

**Correlation of Vascular Endothelial Growth Factor (VEGF)
Immunoeexpression with histopathological grade of astrocytoma
at the H. Adam Malik General Hospital, Medan.**

Eka Bitaria Febrawati, Lidya Imelda Laksmi, Soekimin.

Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Background: Astrocytoma is a neuroepithelial tumor of glial cells consisting of infiltrative tumor cells with increased cellularity in accordance with histopathology grade. Vascular Endothelial Growth factor (VEGF) is one of the important factors in the process of angiogenesis. VEGF plays a role in proliferation, survival, endothelial cell migration and vascular permeability.

Materials and methods: This study is an analytical study with cross-sectional approach with spearman test analysis of 31 samples of paraffin blocks of astrocytoma cases that meet the inclusion criteria then immunohistochemistry examination with VEGF and assessed VEGF expression that will be correlation with histopathology grade of astrocytoma.

Results: A total of 31 samples showed VEGF expression, 7 cases were astrocytoma grade I, 11 cases were astrocytoma grade II, 5 cases were astrocytoma grade III and 8 cases were grade IV astrocytoma. Statistical analysis has a correlation between VEGF expression with histopathology grade of astrocytoma $p = 0.005$ ($p < 0.05$) and shows a sufficient relationship with $r = 0.448$. Conclusions: This study revealed that increased VEGF expression was followed by histopathology grade of astrocytoma.

Conclusion: There is a correlation between the immunohistochemistry expression of VEGF and histopathology grade of astrocytoma, where the increase in VEGF expression is followed by an increase in histopathology grade of astrocytoma

Keywords: Astrocytic tumours, Grade histopathology, VEGF,

INTRODUCTION

Astrocytoma is a neuroepithelial tumor derived from glial cells called astrocytes.(1) The tumor is still the leading cause of death in Indonesia, the determination of the grading of the tumor astrocytoma very useful for estimating the response to the therapy regimen radiotherapy and chemotherapy to determine the survival rate of patients with astrocytoma.(2,3). Based on histopathology features of astrocytoma divided into 4 grades which are distinguished by cell differentiation, cell cellularity, nuclei atypia, mitotic activity, proliferation of vascular snakes and necrosis. (4) In the case of brain tumors an estimated incidence of about 296,851 cases with a mortality rate of 241,037 cases. (5) Central Brain tumor Registry of the United States (CBTRUS) data reports for 2011-2015 the incidence of glioblastoma (56.6%), anaplastic astrocytoma (6.7%), glioma malignant NOS (7.4%) and diffuse astrocytoma (7.4%). The majority were found to be more men than women and the most frequent location was in the supratentorial region (61.2%) especially in the frontal lobe, temporal, parietal and occipitalis.(6) Data in Iraq intracranial tumor rates range from 10-17 per 100,000 people and ranks fourth out of ten other malignant tumors. (4)Epidemiologically, the incidence of astrocytoma in Indonesia has not obtained definitive data, this is probably due to the recording of data that is still less than optimal. In RSUP DR. Wahidin Sudirohusodo Makasar, South Sulawesi in the January-December 2016 period there were 16 cases of astrocytoma with more female sexes and most were at the age of 20-29 years.(7) Data from the Department of Pathology Anatomy Dr. Soetomo Surabaya found 8 cases of astrocytoma and nearly doubled to 14 cases in 2014.(8) In H. Adam Malik General Hospital, data from astrocytoma sufferers in January 2014 to June 2015 were 25 cases. (9)

The brain is an organ that is sensitive to hypoxia. Hypoxia induces a complex intracellular signaling pathway, one of which is hypoxia Inducible Factor (HIF). (10) Oxygen pressure can function as a VEGF regulator. Exposure to hypoxic conditions can induce VEGF rapidly. Adequate vascular response is very important for early development in the growth of solid tumors, so much attention has been focused on the use of angiogenesis inhibitors in addition to other forms of therapy to prevent the development of malignant neoplasms. The process of angiogenesis occurs due to an imbalance of pro-angiogenic and anti-angiogenic factors. One of the proangiogenic factors is Vascular Endothelial Growth Factor (VEGF).(11,12) VEGF is a strong endothelial cell mitogen and a key regulator of physiologic vasculogenesis in the embryogenic circulation system and pathological angiogenesis that leads to the growth of new blood vessels from preexisting blood vessels.(13) VEGF is a disulfide-linked dimeric glycoprotein binding heparin that is secreted in the form of a homodimer with a molecular weight

of 35-45 kDa. Some research results show that heparin interacts with VEGF through the formation of VEGF heparin complex which causes molecular changes so that VEGF becomes more stable, more resistant to inactivation and has a longer half-life. Heparin complex formation VEGF also causes an increase in receptor affinity VEGF were found on the surface of cells to form intracellular signaling as a form of activation for proliferation. One of the functions of VEGF was first recognized as a medium for increased vascular permeability in tumor microvascular underlying tumor growth and formation of tumor peritumoral edema. Therefore, VEGF is also referred to as Vascular Permeability Factor (VPF).(8,14) Proangiogenic signals from VEGF R-2 activated by ligands. Activation of a number of downstream proteins including phospholipase C-gamma / protein kinase C (PKC), Ras pathway members, phosphatidylinositol-3 kinase (P13K), mitogen-activated protein kinase (MAPK) and others for the manifestation of final functions such as cell proliferation, migration, enhancement vascular permeability, and survival. Kinase inserts (KI) or carboxyl-terminal regions, tyrosine kinase receptors (TKRs) have tyrosine autophosphorylation sites, which are important for downstream. Site 1175-PY on VEGF R-2 will bind to PLC γ which will further activate the CCP which will later provide a signal for endothelial proliferation. Akt stimulates the release of nitric oxide from capillary endothelial cells, resulting in an increase in intracellular calcium ion concentration, which activates endothelial nitric oxide synthase (eNOS) in the cell permeability process. Akt / PKB will also activate caspase 9 and BAD which are useful to signal for cell survival. Activation of MAPK-HSP27-FAK and paxillin through focal adhesions will give the signal for migration.(14,15)

Various studies have been conducted to find out how the relationship of VEGF expression with histopathology grade of astrocytoma. Rizk, et al. in his research revealed that there is a relationship between VEGF expression and grading of astrocytoma tumors that were detected in grade I and grade II (70%), grade III (84%) and grade IV (100%). (13) Yang, et al. analyzed the expression of p53 and VEGF in the serum of glioma patients detected by ELISA.(16) Zheng Xu, et al. found a correlation between VEGF R2 polymorphisms and glioma risk factors in populations in China.(10) A European study concluded that VEGF -123 in tumor angiogenesis provides relevant prognostic information in patients with gliomas.(17) Vokuda, et al. found that VEGF expression was associated with grading astrocytoma tumors that gradually increased from grade II, III and IV. (18)

MATERIALS AND METHODS

This study aims to analyze the relationship between VEGF expression and histopathology grade of astrocytoma. 31 paraffin blocks were obtained from grade I, II, III, and IV astrocytoma patients in the Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra and the Anatomic Pathology Unit of the H. Adam Malik General Hospital, Medan during the period of 2016 to 2019. All samples were obtained through surgical procedures. Inclusion criteria were astrocytoma cases with adequate clinical data, available and undamaged formalin-fixed paraffin-embedded tissue block with sufficient tumors tissue. Grade histopathology of astrocytoma is determined based on the WHO classification of CNS 2016. Detail clinical data age and gender obtained from medical records. The location and size of the tumors were obtained from radiological data. Immunohistochemistry smear with antibodies against VEGF was performed in all samples. Histological type and grade were determined independently by researchers through hematoxylin and eosin-stained slides examination.

The tissue sections were deparaffinized and rehydrated before pretreatment. Endogenous peroxidase was blocked with hydrogen peroxide followed by antigen retrieval primary VEGF antibody (Santa Cruz Biotechnology, Inc) mouse monoclonal antibodies were used are primary antibody. Diagnostic BioSystems (Diagnostic Biosystems, Pleasanton, CA, USA) polymer kit was used for detection. The reaction was visualized with diaminobenzidine and counterstained with Mayer's hematoxylin followed by dehydration, clearing, and mounting. Positive control was hepar. VEGF expressions were determined independently by researchers. VEGF expression was assessed in cytoplasm of tumor cells and counted visually with a light microscope (Olympus CX210 with 100x magnification then calculated semi-quantitatively using H-score formula with the formula $\{(1x (\% \text{ of cells expressed by } 1+) + (2x (\% \text{ cells) expressed score } 2+) + (3x (\% \text{ of cells expressed score } 3=))\}$. Intensity scores consist of 0 = negative / not expressing VEGF, score 1 + = weak, score 2 + = moderate, 3 + = strong. Extensive tumor rated H-score between 0-19 = low, 20 -99 = 100-300 = moderate and high.(19)

Statistical analysis was performed using SPSS software package version 22.0 (SPSS INC, Chicago) with a 95% confidence interval and Microsoft Excel 2010. Categorical variables were presented in frequency and percentage. Statistical analysis to determine the correlation of VEGF expression with histopathology grade of

astrocytoma was performed by the Spearman correlation test with a p-value <0.05 were considered significant.

RESULTS

The mean age for astrocytoma was 36.16 years with a standard deviation of 17.33 with the most common age of 21-40 years as many as 13 cases. Eighteen patients (58.1%) were male, only thirteen patients (41.9) were female. Tumors site in astrocytoma most common in the supratentorial region of 28 samples are dominated lobe parietal by 6 samples and frontotemporal lobe by 5 samples. While the infratentorial region found three samples that consist of the posterior fossa 2 samples and cerebellum 1 sample. Based on tumor size distribution, most of the tumor size was 5-7 cm with 8 samples and no tumor size with 17 samples. Most histopathology grade of astrocytoma was found in grading II in 11 cases with subtype histopathology most common is diffuse astrocytoma NOS by 11 in this case. Clinical basic characteristic of astrocytoma patient were summarized in the table 1. Representative H&E section are shown in figure 1.

Table 1. Characteristics of astrocytoma patients

Characteristic	n	%
Age group, mean $\pm$ SD years	Mean: 36,16 SD:17,33	
≤ 20 years	7	22.6
21-40 years	13	41.9
41-60 years	8	25.8
>60 years	3	9.7
Sex		
Male	18	58.1
Female	13	41.9
Tumors site		
Supratentorial	28	90.3
Parietal	6	19.4
Frontal	3	9.7
Temporal	2	6.5
Temporoparietal	4	12.9
Frontoparietal	2	6.5
Occipitoparietal	1	3.2
Frontotemporal	5	16.1
Frontotemporoparietal	1	3.2
Temporofrontoparietal	3	9.7
Ventrikel lateral	1	3.2
Infratentorial	3	9.7
Fossa posterior	2	6.5
Cerebellum	1	3.2
Tumors size		
<5 cm	5	16.1
5-7 cm	8	25.8
>7 cm	1	3.2
No size	17	54.8
Grading		
Grade I	7	22.6
Grade II	11	35.5
Grade III	5	16.1
Grade IV	8	25.8
Subtype		
Pilocytic astrocytoma	5	16.1
Pilomixoid astrocytoma	1	3.2
Subependimal giant cell astrocytoma	1	3.2
Diffuse astrocytoma	11	35.5
Anaplastic astrocytoma	5	16.1
Glioblastoma	8	25.8
Total	31	100

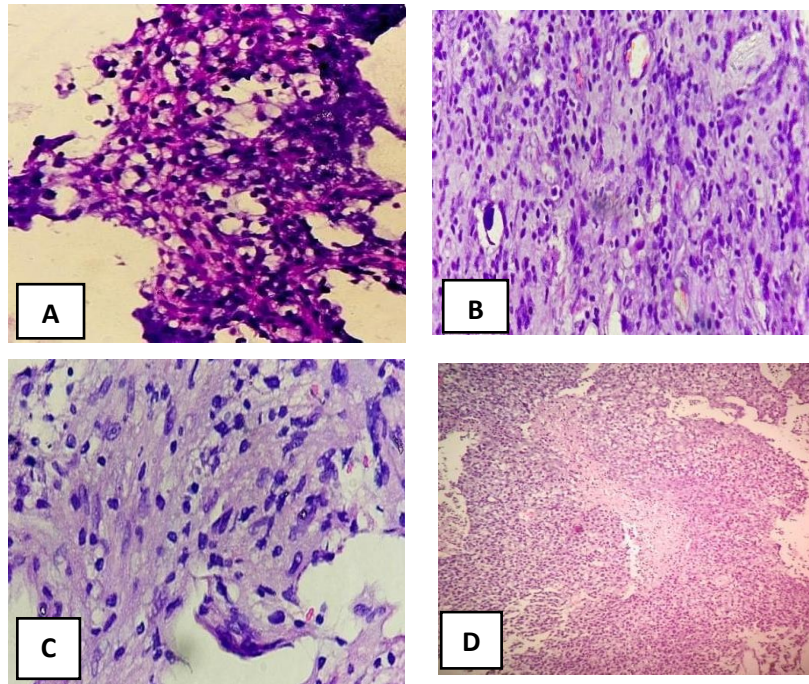


Figure. 1. A. Pilocytic astrocytoma, WHO grade I of astrocytoma (H&E 100x). B Diffuse astrocytoma, WHO grade II of astrocytoma (H&E 100x). C. Anaplastic astrocytoma, WHO grade III of astrocytoma (H&E 100x). D. Glioblastoma, WHO grade IV of astrocytoma (H&E 40x).

VEGF expression in thirty-one based on histopathology grade of astrocytoma case with low H-score, moderate H-score and high H-score (table 2). The intensity of VEGF expression in cytoplasm are shown in figure 2.

Table 2. Astrocytoma on VEGF expression

Expression VEGF (H-Score : 0-300)	Grade I	Grade II	Grade III	Grade IV	Total
Low (0-19)	4	5	2	0	11
Moderate (20-99)	3	5	1	5	14
High (100-300)	0	1	2	3	6
Total	7	11	5	8	31

Astrocytoma grade I found VEGF expression with the low H-score values, namely 4 samples, followed by moderate VEGF H-scores, 3 samples, and no high H-score values were found. In grade II astrocytoma, VEGF expressions with low and moderate H-scores were 5 samples each and VEGF expressions with high H-scores were 2 samples. Then in grade III astrocytoma, VEGF expression was found with low and high H-score values for every 2 samples and moderate H-score for 1 sample. In astrocytoma grade IV obtained the expression of VEGF with the value of the H-score was the highest at 5 samples followed by the value of the H-score high 3 samples and found no value H-score low. Correlation between VEGF expression with histopathology grade of astrocytoma was assessed by the Spearman correlation test. The correlation VEGF expression with histopathology grade of astrocytoma can be seen in table 3.

Table 3. Correlation VEGF expression with histopathology grade of astrocytoma

Variable	Histopathology grade of <i>astrocytoma</i>		
	n	r	p
Ekspression <i>VEGF</i>	31	0,448	0,005

Based on the Spearman correlation test results showed that the correlation between VEGF with histopathology grading astrocytoma has a significant correlation with the value of $p = 0.005$ ($p < 0.05$). Spearman's correlation coefficient shows a sufficient category with a value of $r = 0.448$ ($r = 0.26-0.50$) with a positive/unidirectional direction which means an increase in VEGF expression followed by an increase in astrocytoma histopathology grading. An overview of VEGF expressions is presented in Figure 2

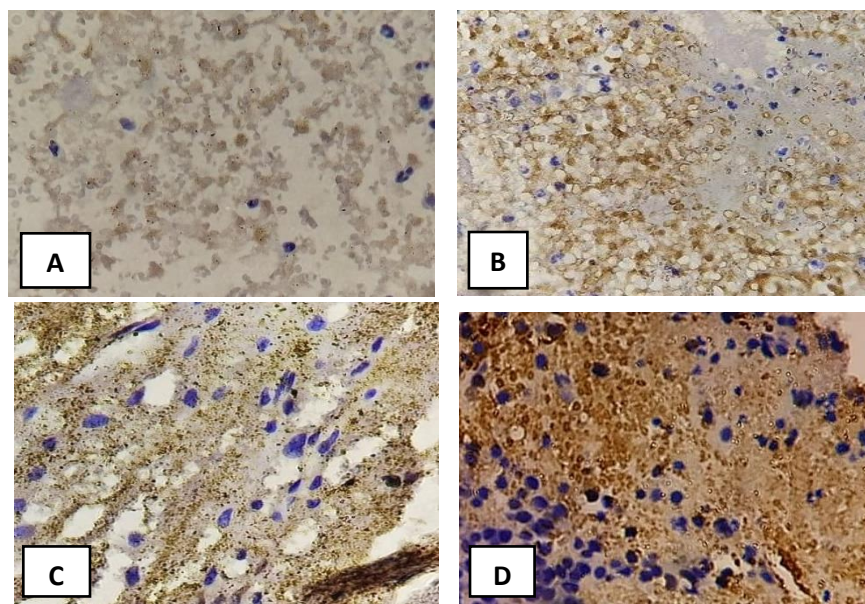


Figure. 2. A. A cases of pilocytic astrocytoma with low cytoplasmic VEGF expression.(IHK 100x) B. A cases of diffuse astrocytoma with moderate cytoplasmic VEGF expression (IHK 100x). C. A case of anaplastic astrocytoma with moderate cytoplasmic VEGF expression (IHK 100x) D. A cases of glioblastoma with high cytoplasmic VEGF expression (IHK 100x).

DISCUSSION

In our study, VEGF expression in grade 1 of astrocytoma was not found with a high H-score value as well as in grade IV astrocytoma also found no VEGF expression with a low H-score value. After the Spearman correlation test, the value of $p = 0.005$ ($p < 0.05$) and the value of $r = 0.448$. Astrocytoma is a solid tumor that is prone to hypoxia, so the angiogenesis process occurs which can induce VEGF expression quickly. VEGF plays a role in neovascularization in tumors cells. It may have an important role in the molecular mechanisms of tumorigenesis, thus, facilitating cell migration and proliferation, endothelial cell survival, and microvascular tube formation. VEGF is also able to stimulate vascular endothelial cell proliferation through binding to specific receptors. Determination of histopathology grade in astrocytoma is assessed based on cell morphology atypia, mitotic activity, features of microvascular proliferation and necrosis. In cancer with high cell proliferation, tumor growth is faster which causes the tumor size to become larger and a higher histopathology grade. Many recent studies have demonstrated the mechanism of additional angiogenic and vasculogenic are associated with tumor growth. Histopathology examination shows more mitosis and a picture of vascular proliferation and necrosis is seen which will increase the histopathology grade of astrocytoma.⁴ tumors are glioma rich in blood vessels that have a proliferation of tumor cells that rapidly, the rate of apoptosis slow da n invasive high level. Phosphorylation of tyrosine depends on ligands to mediate the proliferation and differentiation of vascular endothelial cells. VEGF can cause blood vessel endothelial cell division and increase glioma invasive.

The steps for angiogenic processes in glioma include 1. Glioma cells, in response to gene mutations or hypoxia, release growth factors, such as VEGF, which activate brain endothelial cells. 2. VEGF as a growth factor binds to endothelial cell receptors and activates the signal transduction pathway that leads to endothelial cell proliferation. 3. The growth of blood vessels will stimulate urokinase and metalloproteinase which migrate toward the tumor and bind to specific $\alpha\beta$ integrins. 4. Glioma cells surround the host vessels with co-options that migrate along the blood vessels to form an environment to be protected, or "vascular niche." Grouping the glioma will reach a critical size, about 5×10^5 cells so that they will then recruit additional blood vessels themselves.(22)

In this study, the relationship between VEGF expression and astrocytoma grade histopathology with a positive direction and a sufficient level of correlation. Several previous studies have suggested that high VEGF expression is found in poorly differentiated tumors. Among them is the study Yang et al obtained decreased p53 expression and increased VEGF expression in serum tissue in glioma patients. (16) Vocuda, et al. and Chen, et al. his research also found that VEGF expression was associated with astrocytoma which was gradual according to the histopathology grade of astrocytoma.(18.20) Research Das, et al. suggested that a significant positive relationship was found between mature endothelial cells in the peripheral circulatory system and angiogenic cells in tumor astrocytoma patients.(21) Rizk, et al. stated that VEGF expression was detected around 87.2% in astrocytoma and significantly increased expression was followed by increased grade of astrocytoma tumors.(13) Studies were conducted by Retnani to 30 samples showed that the expression of VEGF can increase the degree of histopathology astrocytoma.(8) Different things were found in the Saragih EB study in 25 samples of astrocytoma patients showing no relationship between VEGF expression and astrocytoma.(9) Although, there are many molecular markers for the diagnosis of glioma, the discovery of novel therapeutic markers is essential for patient management. In this regard, VEGF serves as a novel entity for designing anti-VEGF treatment regimens; however, these are in preclinical trials in USA and other countries. Increased VEGF expression correlates with the degree of malignancy in gliomas.(18)

CONCLUSION

We examined 31 samples of astrocytoma in the Anatomical Pathology Unit of H. Adam Malik General Hospital Medan showed an correlation between the immunohistochemistry expression of VEGF and histopathology grade of astrocytoma, where the increase in VEGF expression is followed by an increase in histopathology grade of astrocytoma.

ACKNOWLEDGEMENT

We acknowledge to all staff members in Department of anatomical pathology, Universitas Sumatera Utara and H. Adam Malik General Hospital, Medan, Indonesia for their help and cooperation.

ETHICAL APPROVAL

Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia approved this study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

AUTHORS DETAILS

First Authors - dr. Eka Bitaria Febrawati, resident of anatomical Pathology Departement, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Second Author – Dr. dr. Lidya Imelda Laksmi, M.Ked(PA), Sp.PA, Lecture and staff anatomical Pathology Departement, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Third Author – dr. H. Soekimin, Sp.PA (K) Lecture and staff anatomical Pathology Departement, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Corresponding Author– Eka Bitaria Febrawati, resident of anatomical Pathology Departement, Faculty of Medicine, Universitas Sumatera Utara Jl. Universitas no. 1 gedung Abdul Hakim, Padang Bulan, Medan, Indonesia

Email ID: ekabitaria@gmail.com

REFERENCES

1. Dorsey JF, Salinas RD, Dang M, Basanta MA, Judy KD, Maity A et al. Cancer of the Central Nervous System. In: Niedrhuber JE, Armitage JO, Kastan MB, Doroshow JH, Tepper JE, Editors. *Abeloff's Clinical Oncology*. Sixth Edition. Philadelphia: Elsevier; 2019.p.967-906..
2. Kumar K, Abbas AK, Aster JC. Buku Ajar Patologi Robbins. Edisi 9. Elsevier; 2017. p.835-799.
3. Louis DN. WHO Classification and *grade* of tumours of the central nervous system. In: Louis DN, Ohgaki OD, Wiestler WK, Cavenee, Editors. *WHO Classification of tumours of the Central Nervous System*. Revised 4th Edition. Lyon: IARC; 2016.p.13-12.
4. Almukhtar ZK, Kmal MS, Alkareem SA. Immunohistochemical expression of Ki 67 & CD 31 in Iraqi patient with *Astrocytoma*, a clinicopathologica; studies. 2019; p.4085-4077.
5. Bray F, Ferlay J, Soejomatarum I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistic 2018: GLOBOCAN Estimates of incidence and mortality world wide for 36 cancer in 185 countries. 2018; p.424-394.
6. Ostrom QT, Gittleman H, Truitt G, Bosci A, Kruschko C, Sloan JS. CBTRUS Statistical Report: Primary brain and other Central Nervous System Tumors diagnosed in the United stated in 2011-2015. 2018; 20(S4) p.86-1.
7. Mutmaima IS, Ihwan A. Karakteristik pasien Astrositoma di RSUP dr. Wahidin Sudirohusodo Makassar periode Januari – Desember2016.2017
8. Retnani DP, Fauziah D. Ekspresi Vascular Endothelial Growth Factor (VEGF) dikaitkan dengan pertumbuhan tumor dan edema Peritumoral Majalah Patologi.2013; 22(3):p.24-9.
9. Saragih EB, Indharty SS, Siahaan AM. Hubungan ekspresi VEGF dengan Ki-67 Labeling index pada penderita *astrocytoma* di RSUP H. Adam Malik Medan. 2017. Tesis Magister FK.USUMedan.
10. Xu Z.G et al. Correlation between VEGFR2 rs2071559 polymorphism and glioma risk among Chinese population. 2015;8(9):16724-16728.
11. Kresno SB. Angiogenesis. In: Ilmu Dasar Onkologi. 3th ed. Jakarta: Badan Penerbit Fakultas Kedokteran Universitas Indonesia. 2012. P 344-68
12. Yadaf L, Puri N, rostagi V, Satpute P, Sahma V. Tumour Angiogenesis and Angiogenic inhibitor: A review. *Journal of Clinical and diagnostic research*.2015;9(6):p.5-1
13. Rizk MK O, Saied M, E, Maqusod El abd ED. Angiogenesis in *astrocytoma*: An immunohistochemical study of VEGF, Factor VIII and COX-2 expression. 2016; 6(4) p. 195-188
14. Roslavtceva VV, Salmina AB, Prokopenko SV, Pozhilenkova, Kobanenko IV,Rezvitskaya GG. The Role of Vascular endothelial Growth Factor in the Regulation of Development and functioning of the Brain: New target molecules for Pharmacotherapy. 2016; 10(4):p.309-300.
15. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and It's Receptor (VEGFR) Signaling in Angiogenesis: a Crucial Target for Anti – and pro – AngiogenicTherapies. 2011; 2(12):p.1105-1097.
16. Yang J, Zhao H, Zhong X. Correlation analysis of the clinicopathological features of glioma and expression of p53 and VEGF. 2017;10(2):p.3606-3611.
17. Rainer E et al. The prognostic value of $\{^{123}\}$ vascular endothelial growth factor $\{^{123}\}$ VEGF in glioma.2018.45: p. 2396-2403.
18. Vokuda SR, Srinivas BH, Manhugiri VS, Verma SK. Vascular Endothelial Growth Factor as an Angiogenic Marker in Malignat *Astrocytoma* and Oligodendroglioma: An Indian Scenario. 2017; 11(2)p.7-5
19. Kraus AJ, Dabbs DJ, Beriwal S, Bhargava R. Semiquantitative immunohistochemistry for estrogen and progesteron receptor : an independent quality assurance study. *Modern Path*. 2012;25:869-76.
20. Jurantil et al. Radiographic assessment of contrast enhancement and T2/FLAIR mismatch sign in lower *grade* gliomas: correlation with molecular group. 2018;p. 9-1
21. Villanueva GK, Rea DN, Krieger DM. Novel surgical and radiologic risk factors for progression or recurrence of pediatric pilocytic *astrocytoma*.2019;p.11-1
22. Brem S et al. Angiogenesis and Brain Tumors Scientific Priciples, Current Therapy and Future Direction in Youmas & Winn. *Neurological Surgery*. Chapter 116. 2017.p.846-825