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"IMMUNOHISTOCHEMICAL CHARACTERISTICS OF PATIENTS WITH MACRO- AND GIANT NON-FUNCTIONAL PITUITARY ADENOMAS"

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Abstract:

The aim of the study is to perform a comparative analysis of immunocytochemistry data from groups of patients with macro- and giant non-functional pituitary adenomas. (NFPA)

Material and methods of research. We studied 20 patients with NFPA (Group 1 - 10 pituitary macroadenomas and Group 2 - 10 giant pituitary adenomas) who underwent transnasal pituitary adenomectomy (TPA).

All patients underwent examinations, including examination of the fundus, measurement of visual fields every 3 months, studies of the levels of STH, IGF-1, LH FSH, ACTH, TSH, prolactin, free thyroxine, cortisol, as well as immunohistochemical studies of operated patients with determination of the levels of expression of Ki67, p53.

Research results. It was found that the presence of aggressive NFPA increases by 8.2 times ($p < 0.001$) when there is an invasive nature of growth (sensitivity 86%, specificity 53%, Youden index 0.45, accuracy 66%). The coefficient is 5.2 ($p < 0.001$) per percentage point of Ki67-positive tumor cell nuclei, and 3.1 ($p < 0.001$) per percentage point of p53-immunopositive nuclei.

Conclusions.1. We identified significant cutoff values for p53 (≥2%; CI: 0.94). The most reliable individual marker for differentiating macro NFPA from giant NFPA was the Ki-67 labeling index ≥ 4% (CI: 0.98).

2. The most reliable individual marker for differentiating macroNFPA and giant NFPA was the Ki-67 labeling index ≥ 4% (CI: 0.98).Tumors with immunoexpression of at least 2 markers were observed in 54% of the cohort and were assessed as aggressive adenomas: for p53 - 4 patients in group 1 and 5 patients in group 2, for Ki 67 - 5patients in group 1 and 6 patients for group 2, in total 11 patients out of 20 IHC examined had a risk of growth relapse.

Keywords: NFPA, IHC, proliferation markers

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INTRODUCTION. Non-fucntional pituitary adenomas (NFPA) are the most common type of macroadenomas, accounting for a quarter to a third of all cases.[1].Although these tumors are generally benign, their invasive growth into adjacent structures such as the sphenoid bone, cavernous sinus, and dura mater often makes complete surgical removal impossible.[2].The remaining tumor cells retain the ability to divide, creating a risk of recurrence requiring retreatment. Most recurrences occur within five years after surgery, negatively impacting patients' long-term prognosis.[3].Currently, surgery is the only treatment method for NFPA, as there are no effective pharmacological drugs.[4].Studies have aimed to identify histological biomarkers such as the proliferation marker Ki-67, cell cycle factors (p27, galectin-3) and other molecules (p53, O-6-methylguanine DNA methyltransferase, matrix metalloproteinase 9) to assess the relationship between invasiveness and recurrence.[5].However, the lack of reliable serum markers to detect residual tumor cells means that the decision on the need for additional treatment is usually based on postoperative imaging results.[6-8].

Convincing prognostic factors for pituitary tumor recurrence have not yet been identified. Clinical factors such as age, gender, tumor size, and tumor invasion have been shown to have limited prognostic value for tumor progression. On the other hand, Ki-67 has been described as an independent cellular marker of tumor progression and recurrence [9]. Recently, Raverot G. et al. [10] proposed classifying pituitary tumors into five grades that can be used by clinicians to predict tumor behavior after surgery. This classification system is based on predictor factors such as tumor invasion on MRI, immunohistochemical profile, mitotic index, Ki-67, and p53 positivity, which can be used to identify patients at high risk of tumor recurrence or progression [10].

There is still debate about the factors that predict the biological behavior of these tumors.

All of the above formed the basis for this study.

The aim of the study is to study the IHC characteristics of patients with macro and giant NFPA.

MATERIAL AND METHODS OF RESEARCH. We studied 20 patients with NFPA (Group 1 - 10 pituitary macroadenomas and Group 2 - 10 giant pituitary adenomas) who underwent transnasal pituitary adenomectomy. The pituitary neurosurgery department of the Republican Specialized Scientific and Practical Medical Center of Endocrinology enrolled 12 patients between 2020 and 2022. Of these, 12 were men (60%) and 8 were women (40%). The average age of men was 48.12 years, while that of women was 46.15 years. The control group consisted of 10 healthy individuals with normal pituitary tissue.

Inclusion criteria: NFPA, TPA, women, men, macro and giant NFPA.

Exclusion criteria: other types of pituitary adenomas, pituitary microadenomas, severe somatic diseases and endocrinopathies

The maximum mean tumor diameter determined by MRI diagnostics was 44.7 ± 13.6 mm in 10 patients with giant NFPA s, and macroadenomas > 30 mm were present in 10 patients.

All patients underwent examinations, including fundus examination, visual field measurements every 3 months, studies of the levels of STH, IGF-1, LH, FSH, ACTH, TSH, prolactin, free thyroxine, cortisol, as well as immunohistochemical studies of operated patients.with the determination of the expression levels of Ki67, p53. In addition, the AKU scale (2022) was introduced for the first time to predict the degree of tumor removal in the preoperative period.

In addition, the postoperative material was subjected to histological diagnostics at the Republican Scientific and Practical Medical Center of the Health Ministry of the Republic of Uzbekistan named after Academician E.Kh. Turakulov (histology department, PhD Issaeva S.S.).

Immunohistochemical studies (IHC) were performed under a contract at the pathomorphology laboratory of IPSUM Pathology LLC. The study was performed under a contract at the IHC laboratory at the Tashkent City Oncology Clinic. Paraffin-embedded blocks with confirmed diagnoses of pituitary adenoma were used. Serial 3-μm-thick sections were deparaffinized, dehydrated, unmasked, and stained with antigens using a specialized automated Ventana Benchmark XT system (Roche, Switzerland). The study was performed with ki-67 (30-9) and P53 (Bp53-11) antibodies.

Ki67. IHC assessment of sections: the proliferative activity of tumor cells in the nuclear compartment was assessed.

P53. IHC assessment of sections: To verify abnormal (mutant) p53 expression, expression in more than 75% of cells in the affected area was considered. Negative expression or weak staining of the nuclear locus in up to 70% of cells was interpreted as the natural (wild) type.

The obtained data were processed using Microsoft Excel and STATISTICA-6 software. The significance of differences in quantitative indicators (n>12) was determined using the Wilcoxon signed-rank test for unrelated ranges. The nonparametric Fisher component randomization test for independent samples was used to determine the significance of small samples (n<12). The Fisher-Irvine exact test was used for qualitative values. Differences between groups were considered

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statistically significant at P<0.05, correlation analysis was carried out using the nonparametric Spearman rank correlation method.

RESEARCH RESULTS. The next step of our research was the analysis of the obtained results of IHC studies.

We aimed to obtain reliable threshold values for both p53,and for the mitotic index. In addition, we analyzed the influence of all individual parameters (invasiveness, Ki67 index, p53) on the selectivity of adenoma subtype differentiation.

Table 1 provides the immunohistochemical characteristics of the study groups.

Table 1
Immunohistochemical characteristics of the study groups

Diagnosis of the disease	**Group 1 – patients with macro NFPA – 10 persons**	**Group 2 – patients with giant NFPA – 10 persons.**
Zero cell	8 (80%)	8 (80%)
P53		
>/3+	4 (40%)	5 (50%)
Ki 67		
>/2+	5 (50%)	6 (60%)
Clinicopathological classification		
1A	1 (10%)	1 (10%)
1B	1 (10%)	1 (10%)
2A	3(30%)	2 (20%)
2B	5 (50%)	6 (60%)

Table 2 shows the statistical values of the main IHC indicators in group 1 of patients with macroadenomas.

Table 2

Statistical values of the main IHC indicators in group 1, n=10.

Indicators	**PZ**	**Ch**	**Sp**	**Index Juden**	**CI accuracy in %**	**OSH,**	**DI**	**r**
Ki-67 positive nuclei, %	≥ 4	0.91	0.95	0.89	95	4.8	3.7-6.9	<0.001
Positive nuclei p-53, %	≥2	0.87	0.92	0.87	93	3.9	1.8-4.5	<0.001
invasiveness	There is	0.86	0.53	0.45	66	4.3	3.9-6.2	<0.001

Note: PV – threshold values, p – significance criterion, S – sensitivity, Sp – specificity, R – correlation with invasiveness, OR – odds ratio, CI – confidence interval, PV – threshold value

Table 3 shows the statistical values of the main IHC parameters in group 2 of patients with giant NFPA s.

Table 3
Statistical values of the main IHC indicators in group 2, n=10.

Indicators	**PZ**	**Ch**	**Sp**	**Index Juden**	**CI accuracy in %**	**OSH,**	**DI**	**r**
Ki-67 positive nuclei, %	≥ 3	0.93	0.90	0.84	93	4.5	3.9-6.5	<0.001
Positive nuclei p-53, %	≥2	0.89	0.88	0.92	96	3.7	1.9-4.8	<0.001

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invasiveness	There is	0.88	0.56	0.50	68	4.8	3.6-5.9	<0.001

Note: TS – threshold values, p – significance criterion, S – sensitivity, SP – specificity, R – correlation with invasiveness, OR – odds ratio, CI – confidence interval,

As can be seen from Tables 2 and 3, the very high specificity value (95%/93%) proves that p53 protein expression ≥2%/≥2% (Youden index 0.87/0.92) is an extremely useful and important parameter. However, even a completely negative staining result does not exclude the possibility of aggressive/invasive tumor growth, as indicated by the relatively low sensitivity.[1].

Next, we performed a correlation analysis of the relationship between MRI and immunohistochemistry indicators. Maximum tumor diameter was associated with stronger immunostaining for Ki-67 (p = 0.009), but no significant association was found for p53 (p = 0.062). Parasellar invasion was present in more than 80% of cases; however, invasion was not associated with proliferative markers.

In conclusion, our results showed that in all patients with giant pituitary adenomas, the absence of parasellar invasion was associated with a higher rate of tumor stability after treatment (p = 0.0389; Pearson residual = +3). However, parasellar invasion was not associated with the outcomes of tumor regrowth/recurrence and cure/shrinkage. Infrasellar invasion and suprasellar extension were not considered good prognostic markers of clinical outcome. However, there was a tendency to associate the absence of extension into the third ventricle with a higher likelihood of tumor stability after treatment. Proliferative tumors, but mainly those classified as grade 2B (invasive-proliferative), showed a significant association with the rate of tumor regrowth/recurrence (p = 0.0127), confirming that these lesions should be considered as highly suspicious for neoplastic proliferation.

Thus, it can be said that the presence of aggressive NFPA increases by 8.2 times (p < 0.001) when an invasive growth pattern is present (sensitivity 86%, specificity 53%, Youden index 0.45, accuracy 66%). The coefficient is 5.2 (p < 0.001) per percentage point of Ki67-positive tumor cell nuclei, and 3.1 (p < 0.001) for each percentage point of p53-immunopositive nuclei.

In all patients with giant pituitary adenomas, the absence of parasellar invasion was found to be associated with a higher rate of tumor stability after treatment (p = 0.0389; Pearson residual = +3).It has been established that the greatest value for prognosticating tumor recurrence in NFPA are the Ki-67 labeling index ≥4% (OR = 3.67), brain invasion (3.34), suprasellar invasion (OR = 3.24), and disease duration (3.18). Genetic predisposition plays a significant role in the development of tumor recurrence. The frequency of the hereditary factor in NAG is 78.78% (OR = 2.51).

And finally, based on the completed studies of risk factors, we carried outMathematical prediction of the risk of recurrence of NAG growth in the postoperative period.

This complex of assessed factors included: patient's gender, age, hereditary predisposition to oncology, presence of bad habits, duration of the disease, brain invasion, suprasellar invasion, retrosellar invasion, parasellar invasion, p53 labeling index ≥2%; Ki-67 labeling index ≥4%; bitemporal hemianopsia, panhypopituitarism (deficiency of more than 3 tropic hormones), tumor size more than 3 cm, brainstem symptoms, amaurosis, etc.

The analysis showed that the most valuable predictors of tumor recurrence in NFPA are a Ki-67 labeling index ≥4% (OR = 3.67), brain invasion (OR = 3.34), suprasellar invasion (OR = 3.24), and disease duration (OR = 2.95). Genetic predisposition plays a significant role in the development of tumor recurrence. The frequency of the hereditary factor in NAG is 78.78% (OR = 2.51).

Based on a combination of possible factors, calculations, and the table, we determined potential risk limits. In this case, the risk limits range from 19.76 to 44.59.

Thus, we calculated the probable risk limits for recurrence of NFPA growth (19.76 - 44.59), as well as the borderline values. For this purpose, we divided the risk limits into three levels: low (up to 19.76), moderate (Caution) - (20.0-44.59), and high (45.0 and above) probability of risk of development. recurrence of pituitary tumor growth.

By using this technique, it is possible to optimize treatment and preventive measures to reduce the incidence of tumor recurrence in patients with macro and giant NAGs in the postoperative period.

Thus, the IHC studies showed that the most reliable individual marker for differentiating macro NFPA and giant NFPA was the Ki-67 labeling index ≥ 4% (CI: 0.98).Tumors with immunoexpression of at least 2 markers were observed in 54% of the cohort

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CONCLUSIONS.1. We identified significant cutoff values for p53 (≥2%; CI: 0.94). The most reliable individual marker for differentiating macro NFPA from giant NFPA was the Ki-67 labeling index ≥ 4% (CI: 0.98).
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