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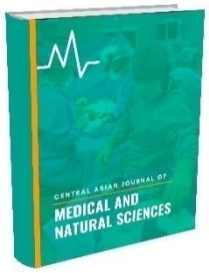

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Immunohistochemical Characteristics of Low-Risk (Grade-II) and High-Risk (Grade-III) Astrocytomas

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Abstract: An immunohistochemical study of astrocytomas of neuroepithelial tumors, which differ from each other in terms of their degree of malignancy, was conducted using the following monoclonal antibodies: VGFR (vascular endothelial growth factor), Ki67 (proliferation marker), Bcl 2 (antiapoptotic factor), p53- (suppressor protein) and glial. immunohistochemical characteristics were analyzed in astrocytomas.

Key words: glioma, astrocytoma, proliferative activity (Ki-67), vascular endothelial growth factor (VGFR), antiapoptotic factor (Bcl 2), p53 protein, immunohistochemistry.

The International Agency for Research on Cancer (IARC) estimates that one in five people worldwide will develop cancer in their lifetime, and 1 in 8 men and 1 in 11 women will die from cancer. In particular, the mortality rate for tumors of the Central Nervous System (CNS) was 1.6-2.5% (Hyun Sung, Jacques Ferlay... 2021). Gliomas (neuroectodermal, neuroepithelial tumors of the brain) are among the primary tumors of the CNS. differs from neuroglia in the development of the central nervous system. Originally, the term "glioma" was proposed by R. Vyrkhov 150 years ago. Among the tumors in which MNT occurs - gliomas make up the main share, accounting for approximately 75-80% of all brain tumors. According to the classification of WHO MNT tumors (2007; 2016), gliomas are classified into 4 levels of dangerousness (Grade) using traditional research methods, staining with hematoxylin and eosin dyes and immunohistochemical reactions. The following criteria are used to determine the degree of danger of gliomas: cellular and nuclear polymorphism, the number of mitotic cells, proliferation of vascular endothelium, and foci of necrosis. Having 2 or more of the above criteria indicates that glial tumors are very dangerous. Taking into account that the share of astrocytomas in the glial line is more than 60%, among them, astrocytomas of the level of danger (Grade) I-II-III - diffuse astrocytoma (fibrillary astrocytoma, protoplasmic astrocytoma, hemistocytic astrocytoma), anaplastic astrocytomas differ from each other by their morphological characteristics stands The most important morphological features in astrocytomas are the following: Elongated glial cells and bundles of glial fibers of various sizes, eosinophilic droplets or Rosenthal fibers due to degenerative processes, some astrocytomas with mucoid discharge, monomorphic and bipolar cells in

myxoma. and an angiocentric location in the form of a pseudo-rosette around the blood vessel is considered characteristic. The cytoplasm of some cells is observed in cases where the eosinophilic stained nucleus is located eccentrically in the cytoplasm. While the morphological picture of anaplastic astrocytoma is reminiscent of fibrillary or protoplasmic astrocytoma, unlike them - in the state of dark clusters of atypical cells and hyperchromicity of their nuclei, chromatin dispersion is expressed by different sizes, irregular shapes, and an abundance of glial fibers. In some cases, it is possible to see that the cytoplasm is dim, the proportion of the cytoplasmic nucleus is shifted towards the nucleus, and in some cells, the nucleus occupies the entire cell. These mentioned morphological scenes repeat each other and immunohistochemical methods have been widely used in recent years as a solution for evaluating tumors histobiologically. Studies on the immunohistochemical properties of gliomas are consistently conducted by different authors, in particular, in the evaluation of immunohistochemical reactions of astrocytomas, it has been shown that the use of two or more antibodies can predict the prognosis of this tumor. Ki-67, a marker of proliferative activity used in astrocytomas, has been shown to be more intense in low-risk astrocytomas than in high-risk astrocytomas, suggesting that this is independent of mitotic activity. This causes difficulties in the interpretation of immunohistochemical reactions. Among safe and dangerous astrocytomas according to the WHO (2016) classification, proliferative activity Ki-67 - less than 1% - more than 5% is given (Louis D.N., 2016). In astrocytomas, depending on the degree of danger, the intensity of the Ki-67 marker is from 1% to 10%, R53 and VGFR are from low to high intensity, and the expression of antiapoptotic factor (Bcl 2) is more than 48%. Compared to diffuse astrocytomas, it was observed that in hemistocytic astrocytomas, the amount of R53 shows intensity in a ratio of 53% to 64-88% (Zrelov A.A. 2019). From the above results, it was determined that the use of 2 or more antibodies (markers) in the immunohistochemical analysis of astrocytic tumors is appropriate for the purpose and the level of danger and importance for prognostic analyses.

Research object and methods:

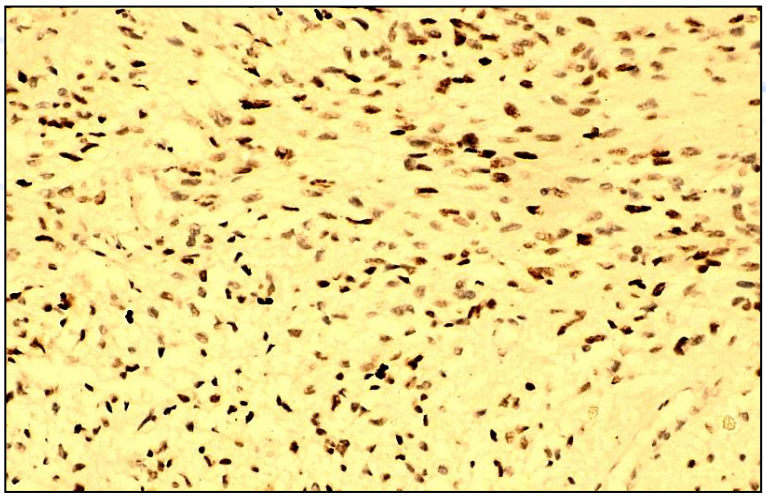
150 patients with different risk levels of astrocytic tumors (diffuse astrocytoma (fibrillary, protoplasmic, hemistocytic), anaplastic astrocytoma) who underwent surgery between 2016 and 2020 at the Republican Neurosurgery Center were randomly selected, their histological results were analyzed, and the tumor of 40 patients tissues were studied from the point of view of immunohistochemistry, in which astrocytomas with malignancy grade II (G) (n=20) and malignancy grade III (G) (n=20) were analyzed.

Methodology of immunohistochemical research: Sections of 5-7 μm thickness were taken from prepared paraffin blocks on a microtome (Leica, Germany), polylysinated objects were mounted on slides and dried on a heat table at 37°C for 10 hours. After deparaffinization and dehydration, the sections were unmasked, demasking in water with a temperature of 98°C for 30-40 minutes, washing solution in Tris buffer (pH=7.5) for 5 minutes, 3% aqueous solution of hydrogen peroxide to block endogenous peroxidase -5 minutes, washing 3 minutes with distilled water, incubation of primary (primary) antibodies, specific antibodies 20-30 minutes, washing in tris-buffer solution (pH =7.5), 5 minutes detection and incubation of primary (primary) antibodies visually 20-30 minutes, washing in tris- buffer solution (ph = 7.5) 5 minutes, detection with diamine benzidine, DAB-chromogen 5 minutes, washing with distilled water for 3 minutes, staining with hematoxylin Mayer for 5 minutes, washing in running water for 1 minute, dehydration Alcohol 96% 2 times for 5 minutes, dealcoholization Ortho- xylol 2 layers for 5 minutes, balsam, coverslip and the window was closed and conclusions were given. For immunohistochemical examination, Bond Leica Australia (Australia) immunohistoprocessor was used to study the expression of cells by means of VGFR, Ki67, Bcl 2 and p53 monoclonal antibodies. In this case, the light was counted in 10 fields of view of the markers (nucleus, cytoplasm, and cell membrane) stained and located in the tumor cell in the lenses of 100,

200, 400 times magnification in a Leica (Germany) microscope. The proliferative activity Ki-67 is taken into account as a percentage, at 0%, 0-4%, 4-10% and above 10%, the intensity of the remaining antibodies in the tumor cell according to the results of IGX and the percentage of stained cells are "signs" It was visually evaluated in points using the "evaluation" method. About 200 cells were counted per case. The obtained results are evaluated in the form of mild, moderate and severe positive reactions.

Obtained results and their discussion: Proliferative activity (Ki-67) in the tissue of diffuse astrocytomas - hyperplasia and cell polymorphism of astrocytes, a small number of pathological mitoses, and in some foci expressing cystic spaces and a changed reticular landscape, the nuclei of which have a small amount of dark brown color. 15 (75%) of 20 patients with diffuse astrocytoma (Grade-2) had a mild positive reaction, with average cell intensity of 3-4% in the total field of view. In 5 (25%) patients, a moderate positive reaction, an average intensity of 7-8% of cells in the total field of vision was determined, a high positive reaction was not observed in diffuse astrocytomas. Proliferative activity (Ki-67) was shown by hyperplasia and cell polymorphism of astrocytes in the tissue of anaplastic astrocytoma (Grade-3) and pronounced atypia, a large number of pathological mitoses and hyperplasia of vascular endothelium, and the intensity of dark brown staining of nuclei in tumor cells, proliferative activity level was shown as follows in anaplastic astrocytomas (table No. 1). In this case, 2 (10%) patients showed a mild positive reaction, an average of 2-3% cell intensity in the general visual field. 5 (25%) patients had a moderate positive reaction, with an average of 7-8% cell intensity in the total field of vision, and 13 (65%) patients had a high positive reaction, with an average of 9-10% cell intensity in the total field of vision (Fig. 1).

Picture 1



In anaplastic astrocytoma (G-III), there is a high-grade positive reaction of the Ki67 marker, an average intensity of cells above 10% in the total visual field.

IGX – Dab Chromagen. Ob10. Ok10.

Table -1.

№	Cell staining intensity in %	Patients (n =20)
1	2-3% mild positive reaction	2 (10%)
2	7-8% moderate positive reaction	5 (25%)
3	9-10% high positive reaction	13(65%)

Proliferative activity level of Ki 67 antibody in anaplastic astrocytoma (n= 20)

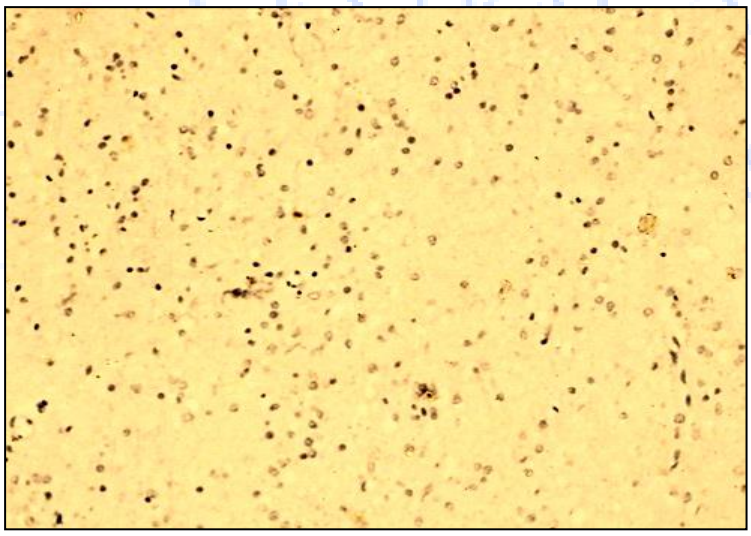
(Bcl 2) - antiapoptotic factor was detected in 10 (50%) of diffuse astrocytomas (Grade-2) with a low-grade positive reaction, 5 (25%) with an intermediate-grade positive reaction, and 5 (25%) with a negative reaction. 3 (15%) of anaplastic astrocytomas (Grade-3) had a low-grade positive reaction (1 point), 5 (25%) had an intermediate-grade positive reaction, and 12 (60%) had a high-grade positive reaction.

High-grade positive reaction of Bcl 2 marker in anaplastic astrocytoma. Cells with an intensity greater than 30% can be seen in the field of view.

IGX – Dab Chromagen. Ob10. Ok10.

p53 protein in diffuse astrocytomas (Grade-2) in 10 (50%) patients with a mild positive reaction, in 2 (10%) patients with a moderate positive reaction, an average of 6-7% cell intensity in the total visual field (Fig. No. 3) and 8 (40%) patients had a negative reaction. Anaplastic astrocytomas (Grade-3) showed mild positive reaction in 2 (10%) patients, moderate positive reaction in 4 (20%) patients, average cell intensity of 4-5% in the total visual field, and high positive reaction in 14 (70%) patients. , an average of 20-25% cell intensity was observed in the total field of view. VGFR (vascular endothelial growth factor) diffuse astrocytomas (Grade-2) showed mild positive reaction in 15 patients (75%), moderate positive reaction in 3 patients (15%) and high positive reaction in 2 patients (10%). In anaplastic astrocytoma (Grade-3), mild positive reaction was observed in 3 patients (15%), moderate positive reaction in 5 patients (25%), high positive reaction in 12 patients (60%).

Picture 2



In diffuse astrocytoma (G-II), there is an average positive reaction of the p53 marker, an average intensity of 6-7% of cells in the total visual field. IGX – Dab Chromagen. Ob10. Ok10.

Conclusion: Nowadays, along with traditional research methods, immunohistochemical methods are widely used in the diagnosis of tumor processes. including in the diagnosis of neuroglial tumors. Based on the above researches, if we take into account that in diffuse astrocytomas, proliferative activity is manifested with a mild positive reaction, it is possible to predict slow growth of this type of tumors. The average intensity of proliferative activity (Ki - 67) in the core of anaplastic astrocytomas indicates danger, while high intensity can lead to its aggressive course and transformation. In astrocytomas with a high level of danger (Grade-3), the presence of high positivity and apoptosis of proliferative active cells, in turn, is the basis for the use of a combined type of treatment. In almost all astrocytic tumors p53 protein was expressed in immunohistochemical reactions. This indicates that there are point mutations of the p53 gene in astrocytomas. In anaplastic astrocytomas, vascular

endothelial growth factor (VGFR) positivity of medium and high level occupies the main share, due to the fact that neoangiogenesis is progressing well in this type of tumor, relapse and regrowth can be predicted.

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