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Ocena stężenia podjednostki alfa po podaniu TRH: przydatny test w przedoperacyjnej diagnostyce *gonadotropinoma*?

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Streszczenie

Wstęp: Klinicznie nieczynne gruczolaki przysadki (*clinically nonfunctioning pituitary adenomas, CNFPAs*) stanowią około 30% makrogruczolaków przysadki i są najczęściej guzami typu *gonadotropinoma*. Celem pracy była weryfikacja użyteczności pomiaru stężenia podjednostki alfa hormonów glikoproteinowych (α-SU) w surowicy w odpowiedzi na pobudzenie egzogennym TRH w rozpoznawaniu gonadotropowego charakteru CNFPAs przed leczeniem neurochirurgicznym.

Materiał i metody: Badania przeprowadzono u 14 pacjentów z klinicznie nieczynnymi gruczolakami przysadki. U każdego chorego przed zabiegiem chirurgicznym oceniono stężenie α-SU w surowicy w odpowiedzi na podanie TRH. Jako odpowiedź znamienną przyjęto arbitralnie wzrost stężenia α-SU o ponad 50% w stosunku do wartości wyjściowej.

Wyniki: Pacjenci zostali podzieleni na dwie grupy, po 7 chorych każda. Grupę pierwszą stanowili pacjenci z guzami typu *gonadotropinoma* (guzy immunopozytywne dla FSH i/lub LH bądź ich wolnych podjednostek). W grupie drugiej znaleźli się chorzy z gruczolakami immunonegatywnymi dla gonadotropin i α-SU. Podstawowe stężenie α-SU powyżej normy stwierdzono u dwóch pacjentów w grupie pierwszej (gruczolaki gonadotropowe) oraz u jednego w grupie drugiej. Wzrost stężenia α-SU po podaniu TRH o ponad 50% obserwowano u wszystkich z wyjątkiem jednego badanego w grupie pierwszej oraz u żadnego z siedmiu chorych z guzami immunonegatywnymi dla FSH, LH lub α-SU. U trzech z siedmiu pacjentów z gruczolakami immunonegatywnymi dla gonadotropin stwierdzono obniżenie stężenia α-SU w surowicy po podaniu TRH.

Wniosek: Pomiar stężenia α-SU w odpowiedzi na podanie TRH może być przydatny w przedoperacyjnej identyfikacji guzów gonadotropowych wśród innych nieczynnych hormonalnie gruczolaków przysadki.

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Słowa kluczowe: *podjednostka α, test z TRH, guzy przysadki, gonadotropinoma*

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Serum alpha-subunit elevation after TRH administration: a valuable test in presurgical diagnosis of *gonadotropinoma*?

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Abstract

Objectives: Clinically nonfunctioning pituitary adenomas (CNFPAs) represent about 30% of pituitary *macroadenomas, gonadotropinomas* being the most frequent among them. The aim of the present study is to re-evaluate the usefulness of the measurement of α-SU serum level in response to TRH stimulation in detecting the gonadotropic nature of nonfunctioning pituitary adenomas before the neurosurgical treatment.

Material and methods: We have studied 14 patients with CNFPAs. The response of α-SU to the administration of TRH was studied in each patient before the surgery. α-SU blood serum level increase over 50% of the baseline level after TRH treatment was considered to be significant.

Results: The patients were divided into 2 groups, each including 7 subjects. The first group included the patients with *gonadotropinomas* (tumors immunopositive for FSH and/or LH or their free subunits). The second group included the patients with adenomas immunonegative for gonadotropins and α-SU. The basal level of α-SU was elevated over the upper limit of normal range in two patients of the first group (gonadotroph adenomas) and in one in the second group. All but one patient from the first group and none of seven patients with tumors immunonegative for FSH, LH or α-SU, had a significant α-SU (over 50%) response to TRH. In three of seven patients with gonadotropins immunonegative tumors a decrease of α-SU serum level after TRH was observed.

Conclusion: The measurement of α-SU serum level in response to TRH administration seems to be useful in preoperative identification of gonadotroph adenomas among other nonfunctioning pituitary adenomas.

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Key words: *α–subunit; TRH test; pituitary tumors; gonadotropinoma*

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Introduction

Clinically nonfunctioning pituitary adenomas (CNFPAs) represent about 30% of surgically removed pituitary *macroadenomas*. Usually, these tumors grow undetected until they become large enough to cause mass effects such as visual impairment, headaches and/or symptoms of hypopituitarism [6, 7, 15]. In spite of no clinical features of pituitary hormones hypersecretion, the presence of pituitary hormones is detected in most of CNFPAs in postoperative immunohistochemical investigations. It has been found that the majority of these tumors (50-75%) show positive immunostaining for gonadotropins and/or their free subunits implying a gonadotroph cell origin [1, 8, 11]. These results are in agreement with data from *in vitro* cell cultures showing gonadotropins secretion in most of CNFPAs [3, 9]. These findings have been also supported by in situ hybridization studies [5].

Gonadotroph adenomas are rarely associated with the increased basal plasma levels of luteinizing hormone (LH) and/or follicle-stimulating hormone (FSH). The elevated free α–subunit (α-SU) concentrations occur only in half of the patients. We have been still looking for a diagnostic tool allowing preoperative identification of gonadotroph adenomas among other nonfunctioning pituitary adenomas. Paradoxical responses of LH, FSH and their free subunits to the intravenous administration of thyrotropin-releasing hormone (TRH) have been previously reported in patients with CNFPAs but their diagnostic value is considered as rather limited [2, 4, 10, 13, 14, 16]. The abnormal response of free α-SU to TRH was reported in approximately 30% of patients only [13, 14] and free β-LH response ranged from 26% [2] to 77,8% [16] of patients with CNFPAs. The aim of the present study is to re-evaluate the usefulness of the measurement of α-SU serum level in response to TRH stimulation in detecting the gonadotropic nature of nonfunctioning pituitary adenomas before the neurosurgical treatment.

Materials and Methods

The study was carried out in 14 patients with pituitary macroadenomas diagnosed before surgery as nonfunctioning. The average age of the patients (9 men and 5 women) was 53.8 yrs. All the patients underwent radiological (MRI) and hormonal examinations including baseline PRL, GH, IGF-1, ACTH, cortisol, LH, FSH, α-SU, TSH, fT_3, fT_4, testosterone (in men) or estradiol (in women). None of the patients had clinical symptoms of hormonal hypersecretion of pituitary hormones and their blood levels were within the normal ranges.

In every patient the histological and immunohistochemical examinations were performed after surgical excision of the pituitary tumor. Tumors were fixed in Bouin-Holland fixative and paraffin sections were stained with Herlant's tetrachrome and immunostained with antisera against pituitary hormones or α-SU. The following antisera were used: polyclonal anti-human PRL (Dako, Denmark), polyclonal anti-human GH (Dako or Immunon, USA), monoclonal anti-human LH (Dako) or polyclonal (Immunon), monoclonal anti-human FSH (Dako), monoclonal anti-human TSH (Immunotech, France), monoclonal anti-human α-SU (Immunotech) and polyclonal anti-ACTH (Sigma, USA).

The response of α-SU to the administration of TRH was studied in each patient before the surgery. The blood samples were collected before, 30 and 60 minutes after the intravenous injection of 200 μg TRH. α-SU blood serum level increase over 50% of the baseline level after TRH treatment was arbitrarily considered to be significant. The data were analyzed statistically using χ^2 test.

Results

The patients were divided into 2 groups, each including 7 subjects. The first group included the patients with tumor immunopositivity for FSH and/or LH or their free subunits. These tumors we classified as *gonadotropinomas*. Four tumors from this group exhibited also the positive immunoreaction for PRL, in spite of the normal PRL serum levels, and 5 tumors also an immunopositivity for GH in spite of the normal level of GH and no symptoms of acromegaly. In one case, the tumor was also immunopositive for ACTH, in spite of no symptoms of Cushing's disease. The second group included the patients with adenomas immunonegative for gonadotropins and α-SU. One tumor showed the immunopositivity for ACTH, two for GH and 2 for PRL. Only in 2 cases the pituitary adenomas were totally devoid of hormone immuno-expression (see table Ia and Ib).

The basal level of α-SU was elevated over the upper limit of normal range in two patients of the first group (gonadotroph adenomas) and in one in the second group. All but one patient from the first group and none of seven patients with tumors immunonegative for FSH, LH or α-SU, had a significant α-SU (over 50%) response to TRH (table Ia and Ib). In three of seven patients with gonadotropins immunonegative tumors a decrease of α-SU serum level after TRH was observed.

Discussion

Our findings confirm the earlier data that clinically nonfunctioning pituitary adenomas express in majority the positive immunoreactions to pituitary hormones and/or their free subunits in spite of their normal blood levels and lack of symptoms of pituitary hyperfunction [8]. The most frequently

Table Ia and Ib

The results of immunohistochemical investigation and serum α-subunit (α-SU) level at baseline and in response to TRH in patients with clinically nonfunctioning pituitary macroadenomas.

Ia. Tumors immunopositive for gonadotropins or their free subunits,

Ib. Tumors immunonegative for gonadotropins or their free subunits.

The normal (N) α-SU values in men: 0-0.8 mIU/ml, in premenopausal women: 0–0,9 mIU/ml, in postmenopausal women: 0-1.6 mIU/ml

% increase after TRH = (maximal response to TRH – mean basal value) × (100/mean basal value). A response greater than 50% was considered significant (S). NS – non significant.

Tabela Ia i Ib

Wyniki badania immunohistochemicznego oraz stężenie podjednostki α (α-SU) w warunkach podstawowych i w odpowiedzi na TRH u pacjentów z klinicznie nieczynnymi gruczolakami przysadki.

Ia. Guzy immunopozytywne dla gonadotropin bądź ich wolnych podjednostek,

Ib. Guzy immunonegatywne dla gonadotropin bądź ich wolnych podjednostek.

Prawidłowe stężenie α-SU w surowicy u mężczyzn: 0-0,8 mIU/ml, u kobiet przed menopauzą: 0–0,9 mIU/ml, po menopauzie: 0-1,6 mIU/ml.

% wzrostu po TRH = (maksymalny wzrost po TRH –średne stężenie podstawowe) × (100/ średne stężenie podstawowe). Wzrost stężenia α-SU o ponad 50% w stosunku do wartości wyjściowej przyjęto za znamienny statystycznie (S). NS – nieznamienny.

Table Ia

Patient	Sex	Age [yrs]	IHC							α -SU serum level [mIU/ml]		
			FSH	LH	α-SU	ACTH	PRL	GH	TSH	basal	after TRH administration	% increase after TRH
G.R.	M	41	-	+	+	-	-	+	-	0.160 (N)	0.880 (↑)	450.0 S
J.T.	M	70	+	-	+	-	+	+		0.040 (N)	0.180 (N)	350.0 S
J.W.	M	71	-	-	+	-	+	+	-	0.370 (N)	0.610 (N)	64.9 S
K.J.	F	54	+	+	-	-	+	+	-	11.300 (↑)	13.800 (↑)	22.1 NS
K.M.	M	37	-	-	+	+	+	+		6.579 (↑)	14.619 (↑)	122.2 S
M.C.	M	39	-	-	+	-	-			0.270 (N)	1.900 (↑)	603.7 S
W.L.	M	41	-	+	-	-				0.010 (N)	1.300 (↑)	12900.0 S

Table Ib

Patient	Sex	Age [yrs]	IHC							α -SU serum level [mIU/ml]		
			FSH	LH	α-SU	ACTH	PRL	GH	TSH	basal	after TRH administration	% increase after TRH
C.J.	M	48	-	-	-					0.390 (N)	0.37 (N)	↓ NS
G.B.	F	53	-	-	-	-	+	+	-	0.690 (N)	0.64 (N)	↓ NS
K.J.	F	61	-	-	-	+	+	-	-	0.460 (N)	0.59 (N)	28.3 NS
K.Z.	F	63	-	-			-	+		1.950 (↑)	0.27 (N)	↓ NS
M.H.	M	53	-	-	-	-	-	-	-	0.260 (N)	0.35 (N)	34.6 NS
S.J.	M	62	-	-		+	-		-	0.417 (N)	1.61 (↑)	46.3 NS
W.M.	F	61	-	-	-	+	-		-	0.230 (N)	0.34 (N)	47.8 NS

the immunopositivity for gonadotropins or their free subunits is observed. We have shown that the frequency of recurrence is higher in tumors expressing gonadotropins [8] and these tumors present the highest values of proliferation markers [12]. Therefore it is important to search for the markers allowing the detection of these tumors before the neurosurgical treatment. In the present paper we have shown that α-SU measurement after TRH administration is a good diagnostic procedure to discriminate the *gonadotropinomas* from the other CNFPAs before surgery, providing that we consider as abnormal α-SU response higher than 50% over the basal level. Nobels et al. [10] proposed an increase of the α-SU over 30% as the limit of the abnormal response. When we analyzed our data according to this criterium, we had to consider 6/7 tests in *gonadotropinoma* group as abnormal. However, 3/7 tests in the group of gonadotropin-immunonegative tumors could be considered as abnormal, too. Thus, the test interpreted with this criterium did not differentiate gonadotropin-expressing and non-expressing tumors.

It can be noticed that 5 adenomas in the *gonadotropinoma* group co-expressed also GH (in spite of the lack of symptoms of acromegaly). A question had arisen whether this co-expression could be responsible for the abnormal response to TRH. Although GH-secreting tumors in acromegalic patients often present the abnormal response of

GH to TRH stimulation, such a presumption seems unlikely. The exaggerated response of α-SU occurred also in monohormonal *gonadotropinomas* not expressing GH. On the other hand, in two monohormonal "silent" *somatotropinomas* included in the second group the administration of TRH failed to stimulate the α-SU level.

Although our observations are based on the limited number of patients, they encourage to use the α-SU measurement after TRH administration in presurgical diagnosis of *gonadotropinomas*.

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