Serum levels of vascular endothelial growth factor and basic fibroblast growth factor in children with brain tumors

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Abstract

Background. Angiogenesis is the process of new vessel formation originating from the existing vascular network. It plays an important role in the growth and spread of malignancies, including brain tumors. The process of angiogenesis is characterized by increased expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), and by the release of their soluble forms into circulation.

Objectives. The aim of the study was to evaluate serum levels of VEGF and bFGF in children with malignant and benign brain tumors.

Material and methods. The study group (group N) included 106 children diagnosed with brain tumors. The children in group N were classified according to tumor pathology into 3 subgroups: N1 (n = 63): patients with malignant tumors, excluding anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM); N2 (n = 25): patients with benign tumors; and N3 (n = 18): patients with high grade gliomas (AA and GBM). VEGF and bFGF were determined by ELISA in blood samples before the initiation of chemotherapy. VEGF and bFGF levels were compared within the subgroups in relation to tumor grading and the extent of surgery.

Results. The median VEGF in patients with brain tumors was significantly higher than in the control group. The median levels of VEGF and bFGF in subgroup N1 were significantly higher than in the control group. The differences in VEGF and bFGF concentrations between the subgroups in relation to the extent of tumor resection were not significant.

Conclusions. Significantly higher plasma VEGF levels in children with brain neoplasms may reflect enhanced angiogenesis in the tumors.

Key words: children, vascular endothelial growth factor, brain tumor, basic fibroblast growth factor

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Angiogenesis is the process of new vessel formation originating from the existing vascular network. It plays an important role in the growth and spread of malignant tumors. Neovascularization is stimulated by environmental factors and mediated by oncogenes and cytokines. Hypoxia is the strongest local environmental factor that induces angiogenesis. It activates the expression of vascular endothelial growth factor (VEGF), thus stimulating tumor cells to produce and release cytokines, which trigger neovascularization.¹⁻³ The activation of some oncogenes, such as KRAS, HRAS, SRC and bcl-2, is responsible not only for malignant transformation, but also for VEGF transcription, which promotes angiogenesis.4 VEGF and basic fibroblast growth factor (bFGF) are crucial for angiogenesis.5,6 Angiogenesis increased by tumors appears to be an important risk factor for the progression, recurrence and metastases of different tumors, including brain tumors. 1-3,7,8 Brain tumors constitute 35-40% of all solid tumors, and approximately 22-28% of all cancers in children.9 Despite significant progress in the treatment of brain tumors, which currently includes neurosurgery, chemotherapy and radiotherapy, the outcomes are still unsatisfactory, and therefore, research for new therapies against brain tumor continues.¹⁰ Recently, numerous researchers have focused on angiogenesis in brain tumors and the use of pro-angiogenic factor inhibitors in treating them, in children as well as adults. 9,11-19 The number of reports on angiogenesis in brain tumors in children is very limited, which indicates a need to address the topic. The aim of the present study was to evaluate serum levels of VEGF and bFGF in children diagnosed with malignant and benign brain tumors.

Material and methods

The study included 106 children (61 boys - 57.5%; and 45 girls - 42.5%), aged from 7 months to 19 years (mean 8.9 years) with brain tumors (group N). The children in group N were divided according to pathological diagnosis into the following three subgroups:

- subgroup N1 (n = 63): patients with malignant tumors, excluding anaplastic astrocytomas (AA) and glioblastomas multiforme (GBM). This subgroup included medulloblastomas (n = 30), anaplastic ependymomas (n = 11), germinomas (n = 5), pineoblastomas (n = 2), choroid plexus carcinomas (n = 2) and morphologically unidentified brain stem tumors (probably malignant gliomas, based on MRIs) (n = 13);
- subgroup N2 (n = 25): patients with benign tumors (pilocytic astrocytomas);
- subgroup N3 (n = 18): patients with high grade gliomas (AA and GBM).

Additionally, based on the extent of surgical resection, the following subgroups were identified: subgroup PR:

partial resection (n = 62); subgroup TR: total resection (n = 18); subgroup STR: subtotal resection (n = 7); subgroup B: biopsy (n = 6); and subgroup NV: not verified (n = 13).

All the patients were treated according to the Uniform Protocol for the Treatment of Brain Tumors in Children developed by the Polish Pediatric Neuro-Oncology Group.⁹ The control group (group C) consisted of 34 children aged from 2 to 17 years (mean age: 8.5 years) diagnosed with functional gastrointestinal disorder.

Serum levels of proangiogenic cytokines, VEGF and bFGF were determined in the study group before chemotherapy. All serum levels were determined using immunoenzymatic assay (ELISA) kits (R&D Systems, Minneapolis, USA).

The statistical analysis of the data obtained was performed using STATISTICA software, v. 10 (SatSoft Inc., Tulsa, USA). The statistical analysis included the Mann-Whitney U-test and Kruskal–Wallis test, followed by the Dunn post-hoc test. A p-value < 0.05 was considered statistically significant.

The study protocol was approved by the Local Bioethics Committee. Each patient's parents or carers signed informed consent for participation in the study. Consent was also obtained directly from older children.

Results

The serum VEGF and bFGF levels in the entire study group of patients with malignant and benign brain tumors (group N) and the subgroups (N1, N2 and N3) as compared to the control group (group C) were analyzed.

The median serum VEGF level was 260.9 pg/mL (range: 17.8-2000 pg/mL) in group N and 194.1 pg/mL (range: 31.4-448.1 pg/mL) in group C. The difference was statistically significant (p = 0.05; Table 1).

The median bFGF levels in group N and group C were similar: 7.64 pg/mL (range: 0.05–79.23 pg/mL) and 7.64 pg/mL (range: 2.7–48.7) respectively (Table 1).

The analysis of VEGF and bFGF within the subgroups according to tumor grading revealed statistically significant differences between the control group and subgroup N1 (p = 0.03 and p = 0.04, respectively; Table 1). The difference in VEGF levels between subgroups N2 and N3 was also statistically significant (p = 0.005; Table 1).

There were no statistically significant differences in VEGF and bFGF concentrations between the subgroups according to tumor resection (Table 2).

Discussion

Gliomas are the most common brain tumors in children, constituting over 50% of all tumor cases. These can be further divided into low grade gliomas (LGG), which

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Table 1. Serum vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) levels in children with brain tumors and in the control group. Subgroup N1: patients with malignant tumors, excluding anaplastic astrocytomas (AA) and glioblastomas multiforme (GBM); N2: patients with benign tumors; and N3: patients with high grade gliomas (AA and GBM). The data are presented as median values with 25–75 percentiles in parentheses

Study groups	VEGF (pg/mL)	bFGF (pg/mL)	
Group N (n = 106) (children with brain tumors)	260.9* (17.8–2000.0)	7.64 (0.05–79.2)	
Subgroup N1 (n = 63) (patients with malignant tumors, excluding anaplastic astrocytomas (AA) and glioblastomas multiforme)	234.9* (28.3–2000.0)	7.53* (0.05–79.2)	
Subgroup N2 (n = 25) (patients with benign tumors)	193.3** (40.1–509.4)	8.05** (0.6–25.2)	
Subgroup N3 (n = 18) (patients with high grade gliomas (AA and GBM)	179.0** (17.8–453.3)	5.92** (0.3–21.4)	
Control group (n = 34)	224.1 (31.4–448.1)	7.64 (2.1–19.5)	

^{*}p < 0.05 vs the control group; **p < 0.05 N2 vs N3.

include pilocytic astrocytomas (WHO grade I) or diffuse astrocytomas (WHO grade II), and high grade gliomas (HGG), which include anaplastic astrocytomas (WHO Grade III) and glioblastomas multiforme (WHO Grade IV). The 5-year survival rates are 80-90% in LGG, 20-40% in anaplastic astrocytoma and 5-15% in glioblastoma. 10,20 Diffused brain stem gliomas are associated with the worst prognosis. The total survival in these cases is 9 months – a truly poor treatment outcome that has remained unchanged for many decades. 9,21,22

Among all types of cancer in children, brain tumors are associated with the worst prognosis and constitute the most common cause of disease-related mortality in children.9 Contrary to the authors' assumptions, the study results did not reveal higher serum levels of VEGF and bFGF in high grade gliomas (WHO III and WHO IV). However, this can potentially be attributed to the low number of patients in the HGG subgroup. Furthermore, the serum levels of the parameters analyzed were higher in the malignant tumor subgroup than in the benign tumor subgroup; the difference, however, was not statistically significant. Additionally, higher pro-angiogenic protein levels were observed in

the entire study group as compared to the control group, but the difference was statistically significant only for VEGF. It is hard to relate these findings to the published data, as the rare publications discussing angiogenesis in brain tumors in children are mostly focused on antiangiogenic cancer therapy in relation to the use of the same agents in adult patients. Therefore, the discussion presented herein aims to explain the importance of investigating the significance of the observed elevated VEGF and bFGF levels.

Table 2. Serum vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) levels and the extent of surgery

Proangiogenic cytokines	Type of resection	Mean	SD	Median	Minimum	Maximum
VEGF pg/mL	total resection	286.73	266.40	220.65	34.90	796.80
	partial resection	192.88	288.87	128.80	14.60	2000.00
	biopsy	215.85	90.82	183.95	150.50	345.00
	not verified	209.91	134.75	185.45	28.30	424.20
	subtotal resection	427.03	506.70	121.40	82.40	1200.00
FGF pg/mL	total resection	11.08	6.09	9.57	2.88	23.18
	partial resection	6.90	7.40	5.05	0.05	47.29
	biopsy	5.34	3.45	4.74	2.15	9.73
	not verified	5.96	4.98	5.43	0.27	13.85
	subtotal resection	11.70	8.81	12.54	1.30	25.51

^{*}p < 0.05 vs the control group; **p < 0.05 N2 vs N3.

The vascular network density in WHO II gliomas resembles that of healthy cerebral tissue. It increases significantly in WHO III gliomas (AA) to achieve peak value in GBM (WHO IV). Many researchers have reported a negative correlation between the vascular network density in tumor biopsy specimens and patient survival. 23,24 In order to improve treatment outcomes in malignancies associated with the poorest prognoses, it is essential to implement new therapeutic approaches. This has led to researchers' interest in the phenomenon of angiogenesis in brain tumors, as anti-angiogenic treatment has become a hope of improving the prognosis in CNS tumors. 4,8-21,23-29 The main source of interest in tumor angiogenesis was the hypothesis presented by Judah Folkman, who assumed that since tumor growth is blood supply-dependent, blocking angiogenesis may be a strategy to inhibit tumor growth.³⁰

VEGF is the strongest factor triggering normal and pathological angiogenesis and stimulating cancer cells to proliferate. It is produced by endothelial cells, myocytes, macrophages, mastocytes, lymphocytes (CD4 cells), plasmocytes and tumor cells, whereas leukocytes and blood platelets are responsible for its transportation. The second best known factor stimulating normal and abnormal angiogenesis is bFGF, which is produced by fibroblasts, macrophages and tumor cells, while leukocytes and blood platelets are responsible for its transpor $tation. {}^{3,5,20,23,25-32}\, VEGF, whose \, expression \, increases \, with \,$ hypoxia, necrosis or p53 gene inactivation, regulates angiogenesis in high grade gliomas and presents their malignant nature. At the same time, bFGF involved in angiogenesis in gliomas shows synergism with VEGF. 19,22,33 Angiopoietins regulate angiogenesis in astrocytomas to the greatest extent, so the involvement of VEGF and bFGF appears to be less significant.³³ In meningiomas, though, VEGF overexpression is observed; however, it is not triggered by hypoxia, as in gliomas, but rather by the concomitant overexpression of platelet-derived growth factor (PDGF) or epidermal growth factor (EGF).7 The pro-angiogenic activity of VEGF in GBM is confirmed by the detection of over 50 times higher VEGF mRNA expression in tumor cells than in healthy cerebral tissue.²² Gupta et al. assessed the density of vascular network in different cases of glioma relative to their VEGF expression, demonstrating a positive correlation between the 2 factors with the highest values in HGG; this was in line with reports by Berkman et al. and Takano et al.^{23,24,30} Maiuri et al. presented interesting findings, demonstrating that in LGGs that transformed into HGGs, the baseline VEGF expression was higher than in other benign tumors, which may constitute an essential risk factor for malignant transformation.^{34,35} Additionally, Krauze et al. demonstrated a significant elevation of urine VEGF concentration in patients with GBM treated with radiotherapy (RT) for tumor progression (69.5% of 202 patients).³⁶ They suggested that regular measurements of urinary VEGF concentration in patients with GBM can facilitate prediction and may be used for monitoring remission.

There are considerably fewer reports addressing the role of bFGF in brain tumor angiogenesis. The observation by Loilome et al. that blocking the FGF signaling pathway in GBM as a monotherapy slows down tumor growth therefore appears to be particularly important.³² The same study also confirms the involvement of bFGF in stimulating tumor growth. As the role of angiogenesis in brain tumor growth is established and indisputable, attempts have been made to use anti-angiogenic agents in cancer treatment. However, these attempts have not yet led to a breakthrough in brain tumor therapy. Additionally, some researchers have reported anti-angiogenic agents as toxic.^{12,13,17,22,26}

Bevacizumab - a humanized monoclonal anti-VEGF antibody – was the first anti-angiogenic treatment to be used in high grade gliomas. Numerous trials of this antibody in glioma therapy are being carried out, and although the preliminary data appear to be promising, we still have to wait for the final conclusions. 11,13,14,17,22 Many authors, for instance Hamerlik et al., have observed that solid tumors respond better to anti-VEGF therapy, including blocking antibodies or tyrosine kinase inhibitors, when it is combined with radiation therapy or administered prior to RT, due to the radiosensitizing effect.²⁷ At the same time, along with hopes regarding anti-angiogenic therapy for brain tumors, doubts have arisen that it may paradoxically promote tumor growth, as the induced tumor necrosis with the associated hypoxia are significant triggers of pro-angiogenic factor release. 5,7,18,37-39 In 2014, an article by a Dutch research team addressing the issue of anti-angiogenic tumor therapy in pediatric brain tumors attempted to determine whether this type of therapy was effective.²⁰ It should be noted that most observations regarding angiogenesis in brain tumors are derived from studies in adult populations, whereas the number of studies addressing this issue in children is very limited. The article raised reasonable doubt regarding the enrolment of pediatric patients in clinical studies that involve the administration of anti-angiogenic agents.²⁰

Given the unsatisfactory treatment outcomes in brain tumors in children, especially HGG and infiltrative brain stem tumors, any attempt to research new therapeutic approaches or strategies matters. This is the idea the present authors had in mind when choosing to address the issue of angiogenesis in brain tumors in children. Since the findings of this study appear ambiguous, it seems appropriate to continue the research on a larger patient sample. The best option would be to conduct a multicenter study.

Conclusions

The significant VEGF level elevation in children with malignant tumors suggests its importance in brain tumor oncogenesis. Further studies with larger groups of patients should be conducted to determine the significance of the observed elevated VEGF and bFGF levels in the diagnostic process.

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