

Non-functioning pituitary adenoma: immunohistochemical analysis of 85 cases

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Abstract

Pituitary adenomas without clinically active hypersecretion are summarized under the term non-functioning pituitary adenoma (NFPA). Since there are no specific serum markers, the differential diagnosis and treatment imply special difficulties. By using immunohistochemical methods we will have new insight into the nature and pathogenesis of these tumours. Ki-67 is a nuclear antigen detected by the monoclonal antibody MIB-1 and its labelling index (LI) is considered a marker of normal and abnormal cell proliferation. The aim of this study was to investigate the possible role of immunohistochemistry and MIB1-LI determination in NFPAs to predict tumoural behaviour and better management.

In this clinicopathological study, 85 cases of NFPAs were analysed immunohistochemically. MIB1-LI was also determined in studied cases. Clinical presentation, treatment and follow-up data were also reviewed and the correlation between clinical and pathologic findings was established.

Eighteen adenomas (21.2%) were immunoreactive to one or two adenohipophysial hormones of which 4 GH positive adenomas had aggressive behaviour (2 significant juxtaseellar extensions and 2 recurrences). MIB-1 LI was more than 5% in only 5 cases including 2 invasive adenomas but with no evidence of recurrence. No significant statistical difference between clinical presentations in immunoreactive and non-immunoreactive NFPAs was observed except for unilateral temporal hemianopia which was more common in immunoreactive adenomas ($P=0.022$).

NFPAs comprise several pathologically different types of tumours, some of which are potentially hormone producing, but some defects in hormone secretion or production of biologically inactive or insufficient amount of hormone may be the culprit in the lack of evidence of rising serum hormone levels. MIB-1 LI may be indicative of invasiveness but not a predictor of recurrence. Silent somatotropinomas may have more aggressive behaviour in comparison with other NFPAs.

Key words: non-functioning pituitary adenoma, immunohistochemistry, MIB-1 LI.

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Introduction

Non-functioning pituitary adenomas (NFPAs) represent about one quarter of all pituitary tumours. Concealed clinically by their endocrinologic silence, NFPAs manifest only after they have grown to sufficient size to produce mass effects. Accordingly headache and visual disturbance are the usual presenting features. When carefully sought, symptoms referable to hypopituitarism can often be elicited and verified on endocrine testing [10,23].

Moderate hyperprolactinaemia (<200 ng/ml) on the basis of stalk compression may also be present, so these adenomas are sometimes erroneously diagnosed as prolactinomas, but prolactin (PRL) level higher than 200 ng/ml is strongly indicative of a PRL secreting pituitary tumour [2].

Immunohistochemistry represents the gold standard method of classifying pituitary adenomas. This method has led to a new classification of pituitary adenomas, one reliably correlating structure with function and cytogenesis with biology [7].

Immunohistochemistry detects pituitary cell gene products at both the light and electron microscopic level and allows classification of pituitary tumours on the basis of their function [6]. Generally, immunohistochemical identification of pituitary hormones correlates with tumour specific messenger ribonucleic acid (mRNA) markers measured either in whole tissue extracts by northern analysis, or at the single cell level by in situ hybridization techniques.

With the exception of the glycoprotein α subunit, immunohistochemical positivity of more than 5% of cell making up the tumour is usually reflective of peripheral circulating hormone levels. Quantification of immunostaining intensity is subjective, and a scale of intensity should also include a description of the extent of staining, that is, whether occasional, scattered, or most tumour cells express the immunodetectable protein [15]. Unlike corticotroph, somatotroph, lactotroph and thyrotroph cell tumours, which hypersecrete their respective hormones, gonadotroph cell tumours are usually clinically silent and do not secrete their gene products efficiently [1,17-19,22].

Ki-67 is a cell-cycle specific nuclear antigen, which is easily detectable by means of the MIB-1 monoclonal antibody, and the labelling index (LI) obtained can be considered a marker of tumour proliferation [11-13,16,21].

MIB-1 LI is a useful marker in the determination of proliferative activity and invasiveness of anterior pituitary adenomas [12,13,16,21].

Materials and methods

Between 1974 and 2000, 107 patients suffering from NFPA were operated on at the University Hospital of Dr. Shariati. We defined NFPAs as patients with pituitary adenoma without any clinical syndromes resulting from excess of each anterior pituitary hormone, such as acromegaly, Cushing thyrotoxicosis or amenorrhea-galactorrhea syndromes. Additionally, preoperative serum PRL level should be less than 200 ng/ml (mild to moderate hyperprolactinaemia due to stalk compression) and also other hormone levels should not be significant.

From 107 patients with NFPAs, medical records were available in 85 cases (61 male, 24 female). Surgical specimens were analyzed using the immunocytochemical method of the Avidin-Biotin Complex (ABC); each specimen was stained with all 6 anterior pituitary hormones (PRL, GH, ACTH, TSH, FSH and LH). For hormone phenotype determination, the primary polyclonal antibody developed in rabbits was used at the following dilutions; 1:1000 for PRL, 1:7000 for GH, 1:2000 for ACTH, 1:2000 for TSH, 1:3000 for FSH and 1:1000 for LH (Dakopatts, Santa Barbara, CA, USA).

Paraffin-embedded surgical specimens were incubated overnight at 4°C in the MIB-1 antibody (Immunotech, Marseille, France) in order to detect Ki-67 antigen [16].

Three fields (hot spots) were selected in regions with the highest concentrations of MIB-1 positive nuclei and were examined at high power magnification ($\times 400$). Each field corresponded to a total number of cells ranging from 700 to 1000, in relation with the cellularity of the tumour specimen. Areas of necrosis, normal adeno-hypophysial cells and endothelial cells were excluded from the evaluation. On considering 1000 cells with "manual" counting, the MIB-1 LI was defined as the percentage of MIB-1 positive cells (dense brown precipitate restricted to the nuclei).

Chi-square and Fisher's exact tests were used to compare the clinical presentations in immunoreactive versus non-immunoreactive adenomas with computer-assisted data analysis using statistical software (STATA-SE 8). Odds ratio and 95% confidence intervals were calculated to assess the

Table I. Clinical presentations of 85 cases of NFPAs

Symptom/Sign	Immunoreactive		Non-reactive	
	Number	Percent	Number	Percent
Decreased visual acuity	18	100	60	89.5
Blurred vision	17	94.4	49	73.1
Visual field defect	9	50	35	52.2
Bitemporal hemianopia	5	27.7	25	37.3
Unilateral temporal hemianopia*	4	22.2	7	10.4
Optic atrophy	2	11.1	14	20.9
III n. palsy	0	0	1	1.4
VI n. palsy	0	0	2	2.9
Headache	10	55.6	42	62.7
Nausea/vomiting	3	16.7	9	13.4
Hypothyroidism	0	0	4	5.9
Impotence	2	11.1	5	7.4

*Unilateral temporal hemianopia was more common in the immunoreactive group ($P=0.022$).

Table II. Hormone phenotypes in 18 cases of immunoreactive NFPAs

Hormone(s)	Percentage of positivity % (n)
GH	5-10% (1)
	10-15% (1)
	20-25% (1)
	30% (1)
	30-40% (1)
PRL	5-10% (1)
	10-15% (2)
	20-25% (1)
GH, PRL	5-10%, 10% (1) 15-20%, 15-20% (1)
ACTH	10% (1)
FSH	10% (1)
	10-15% (1)
	20-25% (1)
FSH, LH	5-10%, 5-10% (2) 10%, 10% (1)

relation between GH positivity of tumours with their aggressiveness. Statistically significance was considered as $p \leq 0.05$.

This study was carried out according to the ethical guidelines of the Declaration of Helsinki.

Results

Patients' ages ranged from 14 to 66 years (mean: 41.6 years). Analysis of sex distribution showed 61 males (71.8%) and 24 females (28.2%).

The most common clinical presentation was visual problems including: decreased visual acuity (91.8%), blurred vision (77.6%) and visual field defects (51.8%). Other than visual complaints, headache was a common symptom (61.2%). Details of symptoms and signs at presentation time are summarized in Table I.

According to old histopathologic classification of pituitary adenomas, based on routine light microscopic evaluation of haematoxylin-eosin stained surgical specimens, most cases were chromophobe adenomas and only a few were mixed chromophobe-eosinophil or chromophobe-basophil adenomas. Two cases were invasive adenomas with cavernous sinus infiltration.

Immunohistochemical analysis of surgical specimens revealed that 67 out of 85 adenomas (78.8%) did not show immunoreaction to all 6 hormones. Therefore the diagnosis of "null cell" adenoma was established in these patients. In the remaining 18 adenomas (21.2%), 13 were immunoreactive to a single hormone and 5 were immunoreactive to two hormones.

Details of hormone phenotypes in these 18 cases are summarised in Table II.

By applying monoclonal antibody MIB-1 to all surgical specimens to detect Ki-67 antigen, in only 5 cases out of 85 patients (5.9%) MIB-1 LI was more than 5%, of which 2 cases were invasive NFPAs with MIB-1 LI of 50-60% and 30-40%.

In the other 3 cases, MIB-1 LI was 5-10%. All of these 5 cases were null cell adenomas (3 males and 2 females; mean age: 29.8 years). In the remaining 80 cases mean MIB-1 LI was 1.5%.

In all cases the neuroradiological diagnosis was obtained using both contrast-enhanced brain CT scan and MRI. In 25 cases (29.4%), pituitary macroadenomas (>10 mm) with suprasellar extension were revealed (dumbbell-shaped suprasellar tumours with bottleneck connection between

intrasellar and suprasellar portions). Cavernous sinus invasion was observed in 2 cases of invasive NFPAs. In the remaining 58 patients (68.2%) pituitary macroadenoma without significant suprasellar extension was diagnosed.

From 85 cases of NFPAs, in 58 patients (68.2%) a trans-sphenoidal approach was used in surgical operation; the remaining 27 patients (28.2%) underwent subfrontal craniotomy with total or subtotal resection of tumour (including 2 invasive adenomas and 2 of 5 silent GHomas).

Although our follow-up data were not complete, most patients were followed more than 5 years and 2 cases of recurrence were identified, both of which were GH positive (10-15% and 30%) with MIB-1 LI less than 5%. These 2 patients first underwent trans-sphenoidal microsurgery, then after recurrence, craniotomy was performed as the reoperation approach. Overall, 4 out of 5 silent somatotropinomas had more aggressive behaviour (juxtacellular extension and recurrence) in comparison with other NFPAs with an odds ratio of 8.8 (confidence interval 95%: 1.3-60.2). Among 7 GH-positive NFPAs (5 pure GH+ and 2 mixed GH+PRL), these 4 silent somatotropinomas with more aggressive behaviour were found in comparison with other NFPAs with an odds ratio of 2.8 (confidence interval 95%: 0.61-13).

Discussion

In the cases studied here, there was a significant predominance of NFPAs in males over females (male/female: 2.55/1). Analysis of sex distribution in different types of pituitary adenomas evaluated by Meindermann and Wilson [14] revealed that prolactinomas and ACTH and TSH-secreting adenomas occurred predominantly in females, while hormonally inactive adenomas and GH-secreting adenomas occurred mainly in males.

Introduction of immunohistochemical assays in histopathological diagnostics of pituitary adenomas resulted in better understanding of the pathology and clinical features of these neoplasms. In 34 of 54 pituitary adenoma patients studied by Golkowski et al. [4], positive immunohistochemical staining results with no increase in corresponding pituitary hormone serum levels were observed. Therefore positive immunohistochemical staining sometimes does not correlate with elevated basal serum pituitary hormone levels. We observed 18 out of 85 NFPAs (21.2%) which were immunoreactive to one or

two pituitary hormones. In all of them more than 5% of the tumoural cell population was immunopositive with no evidence of hormone hypersecretion, of which 5 silent somatotropinomas were identified. Interestingly, in 2 of them 30% and 30-40% of cells were positive, with no acromegalic features. In 4 specimens only PRL became positive and actually these cases may be prolactinoma with falsely low serum prolactin, as in 2 cases reported by Schofl et al. [20]. Silent gonadotroph adenoma was diagnosed in 6 cases (7.1% of all NFPAs), which is not as high a proportion as reported in the literature [1,2,6,7,10,15]. However, 67 cases (78.8%) were truly null cell adenoma with no immunoreaction to all of 6 adenohipophysial hormones; but it should be mentioned that immunohistochemical staining did not include the glycoprotein α subunit, hence truly null cell adenoma would be much less than demonstrated here if it was measured.

Clinical manifestations of NFPAs are caused by compressive effects of the tumour on juxtacellular structures; therefore it is clear that the tumour size is the most important factor in development of clinical presentations. No significant statistical difference between clinical presentations in immunoreactive and non-immunoreactive NFPAs was observed except for unilateral temporal haemianopia, which was more common in immunoreactive adenomas ($P=0.022$). There is no logical explanation for the discrepancy between forms of visual field defects in immunoreactive versus non-immunoreactive adenomas.

Among immunoreactive adenomas, silent somatotropinomas were greater in size with more suprasellar extension, and 2 recurrent cases were also GH-positive; therefore it seems that silent somatotropinomas may have a more aggressive course in comparison with other NFPAs, but more studies with sufficient cases are recommended to reach a conclusion.

MIB-1 LI was more than 5% in only 5 cases (5.9%) and it was very high in 2 cases with significant invasion to the parasellar structure and cavernous sinus (MIB-1 LI: 50-60% and 30-40%), but these 2 cases did not recur after about a 5-year follow-up period. The Ki-67 antigen reflects the state of the tumour cell at the moment of proliferation rather than the rate of cell proliferation. The antigen has been shown to be strongly correlated with thymidine labelling and flow cytometric determinations of

S-phase fractions [8]. Some studies have demonstrated no statistical difference in MIB-1 LI between recurrent and non-recurrent NFPAs [3,5,9,24]. As mentioned above, mean MIB-1 LI in 2 cases of recurrence with GH-positive immunoreaction was 1.5%. Therefore our findings support the idea that MIB-1 LI is a marker of invasiveness but not necessarily a predictor of recurrence.

Initially the distinction between functional and non-functional adenomas was a purely clinical notion. For correlation between hormone production, secretory activity and cytogenesis, immunohistochemical and transmission electron microscopic investigation is needed. In the new classification of pituitary adenomas, immunohistochemical and ultrastructural features of tumour cells should be considered other than clinical and laboratory findings, imaging results and histology.

By immunohistochemical techniques we can find that some pituitary tumours classified as NFPAs are potentially hormone producing and gene expression and some degree of hormone production has occurred at cellular levels, but how can we give reasons for the lack of evidence of rising serum hormone levels in these patients? Three possible causes are hypothesised: 1) some defects in the hormone secretion process, 2) secretion of biologically inactive hormone(s), 3) production of insufficient amounts of active hormone(s).

Further studies using sophisticated molecular biology techniques have been recommended in order to scrutinize these tumours and determine reasons for the discordance between immunohistochemical results and serum hormone profiles in these neoplasms.

Careful pathologic study with immunohistochemistry as well as communication between clinicians and pathologists is vital for better understanding of the tumoural nature of NFPAs and their appropriate management.

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