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The relation of pituitary adenomas invasiveness and the proliferative index measured by immunoexpression of topoisomerase IIα

Związek między inwazyjnością gruczolaków przysadki a indeksem proliferacyjnym mierzonym immunoekspresją topoizomerazy IIα

Borys M. Kwinta[1], Aleksander Wilk[1], Małgorzata Trofimiuk-Müldner[2], Ewelina Grzywna[1], Roger M. Krzyżewski[1], Krzysztof Stachura[1], Dariusz Adamek[3]

[1]Department of Neurosurgery and Neurotraumatology, Jagiellonian University Medical College, Kraków, Poland
[2]Department of Endocrinology, Jagiellonian University Medical College, Kraków, Poland
[3]Department of Neuropathology, Jagiellonian University Medical College, Kraków, Poland

Abstract

Introduction: Cavernous sinus invasion by pituitary adenoma affects surgical procedure radicality and consequently the postoperative course and prognosis in pituitary adenoma treatment. The search for pituitary adenoma aggressive behaviour markers is still a matter of debate.
Material and methods: This study evaluates the relation of pituitary adenoma invasiveness to the expression of topoisomerase IIα in 72 patients who underwent transsphenoidal pituitary surgery. The assessment of tumour growth was conducted according to the Hardy scale as modified by Wilson and the Knosp scale. Topoisomerase IIα expression in tumour specimens was evaluated using immunohistochemical staining.
Results: There was a correlation between the Knosp scale degree and the topoisomerase IIα expression (Spearman $R = 0.3611$, $p < 0.005$). The Kruskal-Wallis H test ($p = 0.0034$) showed that there was a statistically significant topoisomerase IIα expression increase in tumours classified as grade E on the Hardy scale. The topoisomerase IIα expression correlated also with tumour size (Spearman $R = 0.4117$, $p < 0.001$). Higher levels of expression were observed in macroadenomas, as compared to microadenomas ($p < 0.05$, Mann-Whitney test). Topoisomerase IIα expression correlated with cavernous sinus invasion.
Conclusions: The topoisomerase IIα expression correlated more with invasiveness than with extensiveness, which might make it an eminently useful marker in the assessment of aggressive pituitary adenoma behaviour. **(Endokrynol Pol 2018; 69 (5): 530–535)**

Key words: *pituitary adenoma, Knosp scale, topoisomerase IIα expression, cavernous sinuses invasion*

Streszczenie

Wstęp: Naciekanie zatok jamistych przez gruczolaka przysadki wpływa na radykalność zabiegu operacyjnego, a w konsekwencji na przebieg pooperacyjny i rokowanie. Do chwili obecnej nie ustalono jednak, jakie markery najlepiej odzwierciedlają agresywne zachowanie gruczolaków przysadki.
Materiał i metody: W badaniu oceniono związek między inwazyjnością gruczolaków przysadki a ekspresją topoizomerazy IIα w grupie 72 pacjentów po przebytej przezklinowej operacji z powodu gruczolaka przysadki. Zaawansowanie guza oceniano stosując skalę Hardy'ego w modyfikacji Wilsona i skalę Knospa. Ekspresję topoizomerazy IIα w wycinkach guza oceniano w badaniu immunohistochemicznym.
Wyniki: Stwierdzono istotną korelację między zaawansowaniem gruczolaka przysadki według skali Knospa a ekspresją topoizomerazy IIα (współczynnik korelacji R Spearmana 0,3611, $p < 0,005$). Stwierdzono istotny wzrost ekspresji topoizomerazy IIα w guzach w stopniu zaawansowania E wg skali Hardy'ego (test H Kruskal-Wallisa, $p = 0,0034$). Ekspresja topoizomerazy IIα korelowała także z wielkością guza (współczynnik korelacji R Spearmana 0,4117, $p < 0,001$). Wyższe wartości indeksu ekspresji obserwowano w makrogruczolakach w porównaniu z mikrogruczolakami ($p < 0,05$, test Manna-Whitneya). Ekspresja topoizomerazy IIα korelowała z naciekaniem zatok jamistych.
Wnioski: Ekspresja topoizomerazy IIα lepiej koreluje z inwazyjnością gruczolaków przysadki niż z ekstensywnością ich wzrostu. Może więc być bardzo przydatnym markerem agresywności gruczolaków przysadki. **(Endokrynol Pol 2018; 69 (5): 530–535)**

Słowa kluczowe: *gruczolak przysadki, skala Knospa, topoizomeraza IIα, naciekanie zatok jamistych*

Introduction

Pituitary adenomas

Pituitary adenomas are benign tumours that represent 10–20% of all intracranial neoplasms treated surgically, and are the most common lesions found in the sellar area [1–3]. Microadenomas are tumours with diameter less than 1 cm. Larger tumours, referred to as 'macroadenomas', extend suprasellarly causing compression of the optic chiasm, may grow into the third ventricle, damage the hypothalamus, and extend laterally into the cavernous sinuses (CS) [4–7].

Borys M. Kwinta M.D., Ph.D., Department of Neurosurgery and Neurotraumatology, Jagiellonian University Medical College, Botaniczna 3, Kraków Poland, tel.: +48 12 424 8662; e-mail: bmkwinta@gmail.com

Approximately 6–10% of adenomas infiltrate CS [8–10]. CS invasion usually makes complete surgical removal of adenomas impossible, and in such cases alternative therapies, such as radiation, need to be applied. Tumour size itself is not an indicator of aggressive clinical behaviour [11]. Hence, evaluation of CS invasion is crucial for pituitary adenoma treatment planning and further prognosis [12].

Adenomas more prone to aggressive behaviour are: both growth hormone and prolactin secreting (acidophil stem cell adenomas), growth hormone secreting (scant grainy type adenomas), silent corticotroph adenomas, and silent adenomas subtype III [13–15]. Pituitary cancers are extremely rare [16] and represent about 0.2% of all lesions in this location [17].

Markers of aggressiveness

Currently, reliable markers of aggressive adenoma behaviour are lacking [18]. Cytological markers of aggressiveness, i.e. mitoses, polymorphism, or giant cells, are rarely found and are not linked to local invasiveness [2, 11]. The World Health Organisation pituitary adenoma classification discriminates between typical and atypical adenomas based on the Ki-67 labelling index cut-off value of 3% [15, 19]; however, further research indicated low sensitivity of this test [20–22]. The work of Paek et al. did not confirm the relation between the Ki-67 labelling index and infiltration of the CS [18].

Little attention is paid to another potential marker — topoisomerase IIα. This is a nuclear enzyme maintaining chromatin loop homeostasis during DNA replication [23]. It is widely used for the evaluation of the proliferation level of many neoplasms, for example breast cancer, laryngeal cancer, and endometrial, haematopoietic, and central nervous system (CNS) neoplasms [24–28]. Topoisomerase IIα is also a target for oncological therapies [29, 30]. Patients with adenomas with high expression of topoisomerase IIα might be more sensitive to drugs inhibiting this enzyme. It has been noted that the sensitivity of adenoma cells to topoisomerase IIα inhibitors depends on topoisomerase IIα expression in the tumour cells [30].

The presented study evaluates the relation between CS invasion and immunoexpression of topoisomerase IIα in pituitary adenomas — a potential marker of tumour aggressiveness.

Material and methods

Material

This retrospective study included 72 patients who underwent pituitary surgery in the Neurosurgery and Neurotraumatology Department of the JUMC between 2007 and 2015. All patients were qualified for surgical treatment by a multidisciplinary team consisting of neurosurgeons, radiologists, radiotherapists, endocrinologists, and ophthalmologists.

The group consisted of 44 females (61.1%) and 28 males (38.9%) aged 18 to 84 years (mean age 46.9 years; 45.1 years for females and 49.9 years for males). Thirteen patients (18.1%) were diagnosed with acromegaly, seven (9.7%) with Cushing's disease, one (1.4%) with secondary hyperthyroidism, and one (1.4%) with precocious puberty. Twenty-four patients (33.3%) presented significant hyperprolactinaemia, and the remaining 26 (36.1%) were harbouring clinically non-functioning pituitary adenomas. All patients were operated microsurgically via transsphenoidal approach. On immunohistochemistry eight (11.1%) tumours were positive for prolactin only, seven (9.7%) for growth hormone, two (2.8%) for ACTH, one (1.4%) for LH, one (1.4%) for FSH, and three (4.1%) for a-subunit. Twenty-one (29.2%) adenomas were plurihormonal.

This study was conducted in accordance to the Declaration of Helsinki (1964), and its design was approved by the local University Ethical Committee (protocol number KBET/157/B/2012).

Methods

Tumour size, invasiveness, and extension evaluation

To assess pituitary adenoma size and invasiveness computed tomography (CT) and magnetic resonance (MR) images of sellar regions were analysed.

Tumour size was evaluated in three orthogonal planes. The longest dimension in every plane was selected. Tumour volume was assessed using the Di Chiro and Nelson's equation: $V = (\pi/6) * (x * y * z)$ [31], for the volume of a spheroid, where 'x, y, z' are the longest sizes on three orthogonal axes.

The assessment of tumour invasiveness was conducted according to the Hardy scale as modified by Wilson [7] and the Knosp scale, both of which are based on CT and MRI coronal sections [8]. The side of the tumour with higher grading according to the Knosp grading scale was chosen for further analysis (Figure 1).

Morphometric analysis of topoisomerase IIα expression

The pituitary adenoma tissue samples obtained during surgery were evaluated in the Neuropathology Department of the JUMC. Monoclonal immunoglobulin G-class antibodies directed against C-terminal domain of human topoisomerase (NCL-TOPOIIA, Novo-castra, Novocastra Laboratories Ltd., New Castle upon Tyne, United Kingdom) was performed in 1:30 dilution (1:30). The antigen was retrieved at 95°C in citrate buffer (pH = 6.0). Overnight incubation with primary antiserum

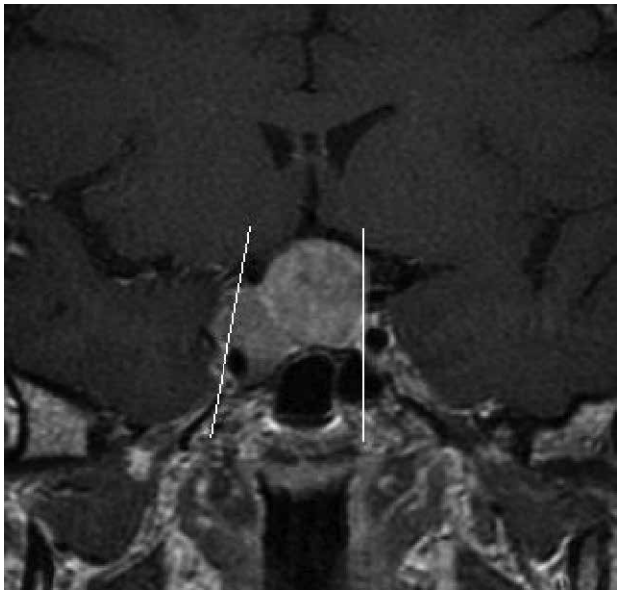

Figure 1. *Sellar MRI T1 C. As an example, the MRI plane shows sections through the CS and both intracavernous and intracranial carotid artery segments. Knosp grading: right side 3, left side 0*

Rycina 1. *Rezonans magnetyczny siodła tureckiego (T1). Na przykładzie uwidoczniono przekrój przez zatoki jamiste oraz śródjamisty i śródczaszkowy odcinek tętnic szyjnych wspólnych. Zaawansowanie wg skali Knospa: strona prawa — stopień 3, strona lewa — stopień 0*

(NCL-TOPOIIA) at 2–8°C was followed by incubation with a secondary biotinylated antibody for 30 minutes. Avidin–biotin complex horseradish peroxidase (30 minutes) with diaminobenzidine tetrahydrochloride was applied as chromogen. Slides were counterstained with haematoxylin.

After immunohistochemical staining, slides were analysed with an optical microscope, Nikon Optishot-2 at 200x magnification. Nondiagnostic fragments (presence of normal pituitary tissue, fibrosis, haemorrhage, etc.) were excluded from further analyses. The morphometric evaluation was performed only if at least one field of view at 200x magnification included adenoma material without thermal or mechanical damage and without massive necrotic or haemorrhagic lesions. If a sample was not suitable for assessment, staining was repeated after cutting another section from the specimen.

Topoisomerase IIα expression was assessed manually using an optical microscope with morphometric grid divided into 16 fields of equal area covering the entire field of view at 400x magnification. The number of stained cells and the total number of cells were counted for each field in five most suitable areas of the immunostained slide. The topoisomerase IIα cell index was calculated as the percentage of cells exhibiting positive immunohistochemical reaction (Figure 2).

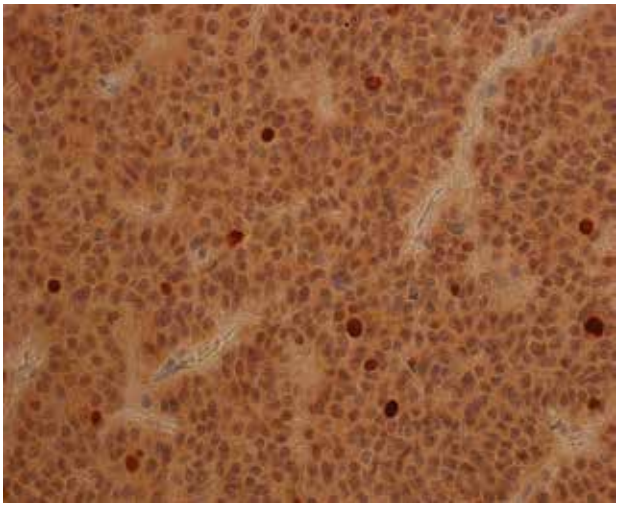

Figure 2. *Immunostaining for topoisomerase IIα in pituitary adenoma (200x magnification)*

Rycina 2. *Barwienie w kierunku topoizomerazy IIα w gruczolaku przysadki (powiększenie 200x)*

Statistical methods

Statistical analyses were performed using STATISTICA 12. Because assumptions for a parametric test were not valid according to Shapiro-Wilk test of normality, all data were evaluated by non-parametric tests. Mann-Whitney U test and Kruskall-Wallis analysis of variance as a multiple-comparison method were used to compare the topoisomerase IIα expression between micro- and macroadenomas and between all of the Hardy scale grades. The Spearman test was used to assess the statistical significance of the correlation between the Knosp scale grading, as well as adenoma size and topoisomerase IIα expression. A p value < 0.05 was considered statistically significant.

Results

The median tumour size was 2793 mm^3 (IQR: 245–6703 mm^3). Macroadenomas (adenomas larger than 1 cm) were diagnosed in 51 cases (70.84%), while microadenomas were found in 21 cases (29.16%).

The results of tumour invasiveness evaluation according to the tumour Hardy-Wilson and Knosp scales are presented in Tables I and II, respectively.

Topoisomerase IIα expression was observed in 48 out of 72 cases. No significant differences ($p = 0.13$) in the topoisomerase IIα expression cell index with respect to patient gender (female 0.31% vs. male 0.56%) were observed.

There was a significant correlation between the Knosp scale degree and topoisomerase IIα expression index (Spearman $R = 0.3611$, $p = 0.0018$. The Kruskal-Wallis H test ($p = 0.0072$) followed by Dunn's test showed that only

Table I. *Pituitary adenoma invasiveness in the study group according to Hardy scale, as modified by Wilson [7]*
Tabela I. *Ocena wielkości gruczolaków przysadki w badanej grupie wg skali Hardy'ego (modyfikacja Wilsona) [7]*

Invasion		Extension		Degree	% of evaluated subjects
Floor of sella intact	I — I sella normal or focally expanded, tumour < 10 mm	Suprasellar	0 — no	I 0	29.17
	II — sella enlarged, tumour ≥ 10 mm		A — expansion into suprasellar cistern: tumour < 10 mm above the sella, taking 25% of chiasmatic cistern	II 0	8.33
				II A	11.11
				II B	6.94
				II D	1.39
Sellar floor occupied	III — localised perforation of sellar floor		B — anterior recesses of 3rd ventricle obliterated, tumour < 20 mm above the sella, taking 50–70% of chiasmatic cistern	III A	5.56
				III B	5.56
				III C	5.56
				III E	1.39
	IV — diffuse destruction of sellar floor		C — displacement of 3rd ventricle, tumour < 30 mm above the sella, reaching foramina of Monro	IV B	4.17
				IV C	5.56
				IV D	8.33
				IV E	6.94
	V — spread via cerebrospinal fluid or blood-borne	Parasellar	D — intracranial, intradural, anterior, middle, or posterior fossa occupation	V	0
			E — extradural, inside or beneath cavernous sinus		

Table II. *Pituitary adenoma invasiveness according to Knosp scale [8]*
Tabela II. *Ocena inwazyjności gruczolaków przysadki według skali Knospa [8]*

Degree	MRI — coronal plane through the centre of sella turcica	No of patients (%)
0	No cavernous sinus involvement, tumour does not exceed line tangent to the medial outlines of intra- and supracavernous internal carotid artery (ICA)	40 (55.56)
1	Tumour does not exceed the line connecting centres of intra- and supracavernous ICA	6 (8.33)
2	Tumour does not exceed the line tangent to the lateral outlines of intra- and supracavernous ICA	11 (15.28)
3	Tumour crosses the line tangent to the lateral outlines of intra- and supracavernous ICA	12 (16.67)
4	Total encasement of the intracavernous ICA	3 (4.17)

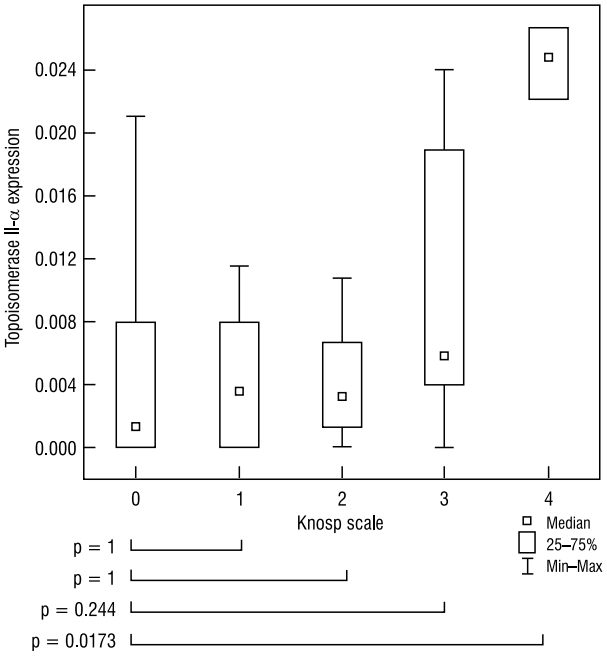

Figure 3. *Topoisomerase II-α expression in relation to the Knosp grading scale*
Rycina 3. *Ekspresja topoizomerazy IIα w zależności od zaawansowania według skali Knospa*

grades 3 and 4, which correspond with the true invasion of the tumour into the CS, tended to correlate with an increased topoisomerase IIα expression indices (Figure 3).

The Kruskal-Wallis H test ($p = 0.0034$) followed by Dunn's test showed that a significant increase in topoisomerase IIα expression was not observed in tumours classified as grade A, B, C, and D on the Hardy scale. However, there was a significant ($p = 0.0143$) expression increase in tumours classified as grade E (Figure 4).

The topoisomerase IIα expression correlated with tumour size (Spearman $R = 0.4117$, $p < 0.001$). Higher values of expression indices were observed in macroadenomas, as compared to microadenomas ($p = 0.0003$).

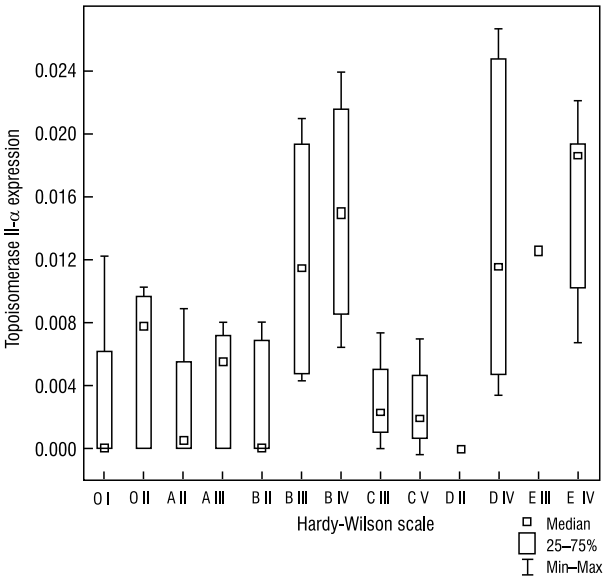

Figure 4. *Topoisomerase II-α expression in relation to the Hardy grading scale*

Rycina 4. *Ekspresja topoizomerazy IIα w zależności od zaawansowania według skali Hardy'ego*

Discussion

The study correlates CS invasion by pituitary adenoma with topoisomerase IIα expression as a predictor of tumour aggressiveness.

In many papers, different MRI criteria were used to assess CS invasion by pituitary adenomas [8, 9, 12, 32]. In this study, we used the Hardy-Wilson classification and the Knosp scale. The radiological evaluation of pituitary adenoma growth allows the assessment of the possible radicality of tumour resection [33]. The Hardy scale indicates the degree of the sellar floor invasion. Wilson's modification of this scale shows the advancement of suprasellar growth. The Knosp scale, on the other hand, specifies the extension of the tumour into the CS and the internal carotid arteries encasement, which directly affect the chances of complete resection. The reference point for the Knosp scale assessment is the coronal plane, which runs through the centre of the sella turcica [8]. Modern studies comparing MRI with intraoperative findings acknowledge the utility of the Knosp group's findings concerning the lateral intercarotid line as a good marker of CS invasion [8, 12, 33]. In the study of Cottier et al., crossing the lateral intercarotid line by a tumour was an indicator of CS invasion (PPV 85%, NPV 95%) [33]. This is consistent with the presented results because it corresponds with degrees 3 and 4 of the Knosp scale.

This study provides evidence that topoisomerase IIα expression was higher in tumours classified as grade 3 and grade 4 on the Knosp scale, which is concordant with CS occupation. A significant increase in topoisomerase IIα expression indices was not observed in tumours classified as grade A, B, and C on the Hardy scale, i.e. in adenomas characterised by intra- and suprasellar growth. However, there was a significant topoisomerase IIα expression increase in tumours classified as grade E (occupying the CSI). It could be said that the topoisomerase IIα expression index is correlated more with invasiveness than with extensiveness).

To date, only a few studies have investigated topoisomerase IIα expression in pituitary adenomas. Sarkar et al. showed that MIB-1 proliferation index, p53 expression, and elevated mitotic index are factors correlated with parasellar invasion [34]. Intrasellar invasion in his study was not related to any immunocytochemical marker [34]. In the studies of Landolt et al., Yilmaz et al., and Moldovan et al. a higher Ki-67 labelling index in invasive pituitary adenomas was found [35–37]. In the study conducted by Wolfsberger et al., the MIB-1 proliferation index, unlike the topoisomerase IIα expression, was significantly higher in the group of invasive adenomas [38]. However, this group was characterised not only by intraoperative signs of CS invasion but also by intraoperative signs of dura and bone infiltration [38]. Simultaneously, in the same paper, a strong correlation between MIB-1 and topoisomerase IIα was reported [38]. Trofimiuk et al. presented a relationship between the topoisomerase IIα expression level and the tumour size and invasiveness [39]. In their study, prospective analysis was performed, and topoisomerase IIα index exceeding 1% was assessed as a prognostic factor of pituitary adenoma recurrence [39]. In the study of Vidal et al., topoisomerase IIα expression was significantly higher in invasive tumours [30], which is also consistent with the results presented in our study.

Conclusions

From the clinical perspective, CS occupation is a very important factor that affects surgical procedure radicality and safety, and consequently the postoperative course and prognosis. As can be concluded from the data obtained from our study, topoisomerase IIα expression correlates with CS invasion and might be a useful marker in the assessment of aggressive pituitary adenoma. A further prospective study concerning topoisomerase IIα expression in recurrent tumours is needed.

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