Vascular endothelial growth factor expression and angiogenesis in various grades and subtypes of meningioma

Priya Dharmalingam1, VR Roopesh Kumar2, Surendra Kumar Verma3,

1 Department of Pathology, Jawaharlal Institute of Post-graduate Medical Education and Research, Pondicherry, India
2 Department of Neurosurgery, Jawaharlal Institute of Post-graduate Medical Education and Research, Pondicherry, India

Correspondence Address:
Surendra Kumar Verma
Professor and Head, Department of Pathology, Jawaharlal Institute of Post-Graduate Medical Education and Research, Pondicherry, India

Abstract

Background: Vascular endothelial growth factor (VEGF) expression has been extensively studied in astrocytoma, whereas relatively less literature exists on VEGF expression in meningioma. Materials and Methods: Patients operated for meningioma from 2006 to 2011 (n = 46) were included. Tumor was subtyped and graded as per WHO grading. Immunohistochemistry was performed for MIB labeling index, VEGF, and CD 34 staining. The patterns of VEGF expression in various histological subtypes and grades and its correlation with microvascular density were analyzed. Results: This series consisted of 40 Grade I meningioma, 4 Grade II tumors, and 2 Grade III tumors. While 14 (30.4%) tumors showed no staining with VEGF antibody, 32 (69.6%) were positive for VEGF. Sixty-five percent of Grade I tumors showed VEGF positivity, while 100% of Grade II and Grade III tumors were VEGF positive (P = 0.015). The mean microvascular density in VEGF-negative tumors was 9.00, while that of VEGF-positive tumors was 17.81 (P = 0.010). 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). Conclusions: 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.

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Introduction

Angiogenesis is a phenomenon of formation of new vessels. Judah Folkman was one of the pioneers to put forward the concept that angiogenesis is a key event in tumorigenesis. [1] Tumor angiogenesis is the result of a shift in the homeostatic balance of proangiogenic and antiangiogenic factors that results in a net gain of proangiogenic stimuli. [2] Increased tumor vascularization and expression of proangiogenic factors have been associated with advanced tumor stage and poor prognosis in various malignancies. [3],[4] Vascular endothelial growth factor (VEGF) has been demonstrated to be an important stimulus for angiogenesis in many tumors. Among tumors of CNS, VEGF expression has been extensively studied in astrocytoma. [5],[6],[7] whereas relatively less literature exists on VEGF expression in meningioma with varying results.

In addition to cellular and molecular factors of proliferation, angiogenesis has been evaluated as a mechanism which influences meningioma growth and recurrence. Among the various proangiogenic factors like VEGF, angiopeptin, endothelin, TGF, etc., VEGF has been shown to demonstrate an important role in promoting migration, proliferation, and tube formation of endothelial cells in meningioma. [8],[9] A few studies have reported on the relationship of VEGF expression with vascularity and peritumoral brain edema in meningioma. [9],[10],[11] An understanding of VEGF expression in meningioma and its influence on tumor grading and vascularity will aid in instituting antiangiogenic therapy as a modality to prevent recurrence. The present study assessed the expression of VEGF in various subtypes and grades of meningioma and evaluated their angiogenic potential by calculating the microvessel density (MVD).

Materials and Methods

This descriptive study included 46 patients who underwent surgical treatment for meningioma at a tertiary care medical institute from South India from 2006 to 2011. The study was approved by the institute ethics committee. The clinical details were collected from the case records and the radiological findings were recorded from CT/MRI scans. Paraffin blocks of all the cases were retrieved and histological features were reviewed. The tumor was graded and subtyped in accordance with established WHO criteria. [12] Serial 4 μm thickness sections were collected on silane-coated slides and subjected to immunohistochemistry by the labeled streptavidin-biotin complex immunoperoxidase methods with the following antibodies: MIB-1 (mouse monoclonal antibody against Ki-67, Dako), CD34 (mouse monoclonal antibody, Dako), VEGF (rabbit polyclonal antibody against VEGF165 isoform, Biogenex). The antigen retrieval was achieved by the heat-induced epitope retrieval method in antihydros citrate buffer pH 6.0 after TRIS buffer wash. The endogenous avidin-binding activity was blocked by immersing in skimmed milk powder. The sections were incubated in primary antibody for 90 minutes. Linked streptavidin biotinylated secondary antibody (Universal LSAB, Dako) was used as secondary antibody and 3,3′-diaminobenzidine as the chromogenic substrate. Appropriate positive and negative controls were run. The proliferation index was calculated using the MIB-1 antibody. The MIB-1 LI was determined by recording the percentage of positively staining tumor cell nuclei out of 1000 tumor cell nuclei. The hot spots (regions with the most immunostaining) were used in the determination of the labeling index and the immunostaining results were evaluated at high magnification (400x).

VEGF expression was assessed by using a scoring system proposed by Raica et al. [13] Both intensity and percentage positivity were analyzed. The pattern of expression, whether focal or diffuse and expression by tumor cells only or both tumor cells and endothelial cells were also recorded.

SCORE 0 - negative
SCORE 1 - weak reaction in less than 10% of tumor cells
SCORE 2 - weak to moderate reaction in 10-50% of tumor cells
SCORE 3 - moderate to strong reaction in more than 50% of tumor cells
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Angiogenetic potential in meningioma was evaluated by measuring intratumoral MVD. The MVD was counted by assessing immunohistochemical expression of CD34 which binds to endothelial cells. MVD was assessed in three "most vascular areas" (hot spots) as described by Weidner et al. [14],[15] The area containing the maximum number of discrete microvessels were identified by using scanner objective (4x). Individual microvessels were counted using high power objective (40x). MVD is defined as the number of manually counted vessel profiles per high power field taken as the average from three hot spot counts.

Data analysis

The expression of VEGF and the angiogenic potential (microvascular density) in meningioma were analyzed. These features were correlated with the subtype and grades of meningiomas. The data analysis was performed with SPSS 15.0 software. The comparison of discrete variables, namely VEGF expression and VEGF score, between groups was done using chi square test/Fisher's exact test. The comparison of continuous variables, namely age, MIB-LI, and microvascular density, between groups was done using independent sample t test/Wilcoxon Mann Whitney test depending upon the normality of the variable. A P value of <0.05 was considered significant. All P values were tested for two-sided hypotheses.

Results

Of the 46 patients, females comprised 78.3% and males 21.7% of the cohort. The age of the patients ranged from 24 to 65 years (mean age = 44.72 yrs; median = 45 yrs). The duration of symptoms before seeking treatment ranged from 1 month to 120 months (mean ± SD = 10.17 ± 18.99 months). 76.1% of patients presented with raised intracranial pressure. Seizures were noted in 19.6% of patients. 43.5% of patients had visual loss, either due to direct compression of optic nerves by the tumor or due to raised intracranial pressure. Cognitive symptoms were noted in five patients. The parasagittal and falx location was the most common location (21.7%), followed by convexity and parasellar location (17.4%) each. The study consisted of 40 Grade I meningiomas, 4 Grade II tumors, and 2 Grade III tumors. Among the Grade I meningiomas, transitional meningioma was the most common histological subtype, comprising about 47.8% of the patients. Meningothelial subtype was the second most common, comprising 19.6% followed by fibroblastic meningioma. There were three cases of meningioma which had features of brain invasion, which were classified as Grade II, though the histological features were characteristic of transitional meningioma.

The MIB-LI ranged from 0.10% to 30%, with the mean ± SD = 1.97 ± 4.79%. The mean MIB-Li in Grade I was 0.8%, while that in Grade II and III were 4.38% and 20.5%, respectively. The difference in mean MIB-Li among various grades was found to be statistically significant, as expected (P < 0.001).

VEGF expression in meningioma

The VEGF protein expression was assessed by staining with anti-VEGF antibody. While 14 (30.4%) tumors showed no staining with antibody, 32 (69.6%) were positive for VEGF. VEGF protein was expressed in tumor cells as well as in the endothelial cells of adjacent microvessels in some cases. In some tumors, the VEGF was focally expressed, while in some the tumor diffusely expressed VEGF protein. VEGF expression was found only in the cytoplasm of tumor cells in 26.1% cases, while both tumor cells and vessels expressed VEGF in 43.5% cases. The VEGF was focally positive in 34.8% (16/46) cases, while another 34.8% (16/46) tumors diffusely expressed VEGF. The VEGF expression in various tumors was scored, as described in methods, based on the intensity and extent of the staining [Table 1]. Fourteen (30.43%) tumors were negative for VEGF, 16 (34.78%) tumors showed weak staining, 13 (28.3%) tumors showed weak to moderate staining, and 3 (6.5%) tumors demonstrated moderate-strong staining.[Table 1]

Analysis of VEGF expression across tumor grades

Twenty-six out of forty (65%) Grade I tumors showed VEGF positivity, while 100% of Grade II (4/4) and Grade III (2/2) tumors were VEGF positive. Considering the site-specific staining of VEGF across the grades, while 15/40 Grade I tumors showed staining in both tumor cells and endothelial cells in blood vessels, 3 out of 4 Grade II tumors and 2 out of 2 Grade III tumors showed staining in both tumor cells and endothelial cells. While 11 out of 40 Grade I tumors showed diffuse VEGF expression, 3 out of 4 Grade II tumors and 2 out of 2 Grade III tumors showed diffusely positive VEGF expression. The VEGF expression of tumors of various grades as classified based on VEGF score is tabulated in [Table 1].

Analysis of VEGF expression across histological subtypes

The VEGF expression in various histological subtypes is tabulated [Table 2]. Among the Grade I tumor subtypes, angiomatous meningioma had the maximum VEGF expression due to the inherent nature of the tumor. Fibroblastic meningioma had the least expression and the positivity was found focally in few tumor cells [Figure 1a,b]. Only one out of eight fibroblastic meningioma expressed VEGF. Among the common Grade I tumors, transitional meningioma had a significantly high VEGF expression as compared to fibroblastic meningioma (81.8% vs.12.5%, respectively; P = 0.0005) [Figure 1]d,e]. Similarly, meningothelial meningioma also demonstrated significantly higher VEGF expression as compared to fibroblastic meningioma (P = 0.02). There was no significant difference in VEGF expression between transitional and meningothelial meningioma (P = 0.36). Among the Grade II and Grade III tumors, a case of papillary and atypical meningioma showed the maximum strong VEGF expression in tumor cells [Figure 1]g,h. [Figure 1][Table 2]

Microvascular density in meningioma and correlation to VEGF

Microvascular density in meningioma was assessed by performing CD34 immunohistochemistry. The calculation of microvascular density with CD 34 IHC was done as described by Weidner et al. [14] It was noted that the microvascular density among meningiomas in the present series ranged from 2 to 50 hpf, with a mean of 15.13 hpf [Figure 1c,f]. The vascular network was well developed in most of the meningiomas. The vessels had thin walls and were lined by a single layer of endothelial cells.

The mean microvascular density in VEGF negative tumors was 9.00, while that of VEGF-positive tumors was 17.81, the difference being statistically significant (P = 0.013). Also, on comparing the microvascular density according to the VEGF score, it was found that there is 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) [Figure 2]. This demonstrates that VEGF-positive tumors had higher microvascular density than VEGF-negative tumors irrespective of tumor grade. Furthermore, the microvascular density correlated with the intensity and extent of the VEGF expression in meningioma.[Figure 2]

Analysis of factors between benign and high grade meningiomas

A comparison of various factors between low and high grade meningiomas was made [Table 3]. As the Grade II and Grade III meningiomas were in small number (n = 4 and n = 2, respectively), they were grouped together to form the high grade meningioma cohort. The VEGF expression was not significantly different between benign and high grade meningioma (P = 0.157). The microvascular density was significantly higher in high grade meningiomas in comparison to benign tumors (P = 0.022). [Table 3]

Discussion

Angiogenesis in meningioma is an important component which has been evaluated in detail to understand the behavior of the tumor in terms of recurrence and eliciting brain edema. Of the many factors postulated to induce angiogenesis in meningioma, VEGF has been shown to promote migration, proliferation, and tube formation of endothelial cells. Its upregulation in meningiomas has been correlated with the development of brain edema secondary to these tumors. [8],[9] The expression of VEGF in meningiomas has been evaluated in some studies both at mRNA levels and protein levels. [11],[16] The present study evaluated the VEGF protein expression and noted that 30.4% tumors showed no staining with antibody, while 69.6% were positive for VEGF. The pattern of VEGF expression has been variably reported in the literature. Some authors reported that VEGF expression was specific to the tumor cells and was not present in tumor stroma, connective tissue, or endothelial cells, [10],[11] while another study noted that VEGF expression was found in the neighboring endothelial cells as well. [17] We noted that VEGF expression was found only in tumor cells in 26.1% cases, while both tumor cells and vessels expressed VEGF in 43.5% cases. A paracrine role of VEGF in inducing angiogenesis can be postulated by its presence both in tumor cells as well as in neighboring cells. Similar expression of VEGF in both tumor cells and surrounding vessels in gliomas has been reported earlier. [18],[19],[20]
The extent of VEGF expression needs to be scored so that accurate classification and reporting is possible. As with any immunohistochemical evaluation, both the intensity of staining and the percentage of positive cells need to be taken into consideration. Various scoring methods have been described for VEGF immunohistochemistry in tumors. While some scoring systems use only the intensity of the staining to classify, [21] some use both the intensity of staining and percentage positivity. [13] The most comprehensive and simple method was described by Raica et al. which included both the intensity and percentage positivity, which has been used in the present study for analysis. [13] 34.8% cases in the present study demonstrated score 1, 28.3% cases demonstrated score 2 and 6.5% cases demonstrated score 3.

Meningiomas are rich in vascularity, which varies within the subtypes and grades. Neangiogenesis in meningiomas can be quantified by evaluation of MVD, which reflects the number of vessels per square mm. Vessels identified on histological sections can be highlighted by staining endothelial cells for endothelial markers with standard immunohistochemistry, which can be contrasted at light microscopy. The variations in microvessel density assessment can occur due to the choice of endothelial marker used based on its sensitivity, the different cut off values used for counting microvessels, the low power magnification used to count microvessels, the number of endothelial markers used, the extent of expression of VEGF and the expression pattern (focal or diffuse) did not significantly differ between the grades. Though all the higher grade (Grade II and Grade III) tumors were VEGF positive in our series and only 26 out of 40 benign tumors were positive for VEGF, the difference still did not reach statistical significance. (P = 0.157) It is possible that the small number of cases in the present series could be the reason for lack of significance among the grades. However, previous studies too have reported similar results, [9],[11] [26] pointing to a conclusion that probably VEGF expression does not correlate with the grade of the tumor.

Interestingly, the VEGF expression differs among the histological subtypes within the WHO Grade I group. We noted that transitional meningioma has a high VEGF positivity (81.8%), followed by meningothelial meningioma (66.7%), while fibroblastic meningioma demonstrated the least VEGF expression (12.5%). VEGF expression in the other histological meningioma subtypes like metaplastic and meningioma with prominent granular inclusions could not be commented because of the small number in series. Angiomatous meningioma expresses a very high VEGF expression due to the inherent feature of the subtype. The VEGF expression in transitional meningioma is similar to the higher grade tumors, while the VEGF expression in fibroblastic subtype is significantly less. Derzivo et al. demonstrated, by a quantitative analysis of VEGF by ELISA, that fibroblastic meningiomas exhibited lower VEGF contents as compared to meningothelial and transitional subtypes. [26] The present study results concur with the previous studies in literature. Thus, it is clearly evident that the angiocytic potential of meningioma is a characteristic of histological subtype and is not governed by the grade of meningioma.

Some studies have earlier reported a significant correlation with the tumor microvascular density and VEGF mRNA expression in meningioma (P = 0.0005). [9],[11] In contradiction to these studies, Trenda et al. could not demonstrate any correlation between the microvascular density and the VEGF expression in the most common types of benign and atypical meningiomas and postulated that angioiagnosis probably depends on factors other than VEGF. [17] Interestingly, we noted in the present series that the mean vascular density in VEGF-positive tumors was 9.00, while that of VEGF-negative tumors was 17.81, the difference being statistically significant (P = 0.013). Also, in addition, we noted that the vascular density gradually increased with the increase in VEGF score. This demonstrates that the MVD of meningioma correlated with the intensity and extent of the VEGF expression in meningioma, irrespective of the tumor grade.

Conclusions

Thus, it is evident that VEGF expression in meningioma has a significant role in inducing the neoangiogenesis in meningioma, as demonstrated by its association with microvascular density (MVD). VEGF expression correlated with the angioiagnostic potential (as measured by MVD) in meningioma irrespective of tumor grade, with a gradual increase in MVD in relation to the VEGF score. The study highlights that the angioiagnostic potential of meningioma is a characteristic of histological subtype and is not governed by the grade of meningioma.

References