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Relationship between Vascular Endothelial Growth Factor (VEGF) with Ki-67 Labeling Index in Intracranial Astrocytoma

Edwin Batara Saragih, RR Suzy Indharty, Andre MP Siahaan

Neurosurgery Department, School of Medicine Universitas Sumatera Utara/ Haji Adam Malik General Hospital, Medan, Indonesia

Abstract : Background.Astrocytoma is a neuroepithelial tumor and divided into four grade based on its malignancy. Recently, angiogenesis was found to be an important factor for tumor growth, invasion grade, metastasis and prognosis. VEGF overexpression has been related to tumor progressivity and bad outcome in several type of tumor. Ki-67 was considered as the best marker in order to look for tumor cell proliferation. The aim of this study is to analyze relationship between VEGF expression and Ki-67 labelling index on astrocytoma patients. **Method**.Subject of this study was astrocytoma patients who had been operated between Januari 2014 and Juni 2015. VEGF and Ki-67 expression were determined using immunohistochemistry study. For statistical analysis, chi square and Spearman correlation was choosen. P value < 0.05 was considered significant statistically. **Result**: There is relationship between astrocytoma grading with mortality ($p = 0.0001$; $r = 0.727$). There is no relationship between Ki-67 labelling index with WHO grading ($p = 0.076$) and mortality ($p = 0.297$). There is no relationship between VEGF expression with WHO grading ($p = 0.106$) and mortality ($p = 0.813$). There is no relationship between VEGF expression and Ki-67 labelling index ($p = 0.508$). **Conclusion**: We concluded that VEGF expression and Ki-67 in astrocytoma patient are not related significantly.
Keywords : Astrocytoma, VEGF, Ki-67.

Introduction

Primary astrocytoma is a neuroepitelial tumor that can be divided into several degrees based on histologic characteristic. Astrocytomas are divided into four classifications grading by WHO. Angiogenesis is found to be very important in tumor growth, the degree of invasion, metastasis and prognosis. Several clinical studies have found that microvascular densities are closely associated with tumor malignancy and prognosis.[1]

VEGF, also called vascular permeability factor (VGF), is an angiogenic factor known to control cerebral angiogenesis in the early stages of embryonic development. VEGF is required to maintain blood vessels newly formed naturally in the vascularization of the retina and in a model of tumor angiogenesis.[2,3]

VEGF expression in tumor cells is stimulated by hypoxia, oncogene (race) and inactivation of tumor suppressor genes (p53) and by a variety of cytokines. Axis activation of VEGF / VEGF receptor (VEGFR) triggers multiple signaling networks that produce endothelial cell survival, mitogenesis, migration, differentiation, and vascular permeability as well as the mobilization of endothelial progenitor cells from the bone marrow into the peripheral circulation.[2,4]

Ki-67 is a monoclonal antibody IgG1 class and was first discovered by Gerdes et al in 1983. Ki-67 can recognize specific antigens on the cell nucleus that are proliferating and none on dormant cells. Ki-67 is rated as a marker that is best used to assess tumor cell proliferation and can be very helpful in cases where histology shows the results of low-grade astrocytomas, while the other parameters are more inclined towards malignancy[5,6].

Several studies have been conducted to find a correlation between the expression of VEGF with Ki-67 in several types of tumors. Studies conducted by the Mineta et al in 2002 on 109 patients with squamous cell carcinoma of the tongue, found that VEGF correlated with Ki-67 significantly. Expression of VEGF is also significantly related to an advanced stage and VEGF as an independent predictor of relapse free survival (RFS). [7,8,9]

The expression of Ki-67 and p53 genes in pilocytic astrocytoma and diffuse astrocytomas are significantly lower than the high-grade astrocytomas. However, there is no significant expression differences between pilocytic astrocytomas and diffuse astrocytoma. Relevance between p53 expression prognosis is still debated. Mutations in the p53 gene that increase the threshold for cell apoptosis with impaired DNA chain affect the loss of ability to induce apoptosis.[8,10]

Material and Method

This was a cross sectional study using analitical reasearch with the purpose to determine the relation between VEGF and Ki-67 expression in patient with astrocytomas.The study was performed in the Neurosurgery Department of Adam MalikGeneral Hospital Medan and Pathology Anatomy Department of Universitas Sumatera Utara in Medan, North Sumatera.The subjects were all patients with astrocytoma who undergo surgical tumor removal at Adam Malik General Hospital between January 2014 to June 2015. Subjects with multiple CNS tumors and recurrent as well as residive tumor were excluded. VEGF and Ki-67 stainings were performed in Anatomical Pathology Department of Universitas Sumatera Utaraby trained laboratory personnel. VEGF and Ki-67 expression analysis were interpretedsemiquantitatively by twopathologists using Johannessen criteria for Ki-67 (low if <10% of cells were stained and high if >10% of cells were stained) and Oehring criteria for VEGF (+1: <10% cells were stained,; +2: 10-50%; +3: 50-90%; +4: >90%). This study was approved by ethical committee in School of Medicine Universitas Sumatera Utara.

Data was analyzed using the data processing software to determine data characteristics. Normality of the data was assessed using the Kolmogorov. Categorical variables were analyzed in the form of frequencies and percentages presented in tabular form. Numeric variables are presented in the form of mean and standard deviation if the distribution is normal. If the distribution is not normal, grouping of data into group was used. Correlation was tested using Spearman or Pearson test, depending on normality.

Result

Twenty-five subjects were enrolled in this study. The mean age of subjects was was 35.96 ± 14.67 years. Most of the research subjects were male gender (15/60%), while the female subjects were 10 (40%). Most of the subjects were WHO grade 1 (36%), followed by WHO grade II, IV, and III respectively (24%, 24%, 16%, table 1)

Table 1. Subjects Characteristic

Characteristic	
Age (year)	35,96 ± 14,67
Gender (n/%)	
Man	15 (60)
Woman	10 (40)
WHO degree (n/%)	
I	9 (36)
II	6 (24)
III	4 (16)
IV	6 (24)
Total	25 (100)

Eighteen subjects (72%) were survived in follow up period. 71,3% of subjects with WHO grade IV astrocytoma were death. All of grade I as well as grade II patients were survived. There is significant correlation between WHO grading and mortality ($p<0.05$, table 2)

Table 2. Association between WHO Classificationand mortality

WHO degree	Live n(%)	Death n(%)	p
I	9 (50)	0 (0)	0.001
II	6 (33,3)	0 (0)	
III	2 (11,1)	2 (28,6)	
IV	1 (5,5)	5 (71,3)	
Total	18 (100)	7 (100)	

Ki-67 Protein Staining

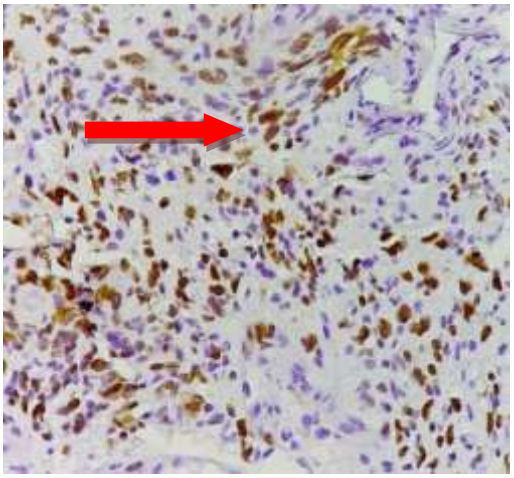

Figure 1. High Ki-67 Protein Expression. Immunohistochemistry staining showed > 10% strong intensity brownish coloration on the nucleus of astrocytoma cell using 40 x magnification

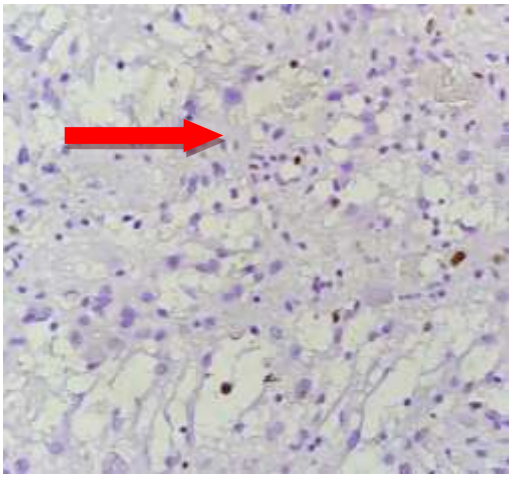

Figure 2. Low Ki-67 Protein Expression. Immunohistochemistry staining showed < 10% weak intensity brownish coloration on the nucleus of astrocytoma cell using 40 x magnification

Ki-67 expression was investigated using immunohistochemistry test. In 40x magnification, the expression was considered low if less than 10% cells were stained. Otherwise, the expression was considered high (Figure 1 and 2)

Negative Ki-67 expression was found in 1 case WHO grade I and 1 case WHO grade II. Almost half of the subjects had strong Ki-67 expression (48%). Statistical analysis showed no significant positive correlation between the expression of Ki-67 with WHO grading (r = 0.362; p = 0.076, table 3).

Table 3. Correlation between expression of Ki-67 andWHO grading

Ki-67	*Grade* I	*Grade* II	*Grade* III	*Grade* IV	*r (p)*
Negative	1 (11,11%)	0 (0,0%)	1 (25,0%)	0 (0,0%)	0,362 (0,076)[a]
Weak	6 (66,67%)	1 (16,67%)	0 (0,0%)	2 (33,33%)	
Strong	2 (22,22%)	5 (83,33%)	3 (75,0%)	4 (66,67%)	
Total	9 (100%)	6 (100%)	4 (100%)	6 (100%)	

Than we correlate the Ki-67 expression and mortality. After statistical analysis, it appears there is no significant correlation between the expression of Ki-67 Li with mortality (p = 0.512, table 4).

Table 4.Association between the expression of Ki-67 Li and mortality

Ki-67 LI	Live	Death	Total	p
Negative	2 (100%)	0 (0%)	2 (8%)	0.512[a]
Weak	7 (77,7%)	2 (22,2%)	9 (36%)	
Strong	9 (64,2%)	5 (35,7%)	14 (56%)	
Total	18 (72%)	7 (28%)	25 (100%)	

Ki-67 and VEGF

We used semiquantitative method to investigate VEGF staining (figure 3 and 4).After statistical analysis, it was concluded that there were no significant correlation between the expression of VEGF against Ki-67 labeling index (Table 5).

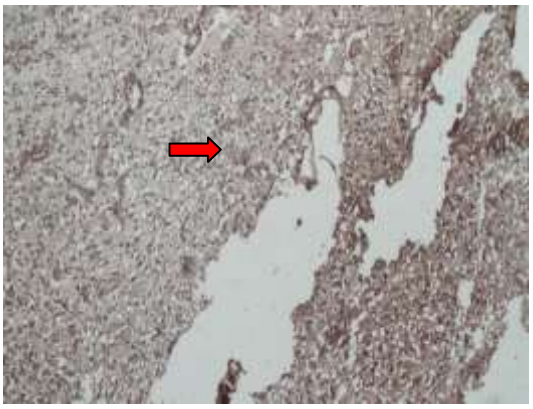

Figure 3. +2 VEGF Expression. Immunohistochemistry staining showed 10 – 50% intensity brownish coloration on the nucleus of astrocytoma cell using 40 x magnification

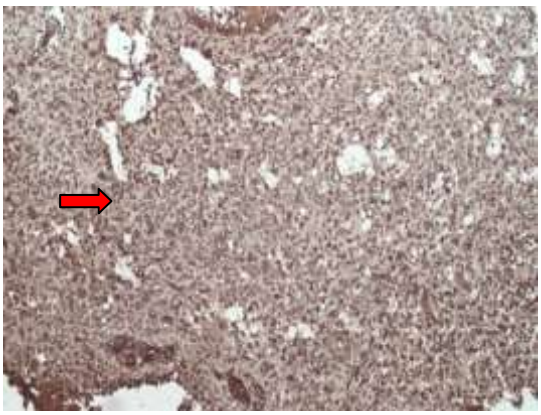

Figure 4. +4 VEGF Expression. Immunohistochemistry staining showed > 10% intensity brownish coloration on the nucleus of astrocytoma cell using 40 x magnification

Table 5. Correlation between VEGF expression and Ki-67 index

VEGF	Ki-67			r (p)
	Negative	Weak	Strong	
+2	1	0	0	0,139 (0,508)
+3	0	2	3	
+4	1	7	11	

Discussion

In this study, we found that astrocytoma WHO grade I classification is the highest type of astrocytoma which is equal to 36%, followed by grade II and grade IV respectively 24% and 14% grade III. These results do not resemble previous studies where the incidence observed at astrocytoma grade II astrocytoma at 39.9% followed by 36.2% gr IV, astrocytoma grade III astrocytoma by 14.3% and 9.5% grade I.[5] It is also due to the lack of epidemiological studies on the incidence of astrocytoma in Indonesia in general, and specifically in the city of Medan.

We also found that the WHO classification has a role in mortality with the results of the significance of p = 0.001 (p <0.05). This shows a significant correlation between the WHO classification with the prognosis of the patient, according to a study conducted by Anvari et al.[11]

Statistic analysisfound that there was no significant relationship between the expression of Ki-67 Li to gender (p = 0483). This is in line with studies conducted by Darweesh et al which concluded that no significant relationship is found between the expression of Ki-67 against gender (p = 0481).[9]

The expression of Ki-67 does not have a role in the classification of astrocytomas with the results of the significance of p = 0.076 (p> 0.05). This is not in accordance with the publications that have been done before concluding that there is a significant correlation between Ki-67 with degree classification of astrocytomas. Publications by Johannessen et al explained that the value of ki-67 increased with increasing degree of the WHO classification.[5]

In this study no significant correlation between the expression of Ki-67 for mortality (p = 0512) was found. The entire supporting publications found that Ki-67 has a good prognostic indicators value of the number of safety as well as to recurrence.[5]

There is no negative expression of VEGF and +1 found in this study, and the majority have an expression +4. After statistical analysis were done, we can conclude that VEGF expression was not related to the degree classification of astrocytomas. This is similar to a study conducted by Oehring et al in 1999, where it is not found that the relationship between the expression of VEGF on the degree of classification of

astrocytomas (p = 0.3749). In contrast to the study conducted by Wang, that concluded that there is a significant correlation between the positive expression of VEGF to the classification of pathology and grade (p <0:01).[12]

In this study we found no significant association between the expression of VEGF on mortality (p = 0813), this in contrast to a study conducted by Oehring et al in 1999 concluded that the expression of VEGF have a meaningful relationship with mortality (p = 0.0034).[4]

In this study we found no significant relationship between the expression of VEGF on the expression of Ki-67 (p = 0.508). In contrast to studies conducted by Ruan in 2003 through his studies of the expression of VEGF and Ki-67 in astrocytoma found that the expression of VEGF and Ki-67 significantly different in each grading astrocytomas and positively correlated with increased progression of tumors. [13]

As a limitation, this study is lack of an age restriction on the uptake of the subject, given that the nature of the underlying biological children are different from adults astrocytomas. The next limitation is the calculation of the subject is calculated by total sampling, so the statistical calculation is not appropriate.

Conclusion and Suggestion

In this study, it can be concluded that no relationship was found between VEGF expression and Ki-67 labeling index in patients with astrocytomas (p = 0.508). VEGF expression has also no connection with the classification of astrocytomas (p = 0704). We also found that there was no correlation between astrocytoma classification of gender by the WHO in astrocytoma patients (p = 1.000). VEGF expression has also no relationship to the mortality of patients with astrocytoma in this study (p = 0.680).

This study found a relationship between the classification of mortality by the WHO in astrocytoma patients (p = 0.001) but there is no significant relationship between proliferation index based on the value of Ki-67 with astrocytoma by the WHO classification (p = 0.076). Proliferation index based on the value of Ki-67 also does not have a relationship with the mortality of patients with astrocytomas (p = 0512).

Advice can be given by writer is the number of subjects taken should be calculated on the basis of the subjects refer to previous research.

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