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Plurihormonality of pituitary adenomas in light of immunohistochemical studies

Wielohormonalność gruczolaków przysadki w świetle badań immunohistochemicznych

Marek Pawlikowski[1], Jolanta Kunert-Radek[1], Maciej Radek[2]

[1]Department of Neuroendocrinology, Chair of Endocrinology, Medical University, Łódź, Poland
[2]Chair of Neurosurgery and Surgery of Peripheral Nerves, Medical University, Łódź, Poland

Abstract

Introduction: Plurihormonality of pituitary adenomas can be defined as the ability of an adenoma to express more than one pituitary hormone**.** The application of immunohistochemistry to diagnose surgically removed pituitary tumours revealed that a great number of pituitary adenomas are in fact plurihormonal. However, data on the incidence and the clinical relevance of the pituitary adenoma plurihormonality are still scarce and controversial.
Material and methods: Hundred fifty-five pituitary adenomas, surgically removed, were studied immunohistochemically with the antibodies against pituitary hormones or their subunits. Additionally, 40 adenomas were immunostained with Ki-67 antibody to evaluate the proliferative potential.
Results: According to the World Health Organization (WHO) recommendations, we did not consider tumours expressing both FSH and LH (gonadotropinomas) or somatoprolactinomas expressing both GH and prolactin as plurihormonal. Even with this limitation, plurihormonality was found to be a frequent finding in both hormonally active and clinically non-functioning pituitary adenomas. It was shown that over one-third (36.1%) of the investigated adenomas expressed more than one hormone. Plurihormonality, especially that which is connected with co-expression of ACTH, seems to be more frequent in the recurrent tumours. Plurihormonal adenomas also possess higher Ki-67 indices, as compared to monohormonal tumours.
Conclusions: Plurihormonality is a frequent phenomenon in both hormonally active and clinically non-functioning pituitary adenomas. It also seems to predict a higher risk of tumour recurrence. **(Pol J Endocrinol 2010; 61 (1): 63–66)**

Key words: *pituitary adenomas, immunohistochemistry, plurihormonality, tumour recurrence*

Streszczenie

Wstęp: Wielohormonalność gruczolaków przysadki jest definiowana jako ich zdolność do ekspresji więcej niż jednego hormonu przysadkowego. Zastosowanie immunohistochemii do diagnostyki usuniętych chirurgicznie guzów przysadki pozwoliło na stwierdzenie, że w istocie znaczną część guczolaków przysadki jest wielohormonalna. Jednak dane o częstości występowania i znaczeniu klinicznym wielohormonalności są skąpe i częściowo sprzeczne.
Materiał i metody: Sto pięćdziesiąt pięć chirurgicznie usuniętych gruczolaków przysadki zbadano immunohistochemicznie z użyciem przeciwciał przeciw hormonom przysadkowym i/lub ich podjednostkom. Dodatkowo 40 gruczolaków zbadano za pomocą przeciwciała Ki-67, w celu ustalenia ich potencjału proliferacyjnego.
Wyniki: Zgodnie z zaleceniami Światowej Organizacji Zdrowia (WHO, *World Health Organization*) nie zaliczono do gruczolaków wielohormonalnych guzów z łączną ekspresją FSH i LH (gonadotropinoma) ani somatoprolactinoma wykazujących łączną ekspresję GH i prolaktyny. Nawet przy tym ograniczeniu, wielohormonalność jest częstym zjawiskiem zarówno w przypadku hormonalnie czynnych, jak i klinicznie nieczynnych gruczolaków przysadki. Wykazano, że w ponad jednej trzeciej (36.1%) badanych gruczolaków przysadki wykrywa się obecność więcej niż jednego hormonu. Wielohormonalność, zwłaszcza związana z współwystępowaniem ACTH, jest częstsza w guzach nawrotowych. Guzy wielohormonalne wykazują także wyższy indeks Ki-67 w porównaniu z guzami monohormonalnymi.
Wnioski: Wielohormonalność jest częstym zjawiskiem zarówno w grupie guzów klinicznie czynnych, jak i grupie guzów klinicznie nieczynnych hormonalnie. Ponadto wielohormonalność wydaje się wiązać z wyższym ryzykiem nawrotu guza.
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Słowa kluczowe: *gruczolaki przysadki, immunohistochemia, wielohormonalność, nawroty guzów*

Marek Pawlikowski, M.D., Ph.D., Department of Neuroendocrinology, Chair of Endocrinology, Medical University of Łódź, 3 Sterling St., 91–425 Łódź, e-mail: marek.pawlikowski@umed.lodz.pl

Introduction

Plurihormonality of pituitary adenomas can be defined as the ability of an adenoma to express more than one pituitary hormone**.** According to the recommendations of the WHO, the gonadotropinomas expressing both gonadotropins, *i.e.* FSH and LH, are not considered plurihormonal. The same concerns adenomas in patients with acromegaly, secreting prolactin in addition to GH [1]. Plurihormonal adenomas leading to the elevated concentrations of more than one pituitary hormone in blood are considered relatively rare. The application of immunohistochemistry to diagnose surgically removed pituitary tumours revealed that a great number of pituitary adenomas, including the so-called "clinically non-functioning" pituitary adenomas (CNFPA), are in fact plurihormonal [2–8]. However, the data on the incidence and the clinical relevance of pituitary adenoma plurihormonality remain scarce.

The aim of this study was to evaluate the incidence of plurihormonality in a non-selected group of immunohistochemically examined pituitary adenomas and to answer the question whether the plurihormonality of the tumours is relevant for their prognosis.

Material and methods

The archival material of 155 surgically removed pituitary adenomas was studied. Paraffin sections of each tumour were immunostained using the primary antibodies against the following pituitary hormones or their subunits: prolactin (PRL, polyclonal, Dako, Denmark), growth hormone (GH, polyclonal, Dako or Immunon, USA), LH (Dako, monoclonal), FSH (Dako, monoclonal), TSH (monoclonal, Immunotech, France), ACTH (polyclonal, Sigma), and alpha-subunit (alpha-SU, monoclonal, Immunotech, France). Additionally, 40 adenomas were immunostained with anti-Ki-67 antigen (MIB-1) antibody (Dako-Cytomation, Denmark). Visualization of the immune reactions was done by means of streptavidin-biotin-peroxidase technique with use of 3,3′-diaminobenzidine as chromogen. The presence of more than 1% of hormone immunopositive cells was considered important. Statistical analysis of the numeric data was done by means of McNemara's and χ^2 tests (recurrence rates) or ANOVA followed by LSD test (Ki-67 indices); statistical significance was at the level $p < 0.05$.

Results

The results are presented in Tables I–III. As can be seen, over one third of pituitary adenomas examined by immunohistochemistry are plurihormonal (Table I). If, following the WHO recommendations, we do not consider GH/PRL adenomas in acromegaly or FSH/LH adenomas as plurihormonal, then plurihormonality is most frequent in clinically non-functioning adenomas (CNFPA) (see Table I). Approximately 1/5 of the investigated adenomas were recurrent. The recurrence rate is highest in case of gonadotropinomas (45.7%). However, if we exclude this type of adenoma from the group of monohormonal tumours, the recurrence rate of plu-

Table I. *Incidence of plurihormonal pituitary adenomas*

Tabela I. *Zachorowalność na gruczolaki wielohormonalne przysadki*

Total	**56/155 (36.1%)**
Acromegaly	
including GH/PRL	18/29 (62.1%)
excluding GH/PRL	7/29 (24.1%)
Cushing's disease	4/14 (28.6%)
CNFPA	38/85 (44.7%)

Table II. *Recurrence rate of pituitary adenomas*

Tabela II. *Wskaźniki występowania nawrotów gruczolaków przysadki*

Total	**32/155 (20.6%)**
Plurihormonal monohormonal	10/56 (17.8%)
Including gonadotropinomas	22/99 (22.2%)
Excluding gonadotropinomas	6/64 (9.4%)
Acromegaly	2/29 (6.9%)
GH/PRL	1/18 (5.5%)
Plurihormonal (GH/alfaSU)	1/7 (14.3%)
Cushing's disease	3/14 (21.4%)
Plurihormonal	3/4 (75%)

Table III. *Clinically non-functioning pituitary adenomas (CNFPA)*

Tabela III. *Klinicznie nieczynne gruczolaki przysadki (CNFPA)*

Total	**85/155 (55.2%)**	**Recurrent 29/85 (34.1%)**
"Pure" gonadotropinomas	35/85 (41.1%)	Recurrent 16/35 (45.7%)
Monohormonal (excluding gonadotropinomas)	7/85 (8.2%)	Recurrent 4/7 (57.1%)
Plurihormonal	38/85 (44.1%)	Recurrent 9/38 (23.7%)
Inactive	=5/85 (5.9%)	Recurrent 0/5 (0%)

rihormonal adenomas is twice as high (17.8% *v.* 9.4%), although the difference is not significant statistically. A statistically significant difference in the recurrence rate is found in corticotropinomas manifesting themselves as Cushing's disease. All the recurrent tumours observed in patients with Cushing's disease were plurihormonal (Table II). In the case of CNFPA, the highest recurrence rate is observed in monohormonal adenomas. However, the number of such tumours is relatively low, and, interestingly, almost all recurrent adenomas in this group (3/4) were "silent" (*i.e.* not manifested as Cushing's disease) corticotropinomas. The next position among the recurrent CNFPAs belongs to "pure" gonadotropinomas. In contrast, no recurrent tumours were found in the group of hormonally immunonegative tumours (Table III). We also analyzed the relationship between tumour recurrence and expression of ACTH in plurihormonal CNFPA. Some tendency towards a higher recurrence rate was observed in the group of plurihormonal CNFPS co-expressing ACTH, as compared to ACTH-negative tumours (6/17, 35.3% *v.* 2/14, 14.2%, respectively). Moreover, the mean Ki-67 index (% of Ki-67-immunopositive cell nuclei), calculated for plurihormonal tumours, was significantly higher than the monohormonal adenoma group (6.0 ± 1.9% vs. 1.7 ± 0.5%, mean ± SEM, $p < 0.05$).

Discussion

Our data show that plurihormonality is a frequent phenomenon in both hormonally active and clinically nonfunctioning pituitary adenomas. Their frequency in our material (36.1%) is very similar to that (31%) reported earlier by Ho et al. [5]. This finding supports the opinion of quoted authors that the immunostaining of all pituitary hormones is mandatory for correct classification of pituitary adenomas. Earlier studies with the application of double immunostaining at the light microscopy and the electron microscopy levels showed that the multiple hormones are expressed within the same tumoural cell, and even within the same secretory granule [9]. Although double immunostaining was not applied in the present study, the sum of the percentages of cells immunopositive for different hormones often exceeded 100. This means that the cells must present double or even triple hormone immunopositivity. These findings indicate that plurihormonal adenomas are constituted from plurihormonal cells rather than from multiple cellular lines expressing different pituitary hormones. The question arises whether the expression of multiple hormones in tumoural cells is relevant for their natural history, and specifically whether it possesses a prognostic value. The recurrence of pituitary adenoma after surgery may depend on early or late diagnosis and the skilfulness of the neurosurgeon, but also depends on tumour aggressiveness. The latter is linked with tumour invasiveness, *i.e.* the ability of the tumour cells to invade the adjacent structures consequently enabling total excision of the tumour, and with its high proliferation potency. The latter is usually measured by calculation of the percentage of cell nuclei positively immunostained with antibody to Ki-67 antigen (Ki-67 index). Generally, Ki-67 index is positively correlated with tumour invasiveness and recurrence, although some exceptions have been observed, and the low Ki-67 index can be noticed in aggressive tumours, and, in contrast, the high proliferation index may occur in indolent adenomas [10]. It was important to see whether plurihormonality increases the risk of the tumour recurrence. Although the high recurrence rate in our material concerns gonadotropinomas, which according to WHO recommendations are considered "monohormonal" adenomas, and monohormonal ACTH-immunopositive adenomas without Cushing's disease manifestation ("silent" corticotropinomas), in the remaining types of adenomas the plurihormonality seems to be linked with the increased risk of tumour recurrence. This suggestion is supported by our finding that the mean value of Ki-67 index in plurihormonal pituitary adenomas is significantly higher than in monohormonal tumours. In our earlier paper we showed that plurihormonal adenomas present higher values of another proliferation index, namely proliferating cells nuclear antigen, PCNA [11]. Our observation of the higher recurrence rate in cases of plurihormonal versus monohormonal corticotropinomas with Cushing's disease is compatible with the findings of Desai et al. [12]. The quoted authors have found that corticotropinomas cosecreting the alpha-subunit are more aggressive and have a higher recurrence rate than corticotropinomas without the expression of this peptide. The increased risk of tumour recurrence seems also to be linked with ACTH co-expression in plurihormonal CNFPA, which corroborates with the data of Bradley et al. [13]. We suggest that plurihormonality of pituitary adenomas, especially that linked with ACTH co-expression, seems to predict a higher risk of tumour recurrence. However, this presumption needs further studies based on higher number of examined tumours.

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