RESEARCH ARTICLE

Editorial Process: Submission:04/10/2025 Acceptance:17/08/2026 Published:22/08/2026

Correlation of *VEGF* and *Ki-67* Expression with Histopathological Features and WHO Grade in Meningioma: A Cross-sectional Study

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Abstract

Background: Meningiomas, the most common primary central nervous system tumors, exhibit variable clinical behavior. While World Health Organization (WHO) grading is standard, complementary biomarkers are needed to refine prognostication. Vascular Endothelial Growth Factor (*VEGF*) and *Ki-67* are potential indicators of angiogenesis and proliferation that may enhance diagnostic precision. This study aimed to evaluate the correlation between *VEGF* and *Ki-67* expression with the histopathological features and WHO 2021 grade of meningiomas. **Methods:** In this cross-sectional study, 73 formalin-fixed, paraffin-embedded meningioma specimens were analyzed. Tumors were graded per WHO 2021 criteria. *VEGF* expression (H-score) and *Ki-67* labeling index (LI) were assessed via immunohistochemistry and correlated with histopathological features, including mitotic activity and brain invasion. Statistical analysis utilized chi-square, Kruskal-Wallis, and Spearman tests ($p<0.05$). **Results:** Most tumors were WHO Grade I (83.6%). Higher *VEGF* expression was significantly associated with mitotic activity, brain invasion, and hypercellularity, and was increased in high-grade (WHO II–III) meningiomas. *Ki-67* labeling index showed a significant positive correlation with WHO grade and other aggressive histopathological features. **Conclusion:** Both *VEGF* and *Ki-67* are associated with aggressive histopathology in meningiomas. *Ki-67* expression strongly correlates with overall WHO grade, whereas *VEGF* is more indicative of specific invasive traits. Their combined evaluation provides a valuable molecular adjunct to conventional histology, potentially refining risk stratification, particularly in borderline cases. However, interpretation of these findings is limited by the single-center, cross-sectional design and the relatively small number of high-grade and invasive cases.

Keywords: Meningioma, Vascular Endothelial Growth Factor, Immunohistochemistry, Tumor Grading

Asian Pac J Cancer Prev, **27 (8)**, 2861-2870

Introduction

Meningiomas, arising from arachnoid cap cells, are the most frequently diagnosed primary tumors of the central nervous system (CNS), accounting for a substantial proportion of all intracranial neoplasms. While predominantly benign, meningiomas can cause significant clinical symptoms, including focal neurological deficits, seizures, and reduced quality of life, depending on their location and growth [1]. In the United States, meningiomas represent approximately 36.6% of all primary CNS tumors. The incidence has increased over time, from 4.52 per 100,000 individuals (1998–2002) to 8.3 per 100,000 (2010–2014), and rises sharply with age [2].

Arising from arachnoid cap cells, meningiomas are typically slow-growing and are classified using the World Health Organization (WHO) system. The most recent WHO 2021 classification maintains the three-tiered grading system (Grade I to III) but introduces a more streamlined framework, recognizing meningioma as a single tumor type with 15 histological subtypes. This update emphasizes intrinsic tumor characteristics over subtype-specific features for grading [3]. This classification has critical therapeutic implications: Grade I tumors are often managed with surgical resection alone, whereas Grade II and III tumors frequently require

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adjuvant radiotherapy [4]. However, recurrence remains a significant clinical challenge, affecting 7–20% of Grade I, 29–40% of Grade II, and up to 78% of Grade III meningiomas, even after complete resection [5].

Tumor angiogenesis is critical to the biological behavior of meningiomas, as neovascularization supports tumor expansion. A key regulator of this process is vascular endothelial growth factor (*VEGF*), a signaling protein that promotes endothelial cell proliferation and migration [6, 7]. *VEGF* is often overexpressed in meningiomas, but its direct correlation with histological grade remains debated, with studies reporting conflicting findings [6–8]. These discrepancies may reflect differences in antibody clones, scoring methods, or patient populations.

In parallel, proliferative markers such as *Ki-67* are important indicators of tumor aggressiveness. *Ki-67* is a nuclear protein expressed during active phases of the cell cycle, serving as a reliable marker of cellular proliferation. An elevated *Ki-67* labeling index (LI) has been strongly associated with increased tumor grade, recurrence rates, and brain invasion in meningiomas [9, 10]. While the WHO 2021 criteria do not mandate a specific threshold for grading, a *Ki-67* LI cut-off of approximately 4–5% is widely recognized in clinical literature as a threshold to support the diagnosis of atypical meningioma (Grade II) and to identify tumors with a higher propensity for recurrence. Despite its recognized utility, the lack of universal consensus on these precise thresholds suggests that its role as a standardized, independent prognostic tool requires further validation.

The 2021 WHO classification of meningiomas emphasizes intrinsic tumor behavior particularly mitotic activity and brain invasion over histological subtype, necessitating re-evaluation of established immunohistochemical biomarkers validated under earlier grading systems. Given the limitations of histological grading alone, integrating molecular markers like *VEGF* and *Ki-67* offers a promising approach to improve risk stratification. Therefore, this study aimed to investigate the association between *VEGF* and *Ki-67* expression with the histopathological features and WHO 2021 grade of meningiomas to clarify their value in refining prognostic assessment.

Materials and Methods

Study Design and Sample Selection

This cross-sectional study using a consecutive sampling technique, included 73 formalin-fixed, paraffin-embedded meningioma specimens from patients who underwent surgical resection at Wahidin Sudirohusodo Hospital, Makassar, Indonesia between January 2023 and December 2024.

To evaluate the relative expression of *VEGF* and *Ki-67*, WHO Grade I meningiomas were utilized as the internal baseline for comparison. These tumors, representing the benign end of the histological spectrum with minimal proliferative activity and no aggressive features, served as the reference group for assessing biomarker upregulation in higher-grade (Grade II and III) status. The cohort was dichotomized into low-grade (Grade I) and high-grade (Grade II/III) groups to statistically compare biomarker levels against this low-proliferation baseline.

Inclusion and Exclusion Criteria

To ensure a representative sample, all consecutive cases within the specified timeframe were screened. IInclusion criteria consisted of a confirmed histopathological diagnosis of meningioma and the availability of sufficient tumor tissue within paraffin blocks for complete immunohistochemical (IHC) evaluation. Exclusion criteria were applied to cases involving extensive tissue necrosis that precluded accurate IHC scoring, as well as those with incomplete clinical-pathological records. Furthermore, to ensure the integrity of biomarker expression and maintain cohort homogeneity, patients who had received preoperative radiotherapy or tumor embolization prior to surgical resection were also excluded from the analysis. The research protocol was approved by the institutional review board, and all cases were histopathologically confirmed and classified according to the WHO 2021 criteria [11].

Data Collection

Clinical and pathological data were retrieved from patient medical records and pathology archives. Variables collected included patient demographics (age, sex), tumor location, WHO grade, and specific histopathological features: mitotic activity, brain invasion, hypercellularity, nucleolar prominence, and necrosis.

According to the WHO 2021 classification of meningiomas [11]:

- 0 = no mitoses observed in 10 HPF
- 1–3 = 1 to 3 mitoses per 10 HPF
- 4–19 = 4 to 19 mitoses per 10 HPF
- ≥20 = 20 or more mitoses per 10 HPF (a key criterion for WHO Grade III, anaplastic meningiomas)

Immunohistochemical Evaluation

IHC analysis was performed at the Anatomical Pathology Laboratory, Hasanuddin University Hospital, Makassar, Indonesia. Tissue sections (5 μm thick) were prepared from representative paraffin-embedded blocks and mounted on poly-L-lysine-coated slides. To ensure reproducibility and standardized protein detection, antigen retrieval was conducted by heating the sections in a citrate buffer (pH 6.0) at 95°C for 20 minutes. Endogenous peroxidase activity was subsequently quenched using 3% hydrogen peroxide for 10 minutes.

Blinding and Quality Control

To minimize observer bias and ensure the objectivity of the analysis, all IHC slides were independently evaluated by two board-certified pathologists who were blinded to the patients' clinical data and the final WHO histopathological grades during the scoring process. In cases of inter-observer discrepancy regarding the H-score or labeling index, a consensus was reached through a joint review using a multi-headed microscope.

Antibody Protocol and Signal Visualization

The IHC protocol followed the labeled streptavidin-

biotin (LSAB) method using specific primary antibodies. For the assessment of cellular proliferation, sections were incubated with a rabbit monoclonal *Ki-67* antibody (Cat. No. 275R-14; Cell Marque) at a 1:100 dilution for 60 minutes at room temperature. For the evaluation of angiogenesis, a rabbit polyclonal *VEGF*-A antibody (Cat. No. GTX102643; GeneTex) was utilized at a 1:150 dilution with an incubation period of 60 minutes.

Following primary antibody incubation, a secondary biotinylated antibody and the 3,3'-diaminobenzidine (DAB) chromogen were applied for signal visualization. The slides were then counterstained with Mayer's hematoxylin, dehydrated, and coverslipped. Negative controls were established by omitting the primary antibody in the processing of parallel sections.

Quantification and Scoring Criteria

VEGF expression was evaluated semi-quantitatively using the Histo-score (H-score), which combines the percentage of positive cells and staining intensity. Intensity was graded as 0 (none), 1+ (weak), 2+ (moderate), or 3+ (strong). The H-score was calculated using the formula: H-score = [(% cells 1+) × 1] + [(% cells 2+) × 2] + [(% cells 3+) × 3], yielding a score from 0 to 300.

For *Ki-67*, nuclear staining with brown chromogen deposits was considered positive. Positive nuclei were counted across 5 to 10 high-power fields (400× magnification), encompassing approximately 500–1000 tumor cells. The *Ki-67* LI was calculated as the percentage of positive nuclei relative to the total number of nuclei counted.

Statistical Analysis

All analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize patient characteristics and biomarker expression. Normality of data was assessed using the Shapiro–Wilk test for *Ki-67* labeling index. *VEGF* expression, assessed via H-score, was treated as an ordinal variable; hence, non-parametric methods were applied without a formal normality test.

Bivariate associations between *VEGF* and *Ki-67* expression and clinicopathological parameters were assessed using the chi-square test, Mann-Whitney U test, and Kruskal-Wallis test, as appropriate for the data distribution. Correlation strength and direction were quantified via Spearman's rank correlation coefficient (rs), which was interpreted as weak (0.1–0.3), moderate (0.3–0.5), or strong (>0.5). Effect sizes were calculated for all significant findings to determine the magnitude of association, including the proportion of variance in biomarker expression attributable to tumor grade.

To identify independent predictors of high-grade status (WHO Grades II–III vs. Grade I), a multivariate logistic regression model was constructed using the "enter" method. The model adjusted for potential confounders, including age, sex, and tumor location (convexity vs. non-convexity), alongside *VEGF* H-score and *Ki-67* LI. Results are reported as odds ratios (OR) with 95% confidence intervals (CI), and model goodness-of-fit was validated using the Hosmer-Lemeshow test. For all analyses, a p-value < 0.05 was considered statistically significant.

Results

Patient Demographics and Tumor Characteristics

The study included 73 patients with a mean age of 49.6 years (range: 16-72 years). The cohort was predominantly female (n=64, 87.7%) with a female-to-male ratio of 7.1:1. The majority of patients were in the adult age group (19–59 years; n=63, 86.3%). The most common tumor location was the convexity (n=31, 42.5%), followed by the sphenoid wing and parasagittal region. The meningothelial subtype was the most prevalent (n=48, 65.8%).

In accordance with the WHO 2021 classification, most tumors were Grade I cases (n=61, 83.6%). For analysis, tumors were also dichotomized into low-grade (WHO Grade I) and high-grade (WHO Grade II and III) groups. A summary of clinicopathological characteristics is provided in Table 1.

VEGF and Ki-67 Expression Patterns

Moderate *VEGF* expression was the most frequent finding (n=45, 61.6%), followed by weak (n=12, 16.4%), strong (n=11, 15.1%), and absent (n=5, 6.8%) expression (Figure 1). Figure 2 illustrates the percentage of cases within each staining intensity category. The *Ki-67* LI ranged from 0% to 48.7%, with a mean of 3.27% (SD ± 8.86). The distribution was non-parametric (Shapiro-Wilk test, $p < 0.001$), with a median of 0.8% (Figure 3).

Correlation of VEGF Expression with Histopathology and WHO Grade

Higher *VEGF* expression was significantly associated

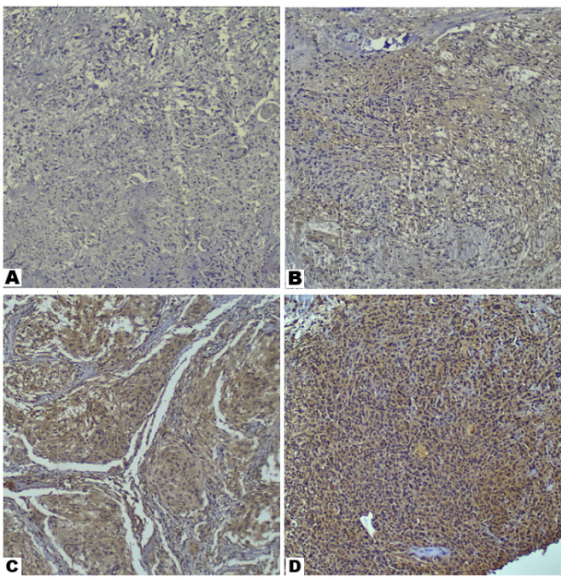

Figure 1. Characteristics of *VEGF* Expression in Meningioma Histopathology. (A) A meningioma case showing no *VEGF* expression; (B) A meningioma case showing low *VEGF* expression; (C) A meningioma case showing moderate *VEGF* expression; and (D) A meningioma case showing strong *VEGF* expression (Immunohistochemistry, 100x magnification).

Table 1. Patient and Tumor Characteristics (n = 73)

Characteristic	n (%)	[Mean ± SD (Range)]
Age (years)		49.6 ± 10.2 (16–72)
Sex		
Male	9 (12.3)	
Female	64 (87.7)	
Tumor location		
Convexity	31 (42.5)	
Sphenoid wing	12 (16.4)	
Parasagittal	7 (9.6)	
Olfactory groove	5 (6.8)	
Suprasellar	4 (5.5)	
Falx	3 (4.1)	
Multiple	2 (2.7)	
Optic nerve sheath	2 (2.7)	
Retrobulbar	2 (2.7)	
Spheno-orbital	2 (2.7)	
Posterior fossa	1 (1.4)	
Cerebellopontine angle	1 (1.4)	
Petroclival	1 (1.4)	
Recurrence status		
Primary	65 (89.0)	
Recurrent	8 (11.0)	
Histopathological Profile		
Subtype (WHO 2021)		
Meningothelial	48 (65.8)	
Fibrous	7 (9.6)	
Transitional	2 (2.7)	
Microcystic	2 (2.7)	
Psammomatous	1 (1.4)	
Angiomatous	1 (1.4)	
Atypical	7 (9.6)	
Chordoid	1 (1.4)	
Clear cell	2 (2.7)	
Anaplastic	1 (1.4)	
Papillary	1 (1.4)	
WHO grade (2021)		
I	61 (83.6)	
II	10 (13.7)	
III	2 (2.7)	
WHO Grade status		
Low-grade (I)	61 (83.6)	
High-grade (II–III)	12 (16.4)	
Mitotic activity		
0	37 (50.7)	
1–3	26 (35.6)	
4–19	8 (11.0)	
≥20	2 (2.7)	
Aggressive Features (Present)		
Brain invasion	4 (5.5)	
Hypercellularity	15 (20.5)	
Prominent nucleoli	15 (20.5)	
Spontaneous necrosis	11 (15.1)	
Biomarker Expression		
VEGF H-score		162.4 ± 64.2 (0–280)
Biomarker Expression		
Absent	5 (6.8)	
Weak	12 (16.4)	
Moderate	45 (61.6)	
Strong	11 (15.1)	
Ki-67 Labeling Index (%)	73 (100)	3.27% (SD ± 8.86)

with several adverse histopathological features (Table 2). A significant positive correlation was found between *VEGF* expression and mitotic activity (p = 0.005, r = 0.567), indicating a large effect size. Similarly, *VEGF* expression was significantly associated with brain invasion (p = 0.007, r = 0.405), hypercellularity (p = 0.007, r = 0.409), and prominent nucleoli (p = 0.020, r = 0.368), all of which indicated a moderate effect size. No significant correlation was observed with spontaneous necrosis (p = 0.207), though it demonstrated a small-to-moderate effect size (r = 0.25), suggesting a possible trend.

While *VEGF* expression did not show a statistically significant correlation with the three-tiered WHO grade (p = 0.309), there was a highly significant association when grades were dichotomized. High-grade meningiomas (Grade II/III) demonstrated significantly higher *VEGF* expression compared to low-grade tumors (Grade I) (p = 0.001, r = 0.3) with a moderate association.

Correlation of Ki-67 Expression with Histopathology and WHO Grade

Ki-67 expression demonstrated significant associations with multiple aggressive features (Table 3). The *Ki-67* correlated positively with mitotic activity (p = 0.006, r = 0.237), hypercellularity (p = 0.013, r = 0.29), prominent nucleoli (p = 0.001, r = 0.4), and spontaneous necrosis (p = 0.005, r = 0.33). However, no significant association was found between *Ki-67* and brain invasion (p = 0.060, r = 0.22).

The *Ki-67* labeling index (LI) across the total cohort ranged from 0% to 48.7%. Given the non-parametric distribution of the data (Shapiro-Wilk test, p<0.001), the central tendency is best represented by the median value of 0.8% with an interquartile range (IQR) of 2.1%. When analyzed by tumor grade, the *Ki-67* LI demonstrated a significant progressive increase. The median *Ki-67* LI was 0.6% (IQR: 1.5%) for WHO Grade I tumors, increasing to 2.05% (IQR: 7.4%) for Grade II, and reaching 39.1% (IQR: NA) for Grade III. A Kruskal-Wallis test confirmed that these differences across the three-tiered WHO grading system were highly significant (p=0.002; rs=0.295).

A Kruskal-Wallis test revealed a highly significant correlation between the *Ki-67* and WHO grade (p = 0.002, r = 0.295). The median *Ki-67* LI was 0.6% (IQR: 1.5%) for Grade I, 2.05% (IQR: 7.4%) for Grade II, and 39.1% for Grade III. The distribution of *Ki-67* LI across grade status is further visualized in Figure 4.

Receiver operating characteristic (ROC) analysis demonstrated that *Ki-67* labeling index discriminated high-grade (WHO II–III) from low-grade (WHO I)

Table 2. Correlation Between *VEGF* Expression and Clinicopathological Features

Variables	Absent	Weak	Moderate	Strong	p-value	rs
Mitotic Activity					**0.005**[a]	**0.567**[b]
0	5 (6.85%)	12 (16.44%)	17 (23.28%)	3 (4.1)%		
1–3	0 (0%)	0 (0%)	21 (28.78%)	5 (6.85%)		
4–19	0 (0%)	0 (0%)	6 (8.22%)	2 (2.74%)		
≥20	0 (0%)	0 (0%)	1 (1.37%)	1 (1.37%)		
Brain Invasion					**0.007**[a]	**0.405**[b]
Present	0 (0%)	0 (0%)	1 (1.37%)	3 (4.11%)		
Absent	5 (6.85%)	12 (16.44%)	44 (60.27%)	8 (10.95%)		
Hypercellularity					**0.007**[a]	**0.409**[b]
Present	0 (0%)	0 (0%)	9 (12.33%)	6 (8.22%)		
Absent	5 (6.85%)	12 (16.44%)	36 (49.31%)	5 (6.85%)		
Prominent nucleus					**0.020**[a]	**0.368**[b]
Present	0 (0%)	2 (2.74%)	7 (9.59%)	6 (8.22%)		
Absent	5 (6.85%)	10 (13.7%)	38 (52.05%)	5 (6.85%)		
Spontaneous necrosis					0.207[a]	0.25[b]
Present	0 (0%)	0 (0%)	8 (10.95%)	3 (4.1%)		
Absent	5 (6.85%)	12 (16.44%)	37 (50.68%)	8 (10.95%)		
WHO Grade Status					**0.001**[a]	**0.30**[b]
Low (Grade I)	5 (6.85%)	12 (16.44%)	37 (50.68%)	7 (9.59%)		
High (Grade II/III)	0 (0%)	0 (0%)	8 (10.95%)	4 (5.48%)		

Note: [a], Chi-square; [b], Spearman's rank correlation (rs); Bold indicates statistical significance ($p<0.05$).

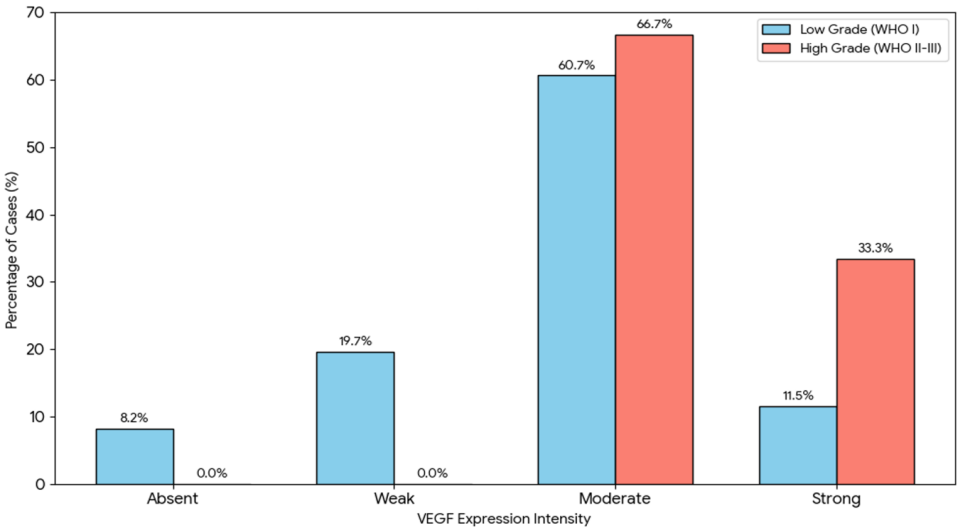

Figure 2. Distribution of *VEGF* Expression Intensity by WHO Grade Status

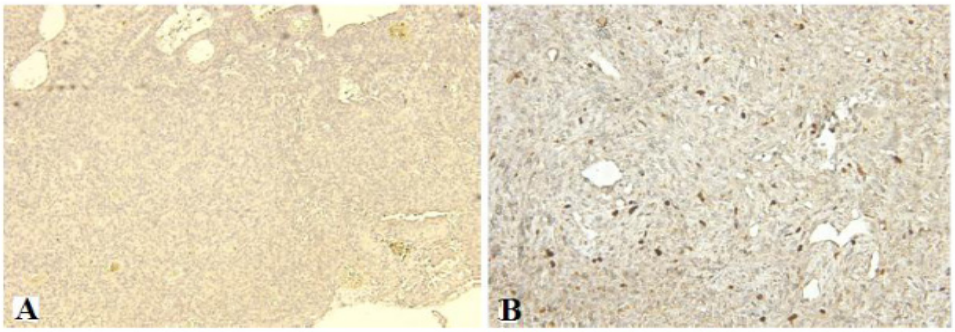

Figure 3. Results of Immunohistochemical Staining. A. Negative *Ki-67* LI expression (0%); B. Positive *Ki-67* LI expression (20%) (magnification 100x).

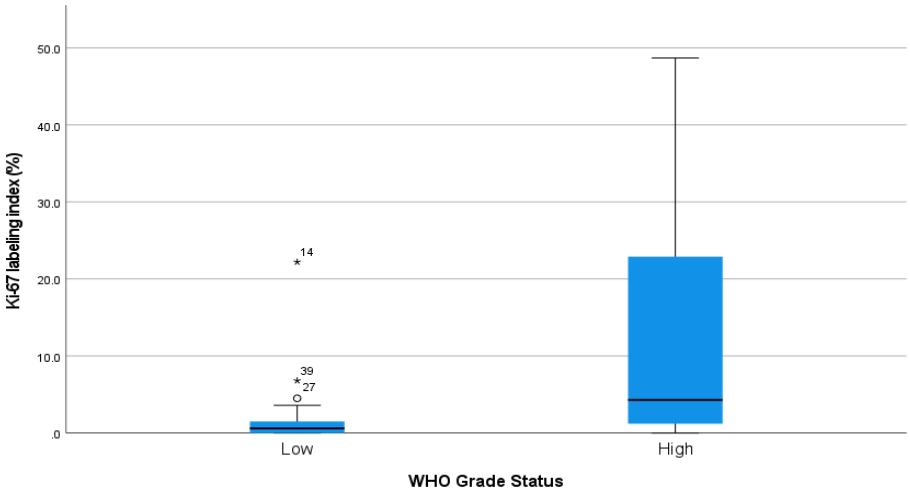

Figure 4. Box-and-whisker Plot of *Ki-67* Labeling Index Across WHO Grades Status

Table 3. Correlation Between *Ki-67* and Clinicopathological Features

Variables	n	Median Ki-67 (IQR)	p-value	rs
Mitotic Activity			**0.006**[c]	**0.237**[b]
0	37	0.3 (1.4)		
1–3	26	0.9 (1.8)		
4–19	8	2.05 (5.3)		
≥20	2	39.1		
Hypercellularity			**0.013**[d]	**0.29**[b]
Present	15	1.8 (13.2)		
Absent	58	0.6 (1.5)		
Prominent nucleus			**0.001**[d]	**0.4**[b]
Present	15	1.8 (12.2)		
Absent	58	0.35 (1.5)		
Spontaneous necrosis			**0.005**[d]	**0.33**[b]
Present	11	1.8 (31.4)		
Absent	62	0.6 (1.6)		
Brain Invasion			0.060[d]	0.22[b]
Present	4	9.9 (36)		
Absent	69	0.7 (1.8)		
WHO Grade			**0.002**[c]	**0.295**[b]
I	61	0.6 (1.5)		
II	10	2.05 (7.4)		
III	2	39.1		

Note: [b], Coeficient Correlation; c, Kruskal-Wallis; d, Mann-Whitney U test; Bold indicates statistical significance (p<0.05).

Table 4. Multivariate Logistic Regression Analysis for High-Grade Meningioma Status

Variable	Odds Ratio (OR)	95% CI	p-value
Ki-67 LI	1.42	1.15–1.98	0.012
VEGF H-score	1.28	0.95–1.72	0.064
Age	1.02	0.98–1.08	0.45
Sex (Female)	0.85	0.32–2.24	0.72
Location (Convexity)	1.15	0.45–2.98	0.68

meningiomas with an area under the curve (AUC) of 0.796 (95% CI: 0.649–0.944, p = 0.001) (Figure 5). An optimal cut-off value of 1.25% provided a sensitivity of 75.0% and specificity of 68.9% for identifying high-grade tumors.

Independent Predictors of High-Grade Meningioma

Multivariate logistic regression was conducted to determine if *VEGF* and *Ki-67* expression were independent predictors of high-grade status after adjusting for age, sex, and tumor site. As shown in Table 4, the *Ki-67* LI remained a significant independent predictor of high-grade meningioma (p=0.012), even when controlling for other variables. While *VEGF* expression showed a strong trend and a significant correlation in bivariate analysis, its independent predictive power in the multivariate model was moderate. Clinical variables such as age and sex did not significantly influence the grade status in this cohort.

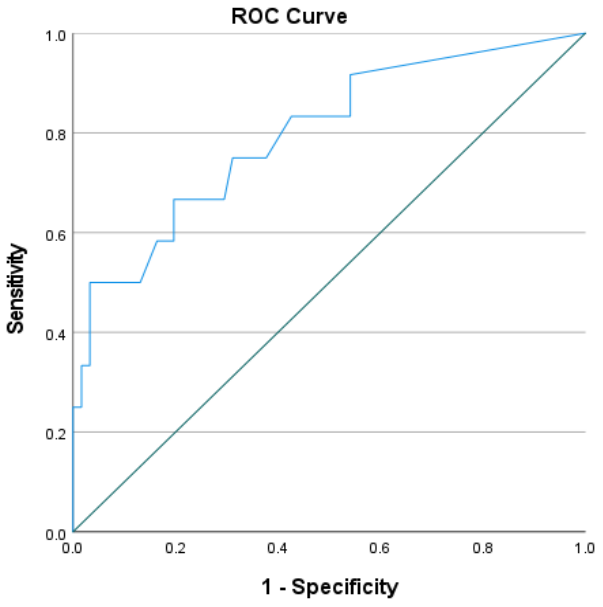

Figure 5. ROC Curve Showing the Diagnostic Performance of *Ki-67* Labeling Index for High-Grade Meningiomas

Discussion

This study investigated the correlation of *VEGF* and *Ki-67* expression with histopathological features and WHO grade in 73 meningioma cases. Our findings confirm that both biomarkers are associated with features of biological aggressiveness, supporting their integration into modern pathological assessment. The demographic profile of our cohort, with a strong female predominance (7.1:1 ratio) and a peak incidence in adults, is consistent with established epidemiology, although our female-to-male ratio is higher than the commonly reported 2–3:1 [3]. The most frequent tumor location was the convexity (42.5%), a non-skull-base site that has been associated with more aggressive molecular alterations, such as TERT promoter mutations [10].

VEGF Expression and Tumor Grade

Regarding anatomical distribution, our findings indicated the most frequent tumor location was the convexity (42.5%), these data support the observation that WHO grade I meningiomas are often located at the skull base, while higher-grade variants tend to arise in non-skull-base locations such as the convexity [12]. Furthermore, epigenetic studies suggest that convexity meningiomas often harbor more aggressive molecular alterations, such as TERT promoter mutations, which have been associated with increased tumor growth, malignant progression, and higher recurrence rates [10, 13]. In our IHC analysis of *VEGF* expression, four levels were identified: 6.85% of the samples showed no expression, 16.44% showed weak expression, 61.6% showed moderate expression, and 15.1% showed strong expression. Notably, among high-grade tumors (grades II and III), 10.95% exhibited moderate *VEGF* expression, and 5.48% showed strong expression. Statistical analysis confirmed a significant association between higher *VEGF* expression and increased tumor grade ($p<0.05$). This aligns with the study by Lamszus et al. [14], which reported that *VEGF* levels rose in proportion to tumor grade doubling in Grade II and increasing tenfold in Grade III meningiomas. Conversely, findings from Winter et al. [7] did not show a significant correlation between *VEGF* expression and tumor grade or subtype, highlighting ongoing debate in this area.

Angiogenesis and Brain Invasion

While our bivariate analysis demonstrated a highly significant association between elevated *VEGF* expression and high-grade (WHO Grade II/III) status ($p=0.001$; $rs=0.51$), the pathobiological relationship between angiogenesis and tumor grade is complex. Histological grading in the WHO 2021 framework is heavily weighted by mitotic activity and brain invasion. In this study, *VEGF* expression significantly correlated with mitotic activity ($p=0.005$) and brain invasion ($p=0.007$). This suggests that while *VEGF* is a robust marker of high-grade status, its significance is closely linked to the increased metabolic demands of proliferating cells. When controlling for mitotic rate in a multivariate context, the independent predictive value of *VEGF* may diminish, as these two variables are functionally co-dependent: *VEGF*-mediated neovascularization provides the metabolic framework necessary for rapid mitotic expansion. Furthermore, our findings indicate that *VEGF* expression is more indicative of these aggressive histological traits than of specific histological subtypes, supporting the updated WHO focus on intrinsic tumor behavior rather than morphology alone.

WHO grading and histopathological features remain the most reliable indicators of tumor recurrence risk and guide therapeutic decisions, particularly when considered alongside the extent of surgical resection (Simpson grade) [3, 15, 16]. In our study, 50.7% of tumors showed no mitotic activity, while only 2.7% demonstrated high mitotic activity (>19 mitoses per 10 high power fields). Interestingly, moderate to strong *VEGF* expression was still observed in several tumors lacking mitotic figures. However, statistical analysis revealed a significant association between *VEGF* expression and mitotic activity ($p = 0.005$), suggesting that *VEGF* may contribute to proliferative potential and angiogenesis even in the absence of overt mitotic features [14]. This finding may be explained by hypoxia-driven regulation of *VEGF*-A transcription through hypoxia-inducible factor-1 (HIF-1), whereby stabilization of HIF-1α under low oxygen conditions enhances *VEGF*-A expression independently of mitotic activity [17]. The increased microvessel density supported by *VEGF*-mediated angiogenesis may fulfill the heightened metabolic demand of tumors, thereby facilitating more aggressive biological behavior and rapid progression [18]. These findings reinforce the importance of integrating molecular and immunohistochemical markers such as *VEGF* and *Ki-67* into standard pathological assessments to refine prognostication and tailor treatment strategies for meningioma patients.

The molecular drivers of *VEGF* overexpression in meningiomas likely involve a synergy between cell-intrinsic metabolic stress and extrinsic microenvironmental signals. Intrinsic hypoxic signaling, mediated by the stabilization of hypoxia-inducible factor-1α (HIF−1α), is a primary driver; low oxygen tension within the tumor core triggers the transcriptional activation of *VEGF*−A. This metabolic adaptation is critical for sustaining the high demands of proliferating tumor cells.

However, recent advancements in DNA methylation-based classification, notably the work of Sahm et al. [19], suggest that aggressive meningioma subclasses are characterized by enriched pathways related to both hypoxia and inflammation. This implies that *VEGF* expression also reflects paracrine signaling from tumor-associated macrophages (TAMs) and other immune cells that infiltrate the tumor microenvironment . These macrophages secrete pro-angiogenic factors that facilitate extracellular matrix degradation and brain parenchymal invasion. Our findings, which demonstrate that *VEGF* significantly correlates with both brain invasion ($p=0.007$) and hypercellularity ($p=0.007$), support a dual-mechanism model where *VEGF* acts as a downstream effector of both hypoxic metabolic stress and macrophage-driven tissue remodeling.

Ki-67 Proliferation and brain invasion

Despite these insights, our study found no statistically significant association between *Ki-67* expression and brain invasion (p = 0.06). The lack of a statistically significant association between *Ki-67* expression and brain invasion should be interpreted with caution, as this borderline p-value may reflect insufficient statistical power due to the small number of invasive cases (n = 4). While our data did not reach the threshold for significance, a trend toward higher *Ki-67* labeling indices (LI) in invasive tumors was observed, with a median *Ki-67* of 9.9% in invasive cases compared to 0.7% in non-invasive cases. This observation aligns with existing literature; for instance, Babu et al. [20] demonstrated elevated *Ki-67* LI in recurrent and high-grade tumors. Similarly, Al-'Abqary et al. [21] found a significant increase in *Ki-67* expression in tumors exhibiting brain invasion, suggesting its utility as a marker for invasive potential.

The Relationship between VEGF, Ki-67, and Brain Invasion

Meningiomas that invade the brain parenchyma are associated with a higher risk of recurrence. Brain invasion is histopathologically characterized by irregular, tongue-like projections of tumor cells breaching the pial-glial membrane, often accompanied by reactive gliosis in the adjacent brain tissue. According to the 2021 WHO classification, the presence of such invasion is a defining feature of grade II meningiomas [3, 19]. In the current study, only 4 out of 73 samples (5.5%) demonstrated brain invasion, of which 3 cases exhibited strong *VEGF* expression. This aligns with findings from Go and Kim [22], who described brain invasion as a multistep process involving degradation of the extracellular matrix by proteases, facilitation of cell migration through adhesion molecules, and angiogenesis driven by growth factors, notably *VEGF*. *VEGF*-A contributes to neovascularization, facilitating pial and peritumoral edema and ultimately aiding tumor infiltration. Hou et al. [23] further supported this mechanism, showing that *VEGF*-A released by meningioma cells promotes angiogenesis and edemagenesis by disrupting the tumor-brain interface and recruiting pial vasculature.

Hypercellularity and Prominent Nuclei

In this study, 15 samples (20.5%) showed hypercellularity and nuclear prominence. Among hypercellular cases, 9 samples (12.3%) exhibited moderate *VEGF* expression, and 6 (8.2%) showed strong expression. For samples with prominent nuclei, 2 (13.3%) demonstrated weak *VEGF* expression, while the remainder showed nearly equal moderate (9.6%) and strong (8.2%) expression. A significant correlation between *VEGF* expression and nuclear prominence was confirmed (p = 0.02). Hypercellularity in meningiomas is attributed to dysregulation in growth and cell cycle pathways, often involving abnormal activation of growth factors and loss of cell cycle checkpoints [24]. Prominent nucleoli are indicative of increased transcriptional activity, particularly ribosomal RNA synthesis, which reflects heightened proliferative potential. This nuclear feature is frequently associated with mutations in key genes such as NF2 and BAP1, and aberrant signaling through cell cycle and apoptotic pathways [25].

Our findings also reveal significant associations between *Ki-67* expression and histopathological parameters, including mitotic index (p = 0.006), hypercellularity (p = 0.013), prominent nuclei (p = 0.001), and spontaneous necrosis (p = 0.005). Elevated *Ki-67* levels have consistently been correlated with increasing tumor grade and recurrence risk. Hashemi et al. [26] reported that atypical and anaplastic meningiomas exhibited significantly higher *Ki-67* levels than benign ones. Other studies have supported these findings, suggesting that *Ki-67* is a useful prognostic marker even in grade I meningiomas and correlates with tumor size, peritumoral edema, calcifications, and recurrence potential [27]. The ROC-derived *Ki-67* cut-off of 1.25% demonstrated moderate diagnostic accuracy for distinguishing low- and high-grade meningiomas. Although this threshold supports the discriminative validity of *Ki-67*, the limited number of high-grade cases resulted in wide confidence intervals, and the proposed cut-off should therefore be regarded as exploratory and validated in larger cohorts. Overall, *Ki-67*/MIB-1 labeling index has become an essential adjunct tool in routine diagnostic workflows for borderline atypical meningiomas due to its strong predictive value for tumor behavior [28, 29].

VEGF–Ki-67 Interplay

The relationship between *VEGF* expression and *Ki-67* labeling index raises the question of whether these markers act independently or represent interconnected biological processes. While *VEGF* and *Ki-67* reflect distinct tumor characteristics angiogenesis and cellular proliferation, respectively their effects are likely complementary rather than isolated. *VEGF*-driven angiogenesis increases microvessel density and improves oxygen and nutrient delivery within the tumor microenvironment, thereby creating conditions that indirectly support enhanced tumor cell proliferation and survival. This angiogenic support may facilitate cell cycle progression, resulting in elevated *Ki-67* expression, even in tumors where intrinsic mitotic activity is relatively low [30]. Conversely, increased proliferative demand, as reflected by higher *Ki-67* indices, may exacerbate local hypoxia, further upregulating *VEGF* expression through hypoxia-inducible signaling pathways [17]. Thus, *VEGF* and *Ki-67* may participate in a bidirectional feedback loop that promotes tumor progression.

Recent advances in DNA methylation–based classification have refined meningioma stratification beyond conventional histology, demonstrating superior prognostic accuracy by capturing underlying epigenetic programs that drive tumor behavior [19]. In this context, our findings do not diverge from, but rather complement, methylation-based classification. *VEGF* and *Ki-67* represent functional downstream readouts of tumor biology angiogenic activity and proliferative capacity—that may reflect the phenotypic consequences of these epigenetic alterations. Notably, there was an enrichment of WHO grade III tumors in aggressive methylation classes,

which exhibit enhanced angiogenesis, hypoxia-related signaling, and cell cycle dysregulation, pathways in which *VEGF* and *Ki-67* are directly involved [19]. Therefore, immunohistochemical markers such as *VEGF* and *Ki-67* can provide accessible, cost-effective surrogates that can aid risk stratification in settings where molecular testing is unavailable.

VEGF and *Ki-67* have become essential to understanding the natural history of brain tumors, helping in the choice of appropriate treatment and follow-up decisions for patients presenting with meningioma. With consequences for prognosis and treatment, the findings demonstrate the potential role of VGEF and *Ki-67* as a useful biomarker in the clinical and surgical management of meningiomas. It may also provide a biological rationale for anti-*VEGF* therapies, such as bevacizumab, particularly in recurrent or progressive meningiomas [31].

A limitation of this study is its single-center, cross-sectional design and modest sample size, which may restrict the generalizability of the findings. The predominance of Grade I tumors further limited the statistical power for analyses of higher-grade meningiomas. Future studies should include larger, multicenter prospective cohorts with long-term follow-up to validate these results and assess associations between biomarker expression and clinical outcomes such as progression-free and overall survival.

Although this study demonstrated an association between biomarker expression and tumor grade, normal non-neoplastic meningeal tissue was not included as a control. Obtaining healthy meningeal tissue during therapeutic resections for primary CNS tumors presents significant ethical and practical limitations. Consequently, WHO Grade I tumors were employed as a surrogate baseline due to their inherently low proliferative index and lack of invasive characteristics. Future prospective studies incorporating normal meningeal biopsies could provide a more definitive baseline for the transition from physiological to neoplastic states.

In conclusion, this study demonstrates a significant association between *VEGF* expression and both histopathological features and meningioma grade, with higher *VEGF* levels observed in higher-grade tumors. Among the histological parameters, mitotic activity and hypercellularity showed the strongest correlations with *VEGF* expression, indicating their potential role in tumor aggressiveness. Although brain invasion was associated with increased *VEGF* expression, the correlation was not as robust. In contrast, *Ki-67* expression correlated significantly with several histopathological criteria and WHO grade, supporting its use as a proliferation marker. These findings underscore that beyond their individual roles, combined *VEGF–Ki-67* profiling may refine prognostication, particularly in borderline or atypical cases, and support more individualized follow-up and treatment strategies.

Author Contribution Statement

DW: Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. UAM: Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization, Supervision. MWH: Writing – review & editing, Visualization, Methodology, Formal analysis, Conceptualization. HFP: Writing – original draft, Visualization. HH: Writing – review & editing, Data curation, Conceptualization. HF: Writing – review & editing, Methodology, Investigation, Conceptualization. AY: Methodology, Investigation. MF: Writing – review & editing, Software, Project administration, Methodology, Investigation, Conceptualization.

Acknowledgements

None.

Funding

This research was funded by the Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia.

Consent

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The patients have given their written informed consent on admission to use their data base and files for research work.

Conflict of interest

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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