Streszczenie w języku angielskim.

Introduction.

Pituitary tumors are quite common tumors of the central nervous system. The most common tumors in this area are pituitary adenomas and, rarely, pituitary cancers. Non-functioning adenomas are one type of pituitary adenoma that do not produce hormones. The incidence of pituitary adenomas is estimated at approximately 15% of all intracranial tumors (the third most frequent tumor, except meningiomas and gliomas), of which approximately 25% are non-secreting adenomas, mostly asymptomatic, and detected by accident (incidentalomas) by CT or MRI.

Pituitary adenomas are benign lesions and rarely transform into a malignant form (pituitary carcinomas). Many different factors are involved in the formation of pituitary adenomas, such as: inactivation of suppressor genes (MEN1) and activation of proto-oncogenes (PTTG).

Also important role play activation of growth factors (FGF4, TGFα and β) controlling the expression of many other genes and factors regulating the cell cycle (p21, cyclin D1).

In many types of cancer, the mechanisms of programmed cell death-apoptosis play an important role in the process of their formation. Both pro-apoptotic proteins (Bax, Bak) and anti-apoptotic proteins (BCL2, BCL XL) take part in the regulation of apoptosis.

Cells of many types of cancer show resistance to apoptotic signals sent by the immune system and produced by stress factors. Defects in the induction of apoptosis may concern various genes involved in the regulation and the course of apoptosis itself.

In response to various factors, e.g. hypoxia, DNA damage, oncogenes, the p53 protein is post-translational modification; with the help of regulatory proteins such as HIF1a. This leads to a change in the affinity of the p53 protein for various promoters, which translates into further activation of specific genes encoding proteins in response to stress. The basic protein involved in p53-dependent apoptosis is the protein Bax. The end effect of p53 activity depends on many factors, e.g. the type of stress and is cell-specific. The KI-67 protein plays an important role in the process of cell proliferation.

KI -67 overexpression is common in many types of cancer, including head and neck cancers and primary tumors of the central nervous system. Hypoxia plays an important role in the formation and progression of many types of cancer. Hypoxia-induced factor (HIF1a) is an important regulator of expression of many genes in response to hypoxia, including genes that regulate angiogenesis and factors responsible for proliferation and cell survival.

One of the main regulators of angiogenesis is vascular growth factor (VEGF), which recruits

epithelial cells into hypoxic vascular regions and stimulates their proliferation, leading to the progression of many types of cancer, including lung cancers and CNS glial tumors.
There are a number of reports in the literature on the expression of apoptotic factors, such as activation of the Fas receptor or caspase 3.
Ki-67 protein expression is related to the biological malignancy of pituitary tumors. Many reports show that the evaluation of p53, Ki-67, HIF1a and VEGF protein expression may be an important diagnostic marker for the identification of potentially malignant pituitary tumors.

Study aims.

The aim of the study is to evaluate the expression of selected apoptosis proteins, p53, KI-67, as well as HIF-1alpha and VEGF in non-secretory adenomas and pituitary carcinomas, and to correlate the obtained results with selected clinical data such as: patient's age, sex, tumor size and the histological characteristic of the lesion. (including the presence of so-called retrograde changes).

Material and methods.

The study included 62 patients diagnosed with pituitary neoplasms of the type of non-functionaly pituitary adenomas (43 patients), atypical adenomas (2 patients) and 1 neuroendocrine carcinoma of the pituitary gland. The age of the patients ranged from 21 to 83 years.
The formalin-fixed material was routinely embedded in paraffin blocks, and then preparations were stained with the H + E method and the immunohistochemical method with the use of appropriate antibodies against P53, Bcl-2, Bax, Ki-67, HIF-1α, VEGF proteins. The preparations were then assessed under a light microscope and an appropriate scale was used to evaluate the expression of the tested proteins (quantitative scale depending on the percentage of cells expressing the tested protein). The obtained results were correlated with clinical data such as age, sex, tumor size and the presence of retrograde lesions. The obtained results were analyzed statistically.
The control group consists of 10 pituitary glands collected during the autopsy.

Results.

The evaluation of the obtained results shows the expression of the antiapoptotic protein Bcl-2 was observed in 61 cases. In 26 cases, expression of the Bcl-2 protein was observed in over 50% of the neoplastic cells, especially in pituitary adenomas with retrograde changes and

in atypical pituitary adenomas. In pituitary neuroendocrine carcinomas, Bcl-2 expression was significantly expressed in almost all neoplastic cells (approx. 90% of cells). No statistically significant correlation was found between the expression of Bcl-2 protein and the histopathology of pituitary tumors ($p = 0.59$).

In the pro-apoptotic protein Bax, expression was observed in 29 cases, mainly in pituitary adenomas without retrograde changes. A statistically significant correlation was observed between Bax protein expression and the morphology of pituitary adenomas ($p = 0.004$). In the group of pituitary adenomas with retrograde changes, Bax protein expression was much less expressed than in adenomas without retrograde changes.

Low expression of p53 protein was observed in 50 cases (in 1% -2% of cells) of pituitary adenoma, in 13 cases p53 expression was observed in 5% or more (10%) of tested cells. Expression of p53 protein was higher in pituitary adenomas with retrograde changes and in atypical adenomas, also in neuroendocrine carcinoma of the pituitary gland. No statistically significant correlation was found between p53 protein expression and the morphology of pituitary adenomas ($p = 0.37$).

The expression of the Ki-67 protein was low, in 51 cases it was 1% -2%, in 8 cases 5%, and in 4 cases more than 10% of the cells expressed Ki-67. No statistically significant correlation was found between the expression of Ki-67 and the tumor histopathology in the group of adenomas. However, higher expression was significant for atypical adenomas and pituitary neuroendocrine carcinoma ($p = 0.27$).

High expression of HIF1-alpha was observed in 59 cases, in 11 cases expression was observed in more than 50% of tumor cells, including atypical adenomas and pituitary neuroendocrine carcinomas. There was a statistically significant correlation between the expression of the HIF-1 factor and the morphology of the examined lesions ($p = 0.007$).

Strong expression of VEGF was achieved in 62 cases, in 9 cases the expression was greater than 50% of neoplastic cells, especially in atypical adenomas and pituitary neuroendocrine carcinomas.

In adenomas without retrograde changes, VEGF expression in more than 50% of cells was observed in 4.6% of cases, in adenomas with retrograde changes, strong VEGF expression was observed in 31.3% of cases ($p = 0.005$).

Moreover, a correlation was demonstrated between the expression of the studied proteins, especially apoptosis and cell proliferation, and the age of the patients and retrograde changes (mainly glazing and fibrosis) of the examined lesion.

Conclusions.

The study confirmed the dependence of Ki-67 expression on the biological aggressiveness of non-functional pituitary adenomas. P53 protein expression is higher in invasive adenomas and in pituitary neuroendocrine carcinomas, and in pituitary adenomas with retrograde lesions.

Bcl-2 protein expression is higher in invasive adenomas and in pituitary neuroendocrine carcinomas, and in pituitary adenomas with retrograde lesions, while Bax expression was more significant in non-retrograde adenomas.

HIF-1a protein expression was more significant in invasive adenomas and pituitary neuroendocrine carcinomas, as was VEGF expression, however VEGF expression was more significant in retrograde adenomas.

The presence of retrograde changes in pituitary adenomas may be related to impaired apoptotic processes and the intensification of the expression of hypoxia and angiogenic factors, however, it is not related to the progression to more aggressive changes (low Ki-67 expression and no atypical division figures).