ORIGINAL PAPERS

Glycoproteic Pituitary Hormones in the Cerebrospinal Fluid of Patients with Pituitary Adenomas

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Abstract

Introduction: The concentrations of pituitary hormones in the cerebrospinal fluid (CSF) are usually much lower than their serum counterpart. However, in patients with pituitary adenomas (PA) significantly higher CSF hormonal concentrations have been described. **Aim:** To study the CSF concentrations of pituitary glycoprotein hormones (GPH) in patients with PA, in relation with the tumor type and immunohistochemical staining for GPH. **Patients and methods:** In 221 cases submitted to transsphenoidal surgery for PA (85 acromegaly - ACM, 92 nonfunctioning pituitary adenomas – NFA, 44 resistant prolactinomas - PRM) the surgical specimen was immunostained for the expression of GPH (FSH, LH and TSH). The GPH were also measured in the serum and CSF in patients and in a control group (79 individuals without endocrine diseases). **Results:** In all types of pituitary tumors the mean CSF levels of GPH (FSH 3.4±0.56 UI/L, LH 1.94±0.28 UI/L and TSH 1.78 mU/L±0.47) were much higher than in the control group: (0.56±0.08, 0.39±0.03, 0.01±0.03, respectively; p<0.05).The serum levels were similar to controls. The GPH-immunopositive tumors were not associated with higher CSF levels than GPH-immunonegative ones. In conclusion, in the presence of pituitary adenomas, pituitary GPH (from tumor or normal pituitary cells) pass easier in the CSF. The mechanisms involved and possible cerebral effects of the increased CSF concentrations of GPH should be further explored

Keywords: pituitary adenoma, glycoproteic hormones, cerebrospinal fluid

Rezumat

Introducere: Concentrațiile hormonilor adenohipofizari în lichidul cefalorahidian (LCR) sunt mult mai mici decât cele din ser. La pacienții cu adenoame hipofizare, însă, concentrații hormonale semnificativ mai mari au fost descrise în LCR. **Scop:** Ne-am propus să studiem concentrațiile în LCR ale hormonilor gliopeptidici (HGP) adenohipofizari la pacienții cu adenoame hipofizare (AH), precum și relația acestora cu tipul tumoral și rezultatul analizei imunohistochimice pentru HGP. **Paicenți și metode:** La 221 de cazuri de adenoame hipofizare la care s-a indicat intervenția neurochirurgicală (85 cu acromegalie ACM, 92 cu adenoame nefuncționale NFA și 44 prolactinoame rezistente la tratament medicamentos) s-a efectuat analiza imunohistochimică a fragmentelor tumorale pentru expresia HGP (FSH, LH și TSH). HGP au fost măsurați în ser și în LCR la pacienți și la un grup control (79 de cazuri fără afecțiuni endocrine). **Rezultate:** La toți pacienții cu AH I valorile medii ale HGP în LCR. (FSH 3.4±0.56 UI/L, LH 1.94±0.28 UI/L and TSH 1.78 mU/L±0.47) au fost semnificativ crescute comparative cu grupul control (0.56±0.08, 0.39±0.03, 0.01±0.03, p<0.05). Concentrațiile serice au fost similare în cele două grupuri. Tumorile imunopozitive pentru HGP nu au prezentat concentrații de HGP în LCR mai mari decât tumorile imunonegative. În concluzie, la pacienții cu adenoame hipofizare, HGP hipofizari (produși fie în tumoră, fie în hipofiza sănătoasă) se găsesc în concentrații crescute în KCR comparative cu grupul control. Mecanismele implicate și posibilele efecte cerebrale ale concentrațiilor crescute în LCR ale HGP necesită studii suplimentare.

Cuvinte cheie: adenom hipofizar, hormoni glicopeptidici, lichid cefalorahidian

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INTRODUCTION

Pituitary adenomas are benign neoplasms originating in the anterior pituitary cells, that represent 15-20% of all intracranial tumors[1].

Pituitary adenomas (PA) can be secreting (producing growth hormone-GH, prolactin-PRL, adrenocorticotropic hormone-ACTH or, very rarely gonadotropins-FSH and LH or thyrotropic hormone TSH) or non-secreting (NPA).

Despite the presence of the blood-brain- barrier (BBB) that prevents the passage of most proteins into the cerebrospinal fluid (CSF), the anterior pituitary hormones (all with peptidic structures) are normally present in the CSF in very low concentrations[2].

However, in patients with PA, the CSF levels are significantly higher compared to controls, especially for gonadotropins[3-5] but also for the other hormones; the CSF concentrations are not correlated to the corresponding serum concentrations[6].

We aimed to further investigate the CSF concentrations of glycoproteic hormones (GPH) produced by the adenohypophysis in patients with PA and their relation to the tumor type (defined by the hormonal pattern and immunohistochemical staining).

MATERIALS AND METHODS

We included in our study 221 patients with PA (85 acromegaly - ACM, 92 nonfunctioning pituitary adenomas –NFA, 44 resistant prolactinomas - PRM) in whom transsphenoidal tumor resection was indicated.

During admission in the neurosurgical department, before surgery, simultaneous blood and CSF samples were collected, centrifuged and stored at -20 degrees C until they were analyzed (within one month from collection). Hormonal analysis of the GPH was performed in these samples using Delfia Fluoroimmunoassay. The results obtained in the control group have been previously published; these data were used for comparison[2].

At the time of the neurosurgical intervention pituitary gland specimens were prelevated, fixed in 10% buffered formalin for 48 hours and paraffin-embedded using routine procedure. Five micrometers thick serial sections were performed from each paraffin block and sections were mounted on silanized slides. For the immunohistochemical study the slides were deparrafinated and rehydrated. We investigated the immunohistochemical expression of GPH using anti- FSH (DakoCytomation, clone C10, dilution 1: 50), anti-LH (DakoCytomation, clone C93, dilution 1: 50) and anti- TSH (DakoCytomation, clone 0042, dilution 1: 50) antibodies. For the interpretation of results we used histopathological examination with the Nikon Eclipse E600 microscope (standard haematoxillyine-eosine staining as well as argentic coloration for the study of reticulin). Pituitary hormones evaluation was done using the following criteria: density below 5%=0, between 5-10% = +1, between 10-50% = +2, between 50-100% =+3. Based on this scoring system we classified as non-secreting tumors those with an intensity of the reaction <2 and as secreting those with an intensity of 2 or 3.

RESULTS

We included in our study 221 patients with PA in whom transsphenoidal tumor resection was indicated. 85 of the cases had acromegaly (ACM-all caused by GH secreting macroadenomas), 92 had nonfunctioning pituitary adenomas –NFA and 44 macroprolactinomas, resistant to medical therapy with dopamine agonists - PRM). All tumors referred to surgery were macroadenomas but the tumor dimension was larger in NFA (3.1±0.99 cm) compared to PRM (2.8±1.08 cm, not statistically significant) and ACM (1.74 ±0.92; p=0.000** compared to NFA).

In all types of tumors the CSF values for gonadotropins and TSH were significantly greater compared to the control group. As for the serum values, these either do not differ from control (TSH) or were lower in the patients group (FSH, LH) (Table 1).

Significantly higher CSF hormonal values were observed in the NFA group compared to the GH- and PRL-secreting tumors, despite corresponding serum concentrations being either similar or even significantly lower compared to the group of patients with ACM and PRM (Figure 1 and Table 2).

The immunohistochemical staining for GPH was analysed in each tyumor type- the results are summarised in Table 3.

We aimed to check whether positive immunohistochemical staining for a particular hormone is reflected into a significantly different concentration either in the serum or the CSF. No statistically significant difference was found between hormonal levels of tumors with positive as compared to negative immunoreactions for these 3 pituitary hormones (Table 4).

DISCUSSION

Anterior pituitary hormones are normally present in the cerebrospinal fluid (CSF) in very low concentrations, due to the low permeability of the blood-brain barrier. (2) In patients with pituitary adenomas (PA),

Table 1. The hormonal concentration in serum, CSF and the CSF/serum ratio in patients and controls

	Controls Mean±SD	Patients SEM	p
Serum LH (mIU/l)	**11.11**±1.55	**4.13**±7.18	**0.000****
CSF LH (mIU/l)	**.394**±0.03	**1.94**±3.67	**0.000****
CSF/serum LH	**0.07**±0.01	**1.46**±2.35	**0.000****
serumFSH (mIU/l)	**14.50**±4.10	**9.37**±16.26	**0.000****
CSF FSH (mIU/l)	**.57**±0.07	**3.44**±7.53	**0.000****
CSF/serum FSH	**0.08**±0.02	**.95**±1.66	**0.000****
Serum TSH (mIU/l)	**1.36**±.17	**1.74**±1.78	0.45
CSF TSH (mIU/l)	**0.01**±0.00	**1.78**±5.48	**0.000****
CSF/ serum TSH	**0.077**±0.009	**1.11**±3.63	**0.000****

Table 2. Statistical significance of the difference in the serum and CSF concentrations of GPH in different tumor types compared to each other and to controls

	ACM-C	NFA-C	PRM-C	ACM-NFA	ACM-PRM	NFA-PRM
Serum LH (mIU/l)	**0.00****	**0.00****	**0.00****	**0.01***	**0.001****	0.3
CSF LH (mIU/l)	**0.00****	**0.00****	0.28	0.12	0.058	**0.004****
CSF/serum LH	**0.00****	**0.00****	**0.00****	**0.00****	0.28	0.09
serumFSH (mIU/l)	**0.00****	**0.00****	**0.00****	**0.04***	**0.00****	**0.035***
CSF FSH (mIU/l)	**0.00****	**0.00****	0.8	**0.005****	0.17	**0.00****
CSF/serum FSH	**0.00****	**0.00****	**0.00****	**0.00****	0.13	0.071
Serum TSH (mIU/l)	0.08	0.57	0.33	0.06	0.55	0.37
CSF TSH (mIU/l)	**0.00****	**0.00****	**0.00****	**0.049***	0.12	**0.01***
CSF/ serum TSH	**0.00****	**0.00****	0.13	**0.00****	0.19	**0.00****

both secreting and non-secreting, significantly higher CSF values for the anterior pituitary hormones have been reported[3,7,8].

We investigated the simultaneously sampled serum and CSF concentrations of glycoproteic pituitary hormones (GPH, namely FSH, LH, TSH) in a cohort of patients with PA and compared the results with those obtained in controls. Also we compared the hormonal concentrations obtained in patients with different tumor types and different immunostaining for GPH.

The serum concentrations of gonadotropins were significantly lower in patients compared to the control group (mainly as a consequence of the presence of the tumoral mass and the compression exerted on the healthy pituitary; in cases with hyperprolactinemia this contributed to the gonadotropin deficiency). The CSF concentrations of gonadotropins were, however, significantly increased in patients (especially in NFA cases) compared to controls. For TSH, the serum levels were similar in patients and controls. However, again,

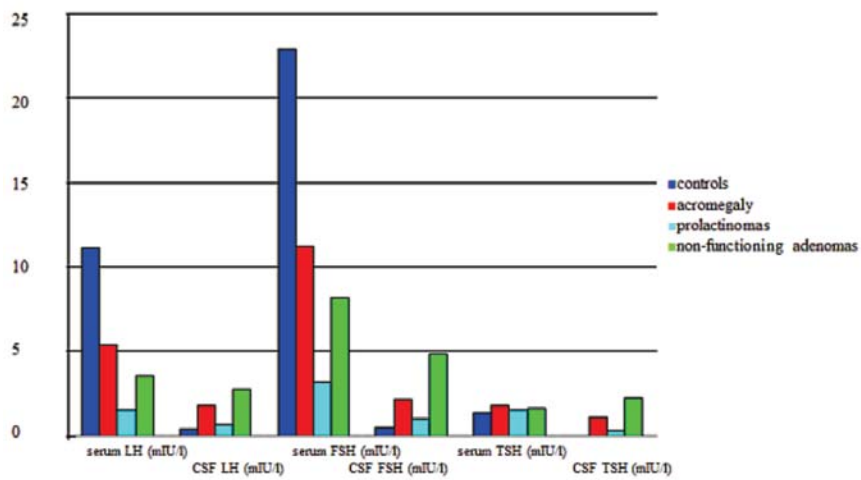

Figure 1. Serum and CSF concentrations of GPH in controls and different types of PA.

Table 3. The results of the immunohistochemical GPH staining for each tumor type

	LH		FSH		TSH	
	+	-	+	-	+	-
ACM	5	80	3	77	7	74
NFA	21	71	19	71	7	82
PRM	1	43	1	41	0	44

the CSF concentrations were markedly increased in patients compared to controls. These results underline the fact that increased CSF concentrations of pituitary hormones in patients with PA can not be explained by simultaneously increased serum levels, in line with historical observations[6,9]. This argues against a mechanism of simple diffusion through BBB.

The mechanism of the increased CSF GPH values in patients with PA remains elusive. The most logical explanation of this phenomenon would be that the excessive hormones present in the CSF come directly from the tumor, by-passing the general circulation. We verified this hypothesis by the analysis of each hormone serum concentration in patients with immunohistochemically-positive and –negative tumors, for that particular hormone. Surprisingly, the immunohistochemical results did not influence the concentration of the examined hormones in either serum or CSF. Therefore, increased CSF concentrations of GPH are equally found in tumors with negative immunohistochemistry for the particular hormone discussed.

An alternative explanation would be that the hormones found in increased concentrations in the CSF of patients are synthesised by the healthy pituitary around the tumoral mass. In this hypothesis the secretion from the healthy surrounding pituitary should not be dependent on the type of pituitary tumor. This would not, therefore, explain why in NFA CSF concentrations are higher compared to the other tumor types. A possible explanation would be that since NFA tumors were larger, in NFA patients alterations of the local relations between anatomical structures were more likely.

Increased CSF concentrations for gonadotropins have also been described in patients with other types of tumors in the hypothalamic-pituitary area[3-5], suggesting that the mass compression exerted by the tumor might be involved in the pathogenesis of the phenomenon, perhaps through increasing BBB permeability. Altenatively, the presence of the tumor mass might lead to changes in the local vascularisation with the alteration of normal circulation of secreted pituitary hormones[10].

An area of interest would also be the potential cerebral effects exerted by the pituitary hormones, present in increased CSF concentrations in these patients. Although evidence for such effects are lacking at present, there are sound theoretical reasons to assume that such effects might indeed be present. Some of the pituitary hormones have receptors in the neural tissue. Most clearly evidenced are the LH receptors[11], most abundantly found in the hippocampus[12], The physiological role of these receptors is unknown but in animal studies they have been involved on the regulation of activity level, wake, stereotypical behavior, sexual behavior[13-16]. The FSH receptors have been described in the cerebral tissue in some animal species[17] but not in others[16,18].

CONCLUSIONS

Patients with various types of PA (acromegaly, prolactinomas, non-functioning adenomas) have increased CSF concentrations of glycopeptidic pituitary hormones compared to controls. The mechanism of this phenomenon, possible associations with tumor characteristics or invasivity, potential effects exerted by the increased CSF glycoproteic hormones upon the central nervous system need to be further explored.

Table 4. Comparative hormonal analysis in cases with positive versus negative immunostaining for each GPH

Hormone	Hormonal concentration (mean ±SD) Immunostaining for the corresponding hormone		p
	positive	negative	
Serum LH (mIU/l)	1.06 ±1.24	4.02±7.16	0.508
CSF LH (mIU/l)	3.01±3.61	1.77±3.66	0.264
serumFSH (mIU/l)	9.99±18.45	9.17±15.56	0.902
CSF FSH (mIU/l)	3.30±5.09	3.46±7.88	0.405
Serum TSH (mIU/l)	1.79±1.56	1.73±1.77	0.607
CSF TSH (mIU/l)	1.27±2.46	1.51±4.05	0.257

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