



Microvascular density in WHO-based histological grading of meningioma

Celia*, Rr. Suzy Indharty, Iskandar Japardi

¹Departement of Neurosugery, Faculty of Medicine University of Sumatera Utara, H.

Abstract : Introduction- Studies about the difference of microvascular density in each grade of meningioma showed conflicting results. Moreover, in Indonesia, there was no study that determine this association.

Objective- To determine the difference of MVD in WHO-based histologicalgrading of meningioma.

Methods- This analytical study was conducted on 33 intracranial meningiomas subjects undergoing surgery at RSUP H. Adam Malik in January 2015-June 2016. The meningioma paraffin block specimens were immunohistochemically processed using the antibody monoclonal PECAM-1 mouse reagent to assess CD31 in the quantification of MVD. The grade of meningioma was determined by WHO classification. Data were collected and analyzed with SPSS 19.

Results-In 33 paraffin blocks, mostly most meningioma were grade 1 (87.9%), followed by grade 2 (9.1%) and grade 3 (3%). Mean of MVD in grade 1 meningioma was 16.30 ± 9.65 while in grade 2 and 3 meningioma was 14.20 ± 5.11 . Using Mann Whitney, this study showed that there was no difference of microvascular density between grade 1 and grade 2/3 of intracranial meningioma ($p=0.869$).

Conclusion-There was no difference of microvascular density in each histological grade of meningioma. Further research with larger samples and various markers is needed.

Keywords : Microvascular density, CD31, Meningioma.

Introduction

Meningiomas are benign brain tumors derived from meninges. Meningioma has the highest prevalence compared to other brain tumors that amounted to 33.8% of all primary brain tumors. Meningioma incidence increases with age with peak at age 70 to 80 years and higher in female by a ratio of 1:2-3 to male.¹

Tumor microcirculation plays a central role in the tumor growth and dissemination. Tumors recruit new vessels from pre-existing vessels induced by factors secreted by the their cells or surrounding stromal cells.²Brain vascular system is a highly specialized structure, composed of distinct cell types forming the blood-brain barrier. Brain vessels play a relevant role in the development of malignant primary tumors.³Previous studies performed in preclinical models of brain tumors demonstrated the irregular morphology of vessel in intracranial meningioma is related to degree of tumour vascularity and the extent of peritumoural vasogenic oedema.^{4,5}

It is no doubt that tumor angiogenesis is a hot topic of research in recent years due to its prognostic value and potential importance in offering novel treatment options.⁶ A peritumoral area of any malignant tumor

has a special biological role. The peritumoral area consisted of histohematic barrier which participate in angiogenesis.⁷

Meningiomas are rich in vascularity, which varies within the subtypes and grades. Neo angiogenesis in meningiomas can be quantified by evaluation of quantification of endothelial cells, which reflects the number of vessels per square mm. This is microvascular density.⁸ A number of endothelial markers like factor VIII, CD31, CD34, and CD105 have been used in various studies to stain endothelial cells.⁹ Microvascular density, as the angiogenic potential, in the central danperitumoral has become the promising marker.¹⁰ However, the effect of peritumoral vessel growth or microvascular density on the tumor outcome has been poorly studied.

Meningioma angiogenesis and MVD were shown to result in the rapid tumor progression and invasivity. The relationship to metastasis was found in lung cancer, leiomyosarcoma, and prostate cancer. However, correlation between the degree of angiogenesis, MVD and proliferative activity of endothelial cells remains incompletely understood.¹¹ Technically, the variety suggestion of panendothelial markers in MVD cause the discrepancy. CD 31 that reacts with plasma cells is sensitive compared to other markers. In addition, CD 31 has also been reported to be present in tumor cells. CD 31 is also a key participant in the adhesion cascade leading to extravasation of leukocytes during the inflammatory process.¹²

Some studies in others tumor about correlation between MVD and tumor grade showed conflicting results. In leiomyosarcoma, Avdalyan et al. (2012) showed that MVD did not depend on the tumor grading ($p=0,07$).¹³ But in prostate cancer, Miyata et al. (2015) showed that MVD correlated significantly with Gleason score that showed the advanced stage of prostate cancer ($p<0,001$).¹⁴

In meningioma, Shi et al. (2016) and Barresi (2007) showed that MVD was higher significantly in higher histological grade of meningioma.¹⁵ Lewy-Trenda et al. (2003) showed higher MVD in atypical meningioma also, but it was not statistically significant.¹⁶ In other study, using CD34 in 30 paraffin blocks of meningioma, Bohra et al (2016) did not find any statistical significant difference in MVD across various grades of meningioma but MVD was surprisingly lower in grade III tumors compared to grade I and II.¹⁷ However, Boari (2013) showed that MVD had another useful function in differentiate meningioma and glioblastoma. Media to lumen ratio was significantly higher in patients with meningioma compared to patients affected by glioblastoma.⁴

Therefore, the role of MVD in predicting histological grade of meningioma was still controversial. Moreover, the studies were conducted mostly in western countries where the population demographics were different from Indonesian population. In Indonesia, the research about microvascular density in meningioma is not was not available. Therefore, this study will aim to determine the difference of MVD in WHO-based histological grading of meningioma.

Methods

Study design

This study was conducted using cross sectional analytic study to determine the MVD in MRI-confirmed meningioma subjects undergoing surgery at H. Adam Malik Hospital Medan from January 2015 to June 2016. This study has been approved by the Committee Local ethics. The specimens were processed into paraffin block and analyzed in anatomical pathology laboratories.

Analysis of microvascular density

MVD (Microvascular Density) is the density of blood vessels measured by its thickness. In this study, the quantification of MVD was done with CD31 immunohistochemitrically using antibody monoclonal PECAM-1 Mouse reagents (Biocare Medical Inc, USA). Paraffin blocks fixed with formalin buffer 10% for <30min and cut with microtome with 3-4mm thickness. The microtome strips are dried first at room temperature and then heated to a hotplate at 60 ° C for 60 minutes. Then, paraffin is deparaffinate, rehydrated, blocked, and added antigen retrieval decloaking chamber. The slides were then ready to be mixed with the primary CD 31 antibody for 60 minutes and processed with trkavidin, chromogen, HE counterstain, and tacha bluing prior to mounting. The classification of CD31 were: 0 = No staining at all or very little partial staining of the membrane in less than 10% of the tumor cells.; 1+ = Vague / barely visible coloration on the membrane in

more than 10% of tumor cells.; 2+ = mild to moderate colored in more than 10% of tumor cells. MVD is measured by the thickness of tunica media-adventitia under 400x magnification of electron microscope. Microvascular vessel count (MVC) was determined as amount of microvascular vessels obtained in 1 field.

Grade of meningioma

According to WHO classification, meningioma was classified divided into 3 grades, Benign: Grade I, Atypical: Grade II, and Malignant: Grade III. According to the histological feature, grade I meningiomas consisted of meningothelial meningiomas, fibrous meningiomas (fibroblastic), transitional meningiomas, psammomatous meningiomas, angiomatous meningiomas, microcystic meningiomas, secretory meningiomas, lymphoplasmacyte-rich meningiomas, metaplastic meningiomas; Class II meningiomas consisted of chordoid meningiomas, clear-cell meningiomas, a typical meningiomas; grade III consisted of papillary meningioma, rhabdoid meningioma, anaplastic meningioma.¹⁸

Data analysis

The data obtained is processed with SPSS 19 (Chicago, IL, USA). Association between CD 31-based MVD and the histopathologic type of meningioma was determined using Mann Whitney. The significance value for this study was a p value less than 0.05.

Results

This study was conducted on 33 paraffin blocks from patients with intracranial meningiomas who had undergone tumor removal surgery at RSUP. H. Adam Malik Medan from January 2014 to December 2015. Mean age of subjects was 44.4 ± 10.5 years with mostly female (69.7%). Based on grading, most meningiomas were grade 1 (87.9%), followed by grade 2 (9.1%) and grade 3 (3%) (Table 1). Meanwhile, based on histopathology results, the most common type were meningothelial (48.5%), followed by fibroblastic (15.2%), atypical (9.1%), and others (21.2%) (Table 1).

Table 1. Grade of meningioma

Meningioma grade	n	%
WHO Grade 1	29	87.9
WHO Grade 2	3	9.1
WHO Grade 3	1	3
Total	33	100.0

Table 2. Histopathology type of meningioma

Histopathology	n	%
Meningothelial	16	48.5
Transitional	2	6.1
Fibroblastic	5	15.2
Clear Cell	1	3
Papillary	1	3
Atypical	3	9.1
Chordoid	1	3
Metaplastic	2	6.1
Psammomatous	2	6.1
Total	33	100.0

Microvascular density was obtained by staining specimens with CD31 to analyze the thickness of the tunica media-adventitia of vessels. CD 31 stained 3+ in all samples of the study. MVD was shown with mean is 16.04 (range 4,03-47,17) (Figure 1).

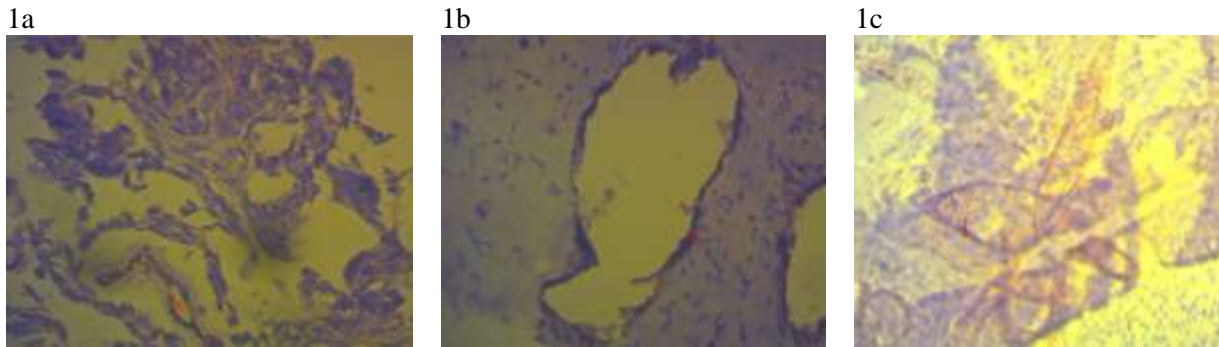


Figure 1. Microvascular density (a) MVD in meningioma grade 1 (b) MVD in meningioma grade 2 (c) MVD in meningioma grade 3

Mean of MVD in grade 1 meningioma was 16.30 ± 9.65 while in grade 2 and 3 meningioma was 14.20 ± 5.11 . Using Mann Whitney, this study showed that there was no difference of microvascular density between grade 1 and grade 2/3 of intracranial meningioma ($p=0.869$) (Table 3). The authors also analyzed microvascular count in each field. Mean of MVD in grade 1 meningioma was 4.69 ± 1.49 while in grade 2 and 3 meningioma was 9.75 ± 1.71 . Using Mann Whitney, this study showed that there was a significant difference of microvascular count between grade 1 and grade 2/3 of intracranial meningioma ($p=0.001$) (Table 4).

Table 3. Association between microvascular density to histological grade of intracranial meningioma

Grade of meningioma	n	Mean	P
Grade 1	29	16.30 ± 9.65	0.869
Grade 2 dan grade 3	4	14.20 ± 5.11	

Table 4. Association between microvascularcount to histological grade of intracranial meningioma

Grade of meningioma	n	Mean	P
Grade 1	29	4.69 ± 1.49	0.001
Grade 2 dan grade 3	4	9.75 ± 1.71	

Discussion

Meningioma is a slow-growing neoplasm derived from the meningothelial cells found in arachnoid granulation. Most tumors were found on the venous sinus wall. Meningioma can be classified by histopathology, tumor location, and tumor growth pattern. The histological grade of meningioma was classified by WHO to 3 grades, benign (grade I), atypical (grade II), and malignant grade 3).¹⁹ This study showed that most meningioma were grade 1 (87.9%), followed by grade 2 (9.1%) and grade 3 (3%) (Table 1).

The thickness of the lumen tunica media-adventisia became the most reliable indicator of small arterial structures, as it was independent of the dimensions of the blood vessels, as compared to other structural parameters such as media thickness, internal diameter or cross-sectional area media, with possible sampling bias.²⁰ However, changes in media lumen may reflected structural changes in various diseases including neoplasms and perhaps paradigmatic of the changes that occur during tumor growth.²¹ Finally, using immunohistochemical techniques, we were able to evaluate microvessel density. Microvascular play plays an important role in the delivery of oxygen and metabolites, therefore it was important to support tumor growth.²²

In leiomyosarcoma, Avdalyan *et al.* (2012) showed that MVD did not depend on the tumor stage, size, and grade. Moreover, they found no significant differences or correlation in the MVD between the tumor and the peritumoral area histology ($p=0,9$) and its degree of malignancy ($r=0,1$; $p=0,07$).¹³ In prostate cancer, Miyata *et al.* (2015) showed that 108 formalin-fixed specimens from patients treated by radical prostatectomy, the mean score of CD31 correlated significantly with Gleason score that showed the advanced stage of prostate cancer ($p<0,001$). However, no association was found regarding pathological features.¹⁴ meningiomas ($p = 0.002$).²³

Meningiomas are rich in vascularity, which varies within the subtypes and grades. Neoangiogenesis in meningiomas can be quantified by evaluation of quantification of endothelial cells, which reflects the number of vessels per square mm, called microvascular density. A number of endothelial markers like factor VIII, CD31, CD34, and CD105 have been used in various studies to stain endothelial cells. In analyzing angiogenesis by VEGF, Dharmalingam *et al.* (2013) has showed that VEGF expression correlated with the microvascular density in meningioma irrespective of tumor grade, with a gradual increase in microvascular density in relation to the VEGF score. There was a gradual increase in microvascular density from tumors which are negative for VEGF to tumors which expressed moderate to strong VEGF, the difference being statistically significant ($P = 0.009$).²³ However, correlation between the degree of angiogenesis and MVD and proliferative activity of endothelial cells remains incompletely understood, and correlation with vessel growth rate has been poorly studied.¹¹

Some studies in about the role of MVD in differentiating histological grade of meningioma showed conflicting results. Shi *et al.* (2016) in 48 patients with pathologically confirmed meningioma (grade I, 38 cases; grade II+III, 10 cases) showed that MVD of benign meningioma strips was 21.16 ± 11.32 , which was also significantly higher than 10.71 ± 5.53 strips of malignant meningiomas ($t=2.325$, $p=0.026$). There was also significant positive correlation between mean of maximum cerebral blood flow and MVD in meningioma ($r=0.718$, $p=0.000$).¹⁵ Using CD105 (endoglin) stained microvessels density in meningioma, Barresi *et al.* (2007) in 45 formalin-fixed meningiomas specimen (28 grade I and 26 grade II), showed that higher CD105 counts were significantly correlated with higher histological grade. No statistical significant correlations were encountered between MVD measured by either gender, age, site, and type of tumour or extent of surgical resection. CD105 also showed an inverse significant correlation with overall survival and recurrence-free survival.¹² In other study, using CD34 in 30 paraffin blocks of meningioma, Bohra *et al* (2016) controversially showed that the MVD was higher in grade I than grade II/III (49.67 ± 22.35 , 41.37 ± 7.45 and 47.86 ± 10.77 , respectively) but without statistical significant difference ($p>0.05$). The CD34 yield was strongest in angiomatous meningioma, due to its blood supply.¹⁷

This study showed that there was no difference of microvascular density between grade 1 and grade 2/3 of intracranial meningioma ($p=0.869$). Mean of MVD in grade 1 meningioma was 16.30 ± 9.65 while in grade 2 and 3 meningioma was 14.20 ± 5.11 . There was one study conducted by Lewy-Trenda *et al.* (2003) in 10 atypical meningiomas (grade 2), and benign meningiomas (grade 1) that showed no statistically significant differences between both groups of tumours and between subtypes of benign meningiomas. They also showed higher number of blood vessels was revealed in atypical meningiomas and much lower in benign ones.¹⁶

Regardless of that, this study showed that there was a significant difference of microvascular count between grade 1 and grade 2/3 of intracranial meningioma ($p=0.001$). Mean of MVD in grade 1 meningioma was 4.69 ± 1.49 while in grade 2 and 3 meningioma was 9.75 ± 1.71 . Using Mann Whitney, (Table 4). In general brain tumor, Assimakopoulou *et al.* (1997) showed that MVC was higher in glioblastomas (50,2), followed by anaplastic astrocytomas (42,3), cerebellar astrocytoma (41,1), Gliosarcoma (40), meningioma (27,9), ependymomas (22,7) medulloblastomas (19,6), astrocytomas grade I/II (14,5), and oligodendrogliomas (14,1). Comparison between grading of astrocytoma showed significant significance. In comparing different type of brain tumor histology, only astrocytic neoplasms showed statistically significant higher mean MVC from meningioma.²⁴

The discrepancy of this study results with most of others can be due to different population characteristics of study. This study also had limitations, that only included 4 cases of grade 2/3 meningioma, causing abnormally distributed data. Further research with larger samples and various markers is needed.

Conclusion

There was no difference of microvascular density in each histological grade of meningioma. Further research with larger samples and various markers is needed.

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Conflict of interest

The authors declared no conflict of interest regarding of this study.

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