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Blood serum concentrations of gonadotropins and α-subunit in patients with gonadotropinomas in relation to the immunoreactivity of pituitary adenoma

Stężenia gonadotropin i α podjednostki w surowicy krwi u pacjentów z gruczolakami gonadotropowymi przysadki w zależności od immunoreaktywności guza

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Abstract

Introduction: Although active gonadotropin-secreting pituitary adenomas are considered very rare, the vast majority of pituitary tumours diagnosed as "non-functioning" express gonadotropins or their free β or α subunits. However, systemic investigations comparing the serum concentrations of follitropin (FSH), lutropin (LH), and α–subunit (αSU) before surgery with the immunoreactivity of the respective substances in the excised tumours are still lacking.

Material and methods: Immunostaining of FSH, LH, and αSU was compared in 43 surgically removed gonadotropin — expressing pituitary adenomas with serum concentrations of the above-mentioned substances before surgery in the same patients.

Results: The serum concentrations of FSH were elevated (> 11.6 mU/mL) in 8/12 (66.7%) cases of FSH-positive adenomas. By contrast, in FSH–negative tumours the elevation of FSH is absent. Moreover, only 1/25 (4%) patients with LH–positive adenoma had the elevated serum concentration of LH (51.5 mU/mL). The overproduction of LH was not observed in adenomas expressing free β LH or in LH-negative tumours. In patients with αSU-positive adenomas elevated serum levels of αSU were observed in 3/15 (20%) cases. No αSU elevations were observed in patients with αSU–negative adenomas. The mean serum FSH, LH, and αSU concentrations were higher in patients with FSH, LH, and/or αSU immunopositive tumours in comparison with immunonegative. However, the differences are not statistically significant.

Conclusions: Although "silent" gonadotropinomas constitute a frequent subtype of pituitary adenomas, the "active" subtype (i.e. manifesting by gonadotropin excess) are rare (approx. 4% of all pituitary adenomas). Gonadotropinomas are difficult to diagnose before surgery. The measurement of gonadotropins including αSU is needed but often not sufficient for presurgical diagnosis. **(Endokrynol Pol 2018; 69 (5): 526–529)**

Key words: *gonadotropinoma, follitropin, lutropin, α-subunit*

Streszczenie

Wstęp: Jakkolwiek „aktywne" gruczolaki przysadki wydzielające gonadotropiny uważane są za bardzo rzadkie to większość guzów przysadki rozpoznawanych jako „nieczynne wydzielniczo" w rzeczywistości wykazuje ekspresję gonadotropin lub ich wolnych podjednostek β lub α. Brak jednak dotąd systematycznych badań, które porównywałyby przedoperacyjne stężenia folitropiny (FSH), lutropiny (LH) i ich podjednostki α (αSU) w surowicy krwi z immunoreaktywnością usuniętych operacyjnie guzów.

Materiał i metody: W usuniętych operacyjnie 43 gruczolakach gonadotropowych przysadki, porównano odczyny immunohistochemiczne na FSH, LH i αSU ze stężeniami tych hormonów oznaczonymi we krwi pacjentów przed operacją.

Wyniki: Podwyższone stężenie FSH we krwi (> 11,6 mU/mL) stwierdzono u 8/12 (66,7%) pacjentów z dodatnim odczynem na FSH w guzie. W grupie chorych z gruczolakami ujemnymi dla FSH nie obserwowano podwyższonego stężenia FSH w surowicy w żadnym przypadku. W przypadku gruczolaków LH-pozytywnych, podwyższenie LH we krwi stwierdzono tylko w 1 przypadku na 25 (4%) (51,5 mU/mL). Podwyższonego stężenia LH nie obserwowano także w przypadkach gruczolaków z ekspresją wolnej podjednostki β-LH oraz LH-negatywnych. W przypadkach gruczolaków αSU-pozytywnych podwyższone stężenia αSU w surowicy krwi (> 1 mU/mL), stwierdzono w 3 przypadkach na 15 (20%), natomiast w guzach αSU-negatywnych podwyższonych stężeń nie obserwowano w żadnym przypadku. Średnie stężenia FSH, LH i a αSU w surowicy krwi były wyższe u pacjentów z guzami odpowiednio FSH, LH i αSU pozytywnymi w porównaniu z pacjentami z guzami immunonegatywnymi dla tych hormonów; różnice nie były jednak statystycznie znamienne.

Wnioski: Jakkolwiek „ciche" *gonadotropinoma* stanowią częsty podtyp gruczolaków przysadki, to „aktywne" (tj. manifestujące się nadmiarem gonadotropin) *gonadotropinoma* są rzadkością (ok. 4% wszystkich gruczolaków przysadki). Gruczolaki gonadotropowe są trudne do zdiagnozowania przed operacją. Oznaczenie gonadotropin oraz αSU są konieczne, lecz najczęściej niewystarczające do rozpoznania przedoperacyjnego. **(Endokrynol Pol 2018; 69 (5): 526–529)**

Słowa kluczowe: *gonadotropinoma, folitropina, lutropina, α-podjednostka*

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Introduction

Initially, gonadotropin expressing tumours (gonadotropinomas) were considered as a very rare subtype of pituitary adenoma. The specific clinical symptoms of these tumours were rarely observed. Precocious puberty in young boys, ovarian overstimulation in young women, and testicular enlargement in adult men were occasionally reported [1–3]. In the aged people no specific clinical symptoms of gonadotropinoma were observed, and those tumours are classified as non-functioning pituitary adenomas (NFPA). However, when immunohistochemical investigation with antibodies against pituitary hormones became a routine and obligatory procedure of pathomorphological examination of excised pituitary tumours, it was found that the vast majority of NFPA express gonadotropins or their subunits [4–8]. Moreover, it was recently shown, on the basis of molecular and *in vitro* studies, that even the adenomas totally immunonegative for pituitary hormones (null cell adenomas — NCA) express gonadotropins [9]. However, according to our knowledge, the systematic studies comparing the presurgical serum concentrations of follitropin (FSH), lutropin (LH), and α-subunit (αSU) with immunoreactivity of the above-mentioned hormones in post-surgical samples are still lacking.

Material and methods

Archival material of 165 immunohistochemically examined pituitary adenomas was included in the study. FSH and LH were detected using anti-FSH and anti-LH monoclonal antibodies (DAKO Denmark or Chemicon MERCK) and αSU with anti-αSU monoclonal antibody (Immunotech France). The visualisation of immunostaining was performed using an Envision kit (DAKO Denmark) with the use of 3,3′ diaminobenzidine (DAB) as chromogen.

In 78 tumours positive immunostaining for FSH, LH, and/or their free β-subunits was found; in 43 cases (21 women and 22 men) clinical documentation, including the results of gonadotropin measurement in blood serum before surgery, was available. FSH and LH were measured by means of chemiluminescence method (OCD Johnson & Johnson, UK). Additionally, in 21 cases (9 women and 12 men) αSU concentration was examined using IRMA (Immunotech, France) method. The female patients were aged 41–73 years (mean age 60.0 ± 9 years), and males were aged 39–77 years (mean age 55.8 ± 11 years). In the patients the confrontation of the results of the immunohistochemical study and of FSH, LH, and αSU measurements in peripheral blood serum was performed. The numerical data were analysed using the programme Statistica 12.

The study was approved by the Ethical Committee of the Medical University of Lodz, decision RNN/351/17/KE dated November 21st, 2017.

Results

All tumours were diagnosed before surgery as non-functioning pituitary adenomas. In one case concerning a 54-year-old woman with extremely high FSH and LH elevation a suggestion of gonadotropinoma diagnosis was included into anamnesis.

As shown in 'Materials and Methods', the gonadotropin-expressing pituitary adenomas affect mainly middle-aged and older patients. There is some tendency towards younger mean age in the males, but the difference with female patients is not statistically significant. The frequency of gonadotropin-expressing adenomas in both sexes is similar. In 36 adenomas the immunopositivity concerned LH and in 13 adenomas the immunopositivity concerned FSH. In six adenomas both gonadotropins were present. Moreover, in 11 cases of LH-immunopositive and in one case of FSH-immunopositive tumour, the immunoreaction for αSU was lacking. We concluded that those adenomas presented the expression of free β subunits. In patients with LH-positive adenomas only one (a 54-year-old woman) had elevated LH in blood serum (51.5 mU/ml). The remaining patients with LH-positive tumours presented normal or even decreased serum LH concentrations (Table I), in spite of the fact that this group included 12 women of menopausal or postmenopausal age. The mean value of LH concentrations in patients with LH-positive tumours is slightly higher than in free β-LH subunit expressing or in LH-negative adenomas, but the differences between the means are not statistically significant. In 12 patients exhibiting FSH immunopositivity of adenoma, in the majority of cases (seven women and one man) serum FSH concentrations were elevated. All these women were in the postmenopausal age (> 50 years). In the remaining four cases of FSH-positive tumours, serum concentration of FSH was normal or decreased (Table II). Excluding one case of a 72-year-old woman, the three remaining cases concerned men. In one case we suspect the expression of free β-FSH subunit because of the lack of αSU immunostaining. In patients with FSH-negative tumours no elevation of serum FSH was observed. In the remaining 30 patients serum FSH concentrations were situated within normal limits, and in five cases they were decreased. Eleven cases concerned women, and all of them in menopausal or post-menopausal age. The mean value of FSH concentrations in patients with

Table I. *Serum LH concentrations in patients with pituitary adenomas*

Tabela I. *Stężenia LH w surowicy chorych z gruczolakami przysadki*

Patients/ Tumours	Serum LH concentrations in patients with LH-positive pituitary adenomas	Serum LH concentrations in patients with free β LH-positive pituitary adenomas	Serum LH concentrations in patients with LH-negative pituitary adenomas
Total number	25 (100%)	11 (100%)	10 (100%)
Elevated*	1 (4%)	0 (0%)	0 (0%)
Normal*	15 (60%)	6 (54.5%)	9 (90%)
Decreased*	9 (36%)	5 (45.5%)	1 (10%)
Mean ± SEM	3.1 ± 0.74 mU/mL	1.7 ± 0.72 mU/mL	3.4 ± 1.1 mU/mL

*reference ranges [0.83–15.5 mU/mL]

Table II. *Serum FSH concentrations in patients with pituitary adenomas*

Tabela II. *Stężenia FSH w surowicy chorych z gruczolakami przysadki*

Patients/Tumours	Serum FSH concentrations in patients with FSH-positive pituitary adenomas	Serum FSH concentrations in patients with FSH-negative pituitary adenomas
Total number	12 (100%)	35 (100%)
Elevated*	8 (66.7%)	0 (0%)
Normal*	3 (25%)	30 (85.7%)
Decreased*	1 (8.3%)	5 (14.3%)
Mean ± SEM	20.6 ± 12.1 mU/mL	7.6 ± 1.74 mU/mL

*Reference ranges [1.38–11.6 mU/mL]

Table III. *Serum αSU concentrations in patients with pituitary adenomas*

Tabela III. *Stężenia αSU w surowicy chorych z gruczolakami przysadki*

Patients/ Tumours	Serum αSU concentrations in patients with αSU-positive pituitary adenomas	Serum αSU concentrations in patients with αSU-negative pituitary adenomas
Total number	15 (100%)	6 (100%)
Elevated*	3 (20%)	0 (0%)
Normal*	12 (80%)	6 (100%)
Mean ± SEM	1.9 ± 0.9 mU/mL	0.33 ± 0.02 mU/mL

*reference ranges [0–1.0 mU/mL]

FSH-positive tumours is over twofold higher than in those with FSH-negative adenomas. However, the difference is not statistically significant because of a high dispersion of the results. In 21 cases, in addition to FSH and LH, the αSU concentration was measured (Table III). The elevated αSU concentration was observed in 3/15 cases (20%) of αSU-positive tumours. In contrast, in anyone of the patients with αSU-negative adenomas an elevated αSU concentration was observed. The mean values of αSU concentrations in αSU-positive adenomas were higher than in cases of α-SU-negative adenomas, but the difference is not statistically significant.

Discussion

The data presented above support the view that while hormonally active gonadotropinomas are rare, the vast majority of non-functioning pituitary tumours (NFPA) express gonadotropins [4–8]. Our findings confirm the statement that FSH hypersecretion is more frequent than that of LH [10]. However, the positive immunostaining for LH, and specifically for the free β-LH subunit, is more frequent in comparison with FSH. Since the frequency of NFPA constitutes approximatively 30% of all pituitary tumours [6, 7, 10], it means that gonadotropinomas can be estimated for about 20% of all pituitary adenomas. This value is compatible with earlier published data (24% according to [10]). A question that remains to be answered is the proportion of "active (over-secreting)" versus the "silent" gonadotropinomas (i.e. the gonadotropinomas not manifesting themselves by enhanced gonadotropin levels). The answer is not simple because reliable determination of whether the elevated FSH is of tumoural origin or generated by the remaining non-tumoural pituitary is not possible. However, the women with FSH-negative tumours, in spite of the postmenopausal age, did not present, in any case, elevation of serum FSH. This finding speaks in favour of tumoural origin of elevated FSH in patients with FSH-positive adenomas. If we consider all nine of the cases of gonadotropin over-secretion observed in our study, the frequency of "active" gonadotropinomas could be estimated for approximately 21% of the all gonadotropin-expressing tumours, and for 4.2% of the pituitary adenomas in general. As expected, the vast majority of gonadotropinomas (mostly expressing LH or free β-LH) are "silent". The causes of this phenomenon are not known, but it could be hypothesised that they may result — at least in part — from the absence of the α-subunit or the lack of the mutual junction between α and β subunits. This

is supported not only by the frequent expression of free β-LH, but also by the disproportion of LH, FSH, and αSU immunostained cells often observed in these adenomas. This presumption is also supported by the observation of the presence of the frequent detection of free subunits: mainly β-LH subunit, more rarely free β-FSH [11]. The possibilities of correct presurgical diagnosis of gonadotropinoma, especially in middle-aged and elderly people, remain limited. The determination of FSH, LH, and αSU in blood before surgery should be obligatory, but is usually insufficient because very low or even undetectable levels of these substances do not exclude the diagnosis of gonadotropinoma. According to some recent publications, presurgical diagnosis might be improved by the measurement of αSU after thyroliberin (TRH) administration [12, 13].

However, the sensitivity of this test may be limited because of the absence of αSU in many tumours.

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