–100% of nonfunctioning pituitary adenomas are macroadenomas, and in different studies it has been found that the frequency of recurrence varies between 19 and 34.8% [19,20] and up to 79% have hormone expression, as evaluated by means of IHC [16], being gonadotroph hormones [21,22].

Conclusion

In our study, 13 (40.63%) PA were nonfunctioning, of which five (38.46%) were seen men. Most of the tumours affect young adult population. IHC correlated well in case of clinically functioning PA. Although clinically nonfunctioning, IHC showed positivity for various hormones in most of the nonfunctioning PA. More advances in molecular techniques as well as proteomic technique that contribute to investigate the proteins involved in the disease process are necessary. Although a small cohort, this study enlightens upon the clinical, hormonal and immunohistochemical profile of patients with PAs in the region of Northeast India. A more detailed study with more number of cases will be useful in understanding the disease in a better way.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Ostrom QT, Gittleman H, Farah P, Ondracek XX, Chen Y, Wolinsky Y, *et al.* CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States 2006-2010. Neuro Oncol 2014; 16:760.

2 Perrin R, Patil S, Perry XX. Pituitary gland. In: Humphrey P, editor. The Washington manual of surgical pathology. 2nd ed. Washington, DC: Lippincott Williams & Wilkins; 2012. p. 446.

3 Fernandez XX, Karavitaki N, Wass JA. Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). Clin Endocrinol (Oxf) 2010; 72:377–382.

4 Kleinschmidt-DeMasters BK. Pituitary gland. In: Rosai XX, editor. Rosai and Ackerman's surgical pathology. 10th ed. Edinburgh: Mosby; 2011. p. 2441.

5 Trouillas XX, Roy P, Sturn N, Dantony E, Cortet-Rudelli C, Viennet G, *et al.* A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case control study of 410 patients with 8 years post-operative follow-up. Acta Neuropathol 2013; 126:123–135.

6 Galland F, Chanson P. Classification and pathophysiology of pituitary adenomas. Bull Acad Natl Med 2009; 193:1543–1556.

7 Hardy XX. Transsphenoidal surgery of hypersecreting pituitary tumors. In: Kohler PO, Ross GT, editors, Diagnosis and treatment of pituitary tumors. International Congress Series No. 303. Amsterdam: Excerpta Medica; 1973. pp. 179–98.

8 Lundin P, Pedersen F. Volume of pituitary macroadenomas: assessment by MRI. J Comput Assist Tomogr 1992; 16:519–528.

9 Knosp E, Steiner E, Kitz K, Matula C. Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. Neurosurgery 1993; 33:610–618.

10 Bratthauer GL, Adams LR. Immunohistochemistry: Antigen detection in tissue. In: Mikel UV, editor. Advanced laboratory methods in histology and pathology. Washington, DC: Armed Forces Institute of Pathology, American Registry of Pathology; 1994.

11 Kovacs K, Scheithauer BW, Horvath E, Lloyd RV. The World Health Organization classification of adenohypophysial neoplasms. A proposed five-tier scheme. Cancer 1996; 78:502–510.

12 Mindermann T, Wilson CB. Age-related and gender-related occurrence of pituitary adenomas. Clin Endocrinol (Oxf) 1994; 41:359–364.

13 Gruppetta M, Mercieca C, Vassallo XX. Prevalence and incidence of pituitary adenomas: a population based study in Malta. Pituitary 2013; 16:545–553.

14 Hennessey JV, Jackson IM. Clinical features and differential diagnosis of pituitary tumours with emphasis on acromegaly. Baillieres Clin Endocrinol Metab 1995; 9:271–314.

15 Laws ER, Randall RV, Kern XX, *et al.* Management of pituitary adenomas and related lesions with emphasis on transsphenoidal microsurgery. New York, NY: Appleton-Century-Crofts; 1982. 277–305.

16 Moreno CS, Evans CO, Zhan X, Okor M, Desiderio DM, Oyesiku NM. Novel molecular signaling and classification of human clinically nonfunctional pituitary adenomas identified by gene expression profiling and proteomic analyses. Cancer Res 2005; 65:10214–10222.

17 Asa SL, Kovacs K. Clinically non-functioning human pituitary adenomas. Can J Neurol Sci 1992; 19:228–235.

18 Milker-Zabel S, Debus XX, Thilmann C, Schlegel W, Wannenmacher M. Fractionated stereotactically guided radiotherapy and radiosurgery in the treatment of functional and nonfunctional adenomas of the pituitary gland. Int J Oncol Biol Phys 2001; 50:1279–1286.

19 De Aguiar PH, Aires R, Laws ER, Isolan GR, Logullo XX, Patil C, Katznelson L. Labeling index in pituitary adenomas evaluated by means of MIB-1: is there a prognostic role? A critical review. Neurol Res 2010; 32:1060–1071.

20 Reddy R, Cudlip S, Byrne JV, Karavitaki N, Wass JA. Can we ever stop imaging in surgically treated and radiotherapy-naive patients with non-functioning pituitary adenoma? Eur J Endocrinol 2011; 165:739–744.

21 Cury ML, Fernandes JC, Machado HR, Elias LL, Moreira AC, Castro M. Non-functioning pituitary adenomas: clinical feature, laboratorial and imaging assessment, therapeutic management and outcome. Arq Bras Endocrinol Metabol 2009; 5:31–39.

22 Hanson PL, Aylwin SJB, Monson JP, Burrin JM. FSH secretion predominates in vivo and in vitro in patients with non-functioning pituitary adenomas. Eur J Endocrinol 2005; 152:363–370.