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VEGF-A and HGF Level in Patients with Brain Tumors Depending on Tumor Location

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Abstract

VEG-A and HGF are angiogenic factors that stimulateblood vessels formationin cancerous tumors and primary brain tumors: gliomas and meningiomas. Their higher activity has been demonstrated for each tumor. The aim of our study was to calculatewhether the angiogenetic activity expressed by the concentration of VEGF-A and HGF differs depending on the location of the brain tumor. In this respect, tumors of the posterior cranial fossa and other location were compared. The study group consisted of 63 patients qualified for surgical treatment in the neurosurgical department. The non-parametric Kruskal-Wallis tests were used in the statistical analysis. It was shown that the concentration of the parameters studied was lower in posteriorfossa tumorscompared to supratentorial and other tumors. This allowed to draw the conclusion thatHGF and VEGF-A plasma levels are related to location of intracranial tumors. Higher concentrations of both factors occur in supratentorial tumors than in posterior fossa tumors. This may mean that angiogenic and mitogenic activity is significantly higher in patients with tumors located in the hemispheres of the brain or in connection with supratentorial dura mater.

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Introduction

Angiogenesis plays an important role in cancer development. It is regulated by biochemical factors that stimulate or inhibit the formation of blood vessels. They are secreted by both cancer cells andimmune system[1]. Theseangiogenesis factors mainly affect the endothelial cells of the vessels in the immediate vicinity of the tumor[1,2].

Optimal neoplastic angiogenesis is possible due to the balance of pro-angiogenic and anti-angiogenic factors[1,2]. The development of neoplastic disease depends on excessive pathological activation of pro-angiogenic factors. Increased activity of various angiogenesis factors has been demonstrated in many neoplastic diseases. In the case of brain tumors, approximately 25 biochemical factors have been identified. Vascular endothelial growth factor A (VEGF-A) plays the most important role, but other factors stimulating the formation of new blood vessels are also known, such as hepatocyte growth factor (HGF)[2,3].

VEGF-A belongs to the family of platelet growth factors. In addition to VEGF-A, this family also includes VEGF-B, VEGF-C, and VEGF-D factors[3,4]. Other factors such as VEGF-E and VEGF F occur in animals, their possible role in the human body is not yet known. Since the form of VEGF-A plays the most important role in the process of angiogenesis and it has the greatest biological significance, it is simply referred to in the literature as the VEGF[2,3]. VEGF is considered to be the most important factor stimulating both the physiological and pathological angiogenesis. It plays a key role in fetal development, during the ovulatory cycle and in the process of wound healing[3]. In pathology, it stimulates the formation of vessels inneoplastic tumors, but is also responsible for vascular neoplasm in diabetic retinopathy, rheumatoid arthritis and psoriasis. VEGF stimulates the synthesis of nitric oxide in endothelial cells, and also acts as a factor extending the life span of these cells and preventing their apoptosis[4,5]. It also stimulates the migration of monocytes and granulocytes and their hematopoiesis by creating colonies of their progenitor cells.VEGF secretion is stimulated by growth factors such as EGF, TGF-α, TGF-β, IFG-1, PDGF, and by inflammatory cytokines such as IL-1a and IL-6[5]. These proteins lead to an increase in the expression of VEGF mRNA and VEGF itself in the cell. VEGF synthesis is also influenced by the concentration of cyclooxygenase-2[5,6]. A very strong factor that stimulates the secretion of VEGF is hypoxia within the growing tumor and the synthesis of the hypoxia induced factor. This is the mechanism that characterizes rapidly growing highgrade gliomas (HGG)[6].

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HGFis a protein that exhibits strong mitogenic properties and regulates the processes of tissue development and regeneration[7]. It is produced by all cells of mesenchymal origin. It is characterized by the ability to disperse colonies of endothelial cells and hepatocytes, hence it has also been called the "scatter factor" (SF)[7,8]. HGF is a heterodimeric protein composed of 728 amino acids and consisting of a 69 kDa heavy α chain and a 34 kDa light β chain linked by a disulfide bond8.HGF binds toc-Met membrane receptor with tyrosine kinase activity[8]. The c-Met receptor protein is encoded by the proto-oncogene of the same name, overexpression of which has been shown in many neoplastic diseases[7,8,9]. Binding of HGF to c-Met activates metabolic pathways, the consequence of which is an increase in the synthesis of metalloproteinase-1 and stromelysin-1 responsible for the degradation of the basal membrane of the endothelium and the extracellular matrix8. HGF exhibits angiogenic properties by promoting the degradation of the endothelial membrane, but also by activating tumor cells to secrete other growth factors[7,9]. HGF inhibits the apoptosis of cancer cells[8,9]. It has also been shown to prevent damage to the DNA of breast cancer cells and high-grade gliomas[7,9]. By stimulating proliferation and angiogenesis, HGF promotes tumor metastasis[9]. Elevated levels of HGF have been demonstrated in the plasma of patients with metastases of breast cancer[9,10]. Many reports in the literature prove the great importance of HGF in the pathomechanism of neoplasms, including intracranial neoplasms[10].

Material and methods

The study included 63 patients with braintumors treated surgically. The group consisted of 39 males (mean age 62.11) and 24 females (mean age69.34).The age distribution ofpatients was statistically normal.The research was approved by the Bioethics Committee of our university No. KB 665/2009. All participants of the study got acquainted with the information about it, understood the idea of the study and signed an informed consent to participate in it.

The study excluded patients with disseminated neoplastic. Patients with concomitant disturbances of consciousness due to high intracranial pressure that prevented consent to the examination were also excluded.In the study group, a blood sample was collectedbeforethe surgery. Due to the fact that the operations were performed in the morning, measurements were also made in the morning in fasting patients.Venous blood was collected by direct puncture of the cephalic vein, median elbow or median forearm after skin disinfection with Kodan® Tinktur Forte bactericidal preparation, using the Greiner Bio-One Vacuette® blood collection system. Venous blood was collected in two plastic tubes with 3.2% sodium citrate

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and one with EDTA 1:10 - 1 part of anticoagulant and 9 parts of blood in the tube. The concentrations of individual factors were determined using reagent kitsfrom R&D and Bender MedSystems.

The following concentrationswere determined inplasma:

1. VEGF-A using Bender MedSystems Human VEGF reagent, with no manufacturer provided reference values for plasma VEGF-A concentration.
2. HGFusing R&D Human HGF reagent, reference values 251 - 742 pg / dL

The ELISA method wasused. In the study group, the examined parameters were analyzed in terms of tumor localization. The patients had tumors of the cerebral hemispheres - supratentorial (n = 27), tumors of the posterior cranial cavity (n = 20) and tumors of other different location (n = 16): suprasellar tumors,skull base tumors with extracranial penetration (named asother location in our study).The types of tumors in our study were gliomas, meningiomas, metastases, neuroblastomas, pituitary adenomas, lymphomas, sub-ependymomas, and chordomas. As, based on our results from previous studies, we did not find significant differences in the concentration of VEGF-A and HGF between different types of tumors, this parameter was not taken into account in the present study, and the distribution of histological types in individual tumor locations was not analyzed.The statistical analysis was performed with the use of the statistical program STATISTICA 8.0 by StatSoft®. The distribution of the analyzed parameters differs from the normal distribution, therefore the non-parametric Kruskal-Wallis test was used in the statistical analysis. The significance level $p < 0.05$ was considered statistically significant. The positional mean was used to describe the variability - the median (Me) and quartiles (lower Q1 and upper Q3).

Results

The results showed that the plasma concentration of VEGF-A and HGF in patients with intracranial tumors is related to tumor localization. It has been observed that patients withcerebral hemispheres tumors had significantly higher VEGF-A plasma concentrationsthanpatients with posterior fossa tumors (Kruskal-Wallis test *1 vs 2* using *post hoc* test $p = 0.0167$). Significantly higher plasma concentration of HGF was also noted in patients with cerebral hemispheres tumorscompared to patients with posterior fossa tumors. A significantly lower HGF plasma concentrationwas also observed in patients with posterior fossa tumors than in patients with tumors of other location (Kruskal-Wallis test *1 vs 2* $p = 0.0167$, *2 vs 3* $p = 0.0253$). These results are presented in Table 1 and additionallyin Figures 1 - 2.

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Table 1. Studied parameters depending on brain tumor location

analyzed parameter	descriptive statistics	Tumor location			*p*
		Cerebral hemispheres n = 27	Posterior fossa n = 20	Other location n = 16	
VEGF-A [pg/ml]	**Q1**	**25,85**	**5,40**	**0,00**	
	Me	**28,15**	**17,90**	**24,84**	**0,0676[1vs2]**
	Q3	**36,03**	**23,30**	**25,50**	
HGF [pg/ml]	**Q1**	**466,67**	**197,62**	**644,14**	
	Me	**557,74**	**325,00**	**697,84**	**0,0167[1vs2]**
	Q3	**699,86**	**369,44**	**730,22**	**0,0253[2vs3]**

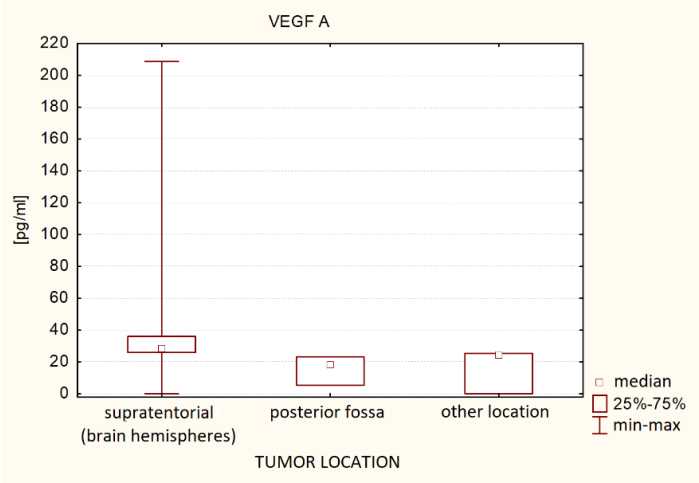

Figure 1. Preoperative plasma VEGF-A concentration in patients depending on brain tumor location

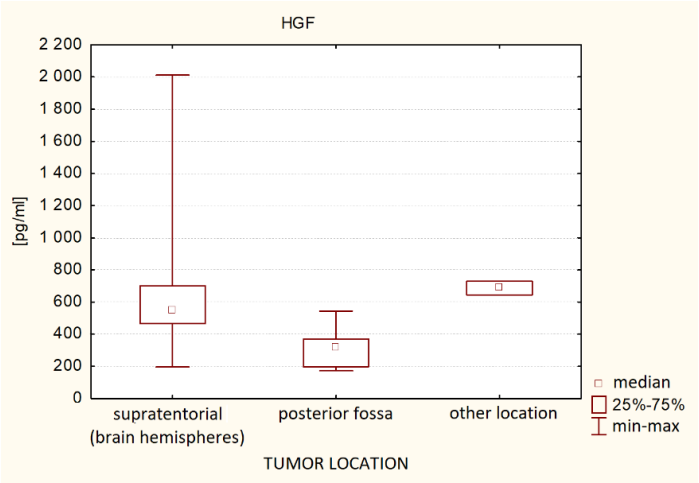

Figure 2. Preoperative plasma HGF concentration in patients depending on brain tumor location

Discussion

Our study hasshown that VEGF-A andHGF plasma concentrationis significantly lower in patientsposterior cranial fossatumors compared to patients with supratentorial tumors and

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tumors of other locations. There are no similar reports in the available literature. We found neither brain tumors nor cancers of other parts of the body. One report about this subject is the publication of Maemura et al. (1998) analyzing HGF serum concentration in patients with breast cancer metastases[11]. Authors examined 34 patients with metastatic breast cancer. Maemura et al. showed a general relationship between HGF concentration and the location of the metastatic site, although serum HGF concentrations were slightly higher in patients with liver metastases[11]. Additionally, Maemura et al. showed that in patients with multiple sites of metastasis, the level of HGF was higher than in patients with other lesions, which, however, results from the severity of the disease and not from the location[11]. The publication of Maemura et al. is the only literature data found on the potential relationship between the factors of angiogenesis and tumor localization[11]. This encouraged us to research this topic and look for such an association in tumors of the brain, brainstem, cerebellum and skull base.

Moreover, our study has shown that angiogenetic activity in the space ismore significant supratentorialthan in posterior fossa. There are no reports in the available literature on the potential differences in the vascularization of anatomical structures in the cranial cavity depending on whether they are located supratentorial or in the posterior fossa. The difference in dura mater vascularization between the posterior fossa and the supratentorial space is noteworthy[12]. The dura mater isvascularized mainly supratentorial bymiddle meningeal artery, while in posterior fossa the vascularization is worse[12,13,14]. However, this does not seem to translate into the results of our study, as in the case of meningiomas, the vascularization of which also originates from the meningeal arteries, no differences in angiogenesis factors were found compared to other tumors.

This makes our resultsinnovativeeven more, without previous research on this topic. It may also have important clinical implications and influence the treatment of tumors, as anti-angiogenesis drugs such as Bevacizumab[15] may prove to be more effective in treating supratentorial tumors[16,17], although the conclusions beyond the scope of this study are far-fetched. They would require confirmation by further prospective studies.

Conclusions

HGF and VEGF-A plasma levelsare related tolocation ofintracranial tumors. Higher concentrations of both factors occur in supratentorial tumorsthan in posterior fossa tumors. This may mean that angiogenic and mitogenic activity issignificantlyhigherin patients with tumors located in the hemispheres of the brain or in connection with supratentorial dura mater.

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Abbreviations

- VEGF-A - Vascular endothelial growth factor A
- HGF - hepatocyte growth factor
- HGG - high grade gliomas
- SF - scatter factor

Declarations

1.Ethics approval and consent to participate: The experiment was approved by the Bioethics Committee of the Ludwik Rydygier Collegium Medicum in Bydgoszcz (KB-665/2009). Written informed consent was obtained from the patients and control group for publication of this masuscript and any accompanying data. A copy of the written consent is available for review by the Editor of this journal.

2. Consent for publication: Written informed consent was obtained from the all participians for publication of this masuscript and any accompanying data.

3. Availability of data and materials: All relevant data are within the paper.

4. Competing Interests: The authors declare that they have no conflict of interest.

5. Funding: The study was financed from own funds of the Neurosurgery of Collegium Medicum in Bydgoszcz. The authors received no specific founding for this work.

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