HORMONAL MARKERS IN THE STUDY OF PITUITARY ADENOMAS

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ABSTRACT

Twenty pituitary adenomas were studied using light microscopy, immunohistochemistry and electron microscopy. Based on clinical manifestations of hormonal hypersecretion they were divided into two groups, i.e. group-I (12 patients) with no endocrine dysfunction and group-II (8 patients) with endocrine dysfunction. Group-I could be further sub-divided into two groups based on IHC: group IA in which no hormone could be localised in the tissue (null cell adenomas) and group IB where hormones could be localised in the tissue (1). Tumours in group II, with light microscopy, were acidophil (n=5), basophil (n=1) or mixed (n=2) adenomas. Eight of these 20 cases were plurihormonal. Growth hormone, and prolactin were the commonest combination. This study has helped in the reclassification of the pituitary adenomas according to the hormone they produce.

MJAFI 1996; 52 : 27-31

KEY WORDS : Pituitary neoplasms; Diagnosis; Immunohistochemistry; Electron microscopy.

Introduction

Pituitary adenomas, a subject full of controversies, represents a challenge both to clinicians and to pathologists. Clinically they differ from other intracranial neoplasms because beside causing local changes they produce different hormones resulting in a wide range of systemic symptoms. Diagnosis of pituitary adenoma per se is not a problem. However, it is often difficult to ascertain their precise hormone-producing capability by routine light microscopy (LM) based on the staining characteristics of different secretory granules in haematoxylin and eosin (H&E) sections.

Pituitary tumours are the commonest neoplasms of the sellar region. The incidence of pituitary adenomas in India varies between 3.8 to 10.5% of all intracranial space occupying lesions (1).

The earliest classification of pituitary adenomas based on tinctorial characteristics into acidophil, basophil, and chromophobe type is an oversimplification. Recent studies have unequivocally shown that this does not give, in all cases, a true indica-

tion of the hormone producing status of these tumours. The chromophobe adenomas are regarded as tumours with no endocrine function. Acidophil adenomas are associated with acromegaly and assumed to secrete growth hormone. The basophil adenoma are implicated in the causation of Cushing's disease and are thought to produce ACTH.

Unprecedented progress has been achieved with the introduction of three new techniques - radioimmunoassay, electron microscopy (EM) and immunohistochemistry (IHC) [1,2]. Radioimmunoassay has made it possible to measure minute quantities of various hormones in blood and tissue. With EM it is possible to study the fine structural details of the adenoma cell. IHC permits the specific identification and localization of different hormones on the cells. By using monoclonal antibodies against the various pituitary hormones it has been proved that the so called non-functioning pituitary adenomas produce hormones and that some of these adenomas have the capacity to produce more than one hormone, and may be associated with acromegaly, Cushing's syndrome, hyperprolact-

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inemia, hyperthyroidism or increased gonadotrophin secretion [1,2]. This study was conducted with a view to classify pituitary adenomas according to the type of hormones the tumour cells synthesised based upon immunohistochemical and ultrastructural characteristics.

Material and Methods

This study was carried out at the Department of Pathology, AFMC Pune during the period 1992-94. Twenty surgically removed pituitary adenomas were examined. For LM the specimens were fixed in 20% formal saline, embedded in paraffin and stained by the routinely used H&E method. For IHC, Slidder's orange-fuschin-green method was used. This stains the acidophils orange-yellow, the basophils reddish purple and chromophobes appear gray.

Specimens reported as chromophobe adenomas by LM were subjected to EM examination. The tissues obtained in 2% glutaraldehyde were post-fixed in 1% osmium tetroxide in phosphate buffer and embedded in epoxy resins. Ultrathin sections stained with lead citrate and uranyl acetate were examined under a JEOL-100 CX-II transmission electron microscope. The cases of chromophobe adenomas were also subjected to immunoelectron microscopy (IEM) to analyze the precise location of the hormone inside the cell [3,4].

For IHC 5 µm thick paraffin embedded sections were cut and stained for the various pituitary hormones using the peroxidase-antiperoxidase (PAP) technique [5]. The antisera kits were obtained from DAKO Products. Normal human pituitary glands obtained at autopsy were used as positive controls.

Result

Clinically the patients could be divided into two broad groups: Group-I with "non-functioning adenomas" (12 patients), and group-II with "functioning adenomas" (8 patients).

The main clinical symptoms in all patients in group-I were visual disturbances and those due to raised intracranial pressure. This group showed a male preponderance. The ages ranged from 19 to 48 years with a median of 43 years. The age and sex distribution of the patients is given in Table 1.

TABLE 1
Age and sex distribution of the patients

Clinical group	Total	Male : Female	Median age in years
Group I (Clinically non-functional)	12	11:1	43 (19-53)
Group IA	6	5:1	44.5 (32-53)
Group IB	6	6:0	38.5 (19-46)
Group II (Clinically functional)	8	6:2	28.5 (24-34)
Acromegaly	5	5:0	28 (25-32)
Cushings	1	1 Male	34
Galactorrhoea	1	1 Female	28
Acromegaly + Galactorrhoea	1	1 Female	24

On histopathological examination in group I, four of the tumours revealed a diffuse pattern, 4 had a sinusoidal pattern, 3 had a mixed pattern (diffuse + sinusoidal-2, diffuse + papillary-1) and one had a trabecular pattern. On the basis of immunohistochemical analysis, tumours in this group could be further divided into two subgroups i.e. group IA or the 'null cell adenomas' with no evidence of hormone production (6 cases) and group IB which showed evidence of hormone production. Two were positive for GH, one for PRL, one showed positivity for GH, PRL and ACTH, and two for GH, PRL with occasional positivity for ACTH (Table 2).

The clinically hyperfunctioning group (Group-II) could be subdivided into four groups: (i) 5 patients had acromegaly, (ii) 1 had Cushing's syndrome, (iii) 1 had galactorrhoea, and (iv) 1 had acromegaly and galactorrhoea. The histopathological analysis showed in 4 cases a diffuse pattern, while 2 had a trabecular pattern, and 2 had a glandular pattern, none had a sinusoidal pattern.

All five acromegalic patients were males and they were of relatively younger age. On light microscopy, 4 of them had a diffuse pattern (Fig 1) and one had a trabecular pattern. Three were acidophil and two were mixed adenomas. Immunohistochemically three of these cases showed strong positivity for GH (Fig 2) and two were positive for GH and PRL. Thus in this group, three were pure somatotroph adenomas and two plurihormonal adenomas.

TABLE 2
Outcome of light microscopy and immunohistochemistry (IHC) on 24 cases of pituitary adenoma

Clinical group	Staining pattern on light microscopy	Hormones identified on IHC
I (Non-functioning adenomas) (12)	— (6)	Group Ia null cell type Group Ib Hormone producing GH (2) PRL (1) GH+PRL+ACTH (1) GH+PRL(+ACTH) (2)
II (Functioning adenomas) (12)		
Acromegaly (5)	Acidophilic (3) Mixed (2)	GH (3) GH+PRL (2)
Cushing syndrome (1)	Basophilic (1)	PRL (1)
Galactorrhoea (1)	Acidophilic (1)	GH+PRL (1)
Galactorrhoea with Acromegaly (1)	Acidophilic (1)	GH+PRL (1)

Fig. 1 : H & E section of the pituitary showing acidophil adenoma (diffuse pattern).

Fig. 2 : Growth hormone secreting pituitary adenoma.

One of the 4 pleurihormonal adenomas appeared as an acidophil adenoma with a glandular pattern on LM. Immunohistochemistry revealed a

Fig. 3 : Prolactin secreting pituitary adenoma (x 400).

strong positivity for GH and PRL indicating it as a pleurihormonal adenoma. This patient was a young female who had presented with acromegaly and galactorrhoea. The other patient was a 28-year-old female and only had galactorrhoea. The tumour was an acidophil adenoma with a trabecular pattern under the LM. IHC showed positivity for both GH and PRL.

The patient with Cushing's syndrome was a 34-year-old male. Under the LM, the tumour was a basophil adenoma with microglandular pattern while IHC revealed positivity for PRL (Fig 3). It was thought that it was a pleurihormonal adenoma which was incompletely removed, the ACTH secreting part of the tumour being left behind.

Electron microscopy was done on 5 out of 12 specimens of group I. One was a null cell

adenoma, which showed cells with varying number of secretory granules, prominent golgi apparatus (GA) and poorly developed rough endoplasmic reticular (rER). The other chromophobe adenomas showed well-granulated and poorly-granulated cells in various proportions. Sparsely-granulated cells showed prominent rER and GA. Using IHC the second case was a mixed GH and PRL adenoma. The third case was positive for GH (Fig 4) and the fourth for PRL. The fifth case was positive for GH, PRL and ACTH. The IEM revealed that the granules of the various hormones were localized in different cell populations. However, in one case, both hormones could be seen in a single granule in the same cell.

Discussion

It is now well established that routine light microscopy is not very sensitive in predicting the functional activity of pituitary adenomas. In this study, 50% patients with clinically 'non-functioning' adenoma showed evidence of hormone secretion by immunohistochemistry.

Plurihormonal adenomas in this study constituted 35% of all adenomas. This correlated well with other reports [1,2]. Varying combinations of hormones like GH and TSH, GH with PRL and TSH, GH with PRL and ACTH, GH with PRL, LH, GH and ACTH have been demonstrated by various workers [1,2].

Studies using immunoelectron microscopy have shown that in some tumours a single cell may contain both GH and PRL and sometimes both hormones were found in the same granules in a cell [3]. Tumours with two types of hormonally active cells were also described, one producing GH while another produced PRL [4]. In this study, immunoelectronmicroscopy using colloidal gold particles and double labelling had a special role to play. The individual hormones could be localised inside the granules of a single cell. This unequivocally demonstrates the existence of plurihormonal adenomas and helps in understanding the histogenesis of pituitary adenomas.

Clinical manifestations were usually due to excessive secretion of only one of the many hormones produced, e.g. patients with plurihormonal adenomas producing GH and PRL presented purely with features of acromegaly.

Moreover, the localisation of the hormones was not always associated with corresponding rise in serum level of the hormones nor was it reflected in the clinical picture. It is not understood why some hormones localised by IHC do not manifest clinically. The various reasons that have been ascribed [2] are :

1. The amount of secretion is too small.
2. Antigenic similarity of hormones causing crossreactivity.
3. Block in the release of the hormone into the circulation.
4. A block at tissue receptor level.
5. The presence of antihormones nullifying the effects of hormones.

It was proposed by Kovacs in 1986 that pituitary adenomas arose from uncommitted stem cells with the potential to differentiate into different cell types and produce several hormones [2].

It will be evident that with the advent of IHC, knowledge on pituitary adenomas has made many important advances. Based on these observations Horvath and Kovacs (1976) [6], and Kovacs [2] proposed a new classification of pituitary adenomas, as follows : Null cell adenomas, PRL cell adenomas, GH cell adenomas, corticotroph cell adenoma, thyrotroph cell adenomas, gonadotroph cell adenomas, plurihormonal adenomas.

Such a classification has been developed based on histologic and ultrastructural features as well as cellular hormone content [6]. This classification permits the separation of pituitary adenomas into distinct biological groups based on their capacity to synthesise hormones. EM helps in confirming the secretory nature of those chromophobe adenomas which were found to be hormone producing using IHC.

This study thus shows that the use of a combination of techniques like LM, IHC and EM helps us to have a better understanding of the hormonal status of pituitary adenomas. From a clinical standpoint, it is important to know whether these morphologically distinct tumour types differ in relation to biological behaviour, rate of growth, recurrence and therapeutic responsiveness. It appears that null cell adenomas are slowly growing tumour, whereas silent corticotroph adenomas have a more rapid growth and exhibit a more frequent

rate of recurrence [7]. Clinically nonfunctioning adenomas which were found to be secreting PRL by IHC could be diagnosed as prolactinomas only by IHC. It is rather important to diagnose them as they may be amenable to treatment with bromocriptine [2].

Thus all pituitary adenomas, especially in young patients, should be examined not only histologically but also by IHC and EM as they provide useful information to the neurosurgeon and endocrinologist in assessing prognosis and making decisions related to treatment.

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