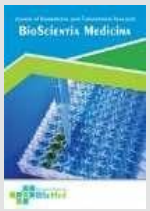
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Programmed Death-Ligand 1 (PD-L1) and Ki-67 Labeling Index: Correlation with Tumor Grade and Recurrence

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ARTICLE INFO

Keywords:
Ki-67
Meningioma
PD-L1
Recurrence
Tumor grade

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v9i1.1165

A B S T R A C T

Background: Meningiomas are common primary brain tumors with variable clinical behavior. While most are benign, a subset exhibits aggressive characteristics and a high recurrence rate. This study aimed to investigate the association of Programmed Death-Ligand 1 (PD-L1) and Ki-67 labeling index with histopathological grade and recurrence in meningioma. **Methods:** A retrospective study was conducted on 139 cases of meningioma diagnosed between January 2020 and December 2023. PD-L1 expression was evaluated using immunohistochemistry (IHC) and scored using the combined positive score (CPS). Ki-67 labeling index was determined as the percentage of tumor cells with positive nuclear staining. The association of PD-L1 and Ki-67 with histopathological grade and recurrence was analyzed. **Results:** PD-L1 expression was positive in 66.7% of cases and was significantly associated with recurrence ($p = 0.006$). A higher Ki-67 labeling index (>4%) was observed in 68.9% of cases and was also significantly associated with recurrence ($p = 0.013$). No significant association was found between PD-L1 expression or Ki-67 labeling index and histopathological grade. **Conclusion:** PD-L1 expression and Ki-67 labeling index may serve as potential prognostic markers for predicting recurrence in meningioma. Further studies are needed to validate these findings and explore their potential therapeutic implications.

1. Introduction

Meningiomas stand as the most prevalent primary intracranial neoplasms, originating from the arachnoid cap cells within the meninges, the protective layers enveloping the brain and spinal cord. While the majority of meningiomas exhibit benign characteristics, a subset displays aggressive behavior, characterized by a high propensity for recurrence, even following surgical interventions and radiotherapy. This aggressive variant poses a significant clinical challenge due to the absence of a standardized systemic treatment modality and the intricate nature of post-surgical management, often confounded by the lack of accurate prognostic markers to predict tumor behavior and guide therapeutic decisions. A deeper comprehension of the biological underpinnings driving tumor progression holds the key to identifying effective systemic therapies and ultimately improving outcomes for meningioma patients grappling with these formidable tumors. The World Health Organization (WHO) has established a grading system that serves as the cornerstone for meningioma assessment. The most recent iteration of the WHO guidelines, released in 2021, delineates 15 distinct subtypes of meningiomas, categorized into 3 grades based on meticulous histological criteria: benign (WHO grade I), atypical (WHO grade II), and anaplastic (WHO grade III). This grading system has profound clinical implications, as it intricately correlates with the risk of recurrence, overall survival prospects, and the strategic tailoring of treatment approaches. The estimated 10-year

relapse-free survival (RFS) rates for WHO grade I, II, and III meningiomas are 75-90%, 23-78%, and 0%, respectively, underscoring the gravity of tumor recurrence as a formidable clinical hurdle, even in the context of WHO grade I meningiomas.[1-4]

The intricate interplay of factors contributing to meningioma recurrence remains an enigma, fueling extensive research efforts. Several factors have been implicated in elevating the risk of recurrence, including high tumor grade, incomplete surgical resection, and specific genetic aberrations. However, the precise molecular mechanisms orchestrating meningioma recurrence remain elusive, prompting ongoing investigations. In recent years, the spotlight has been cast on the pivotal role of the immune system in tumorigenesis and the intricate dynamics between tumor cells and the host's immune response. Programmed Death-Ligand 1 (PD-L1), a transmembrane protein expressed on both tumor cells and immune cells, has emerged as a central player in the intricate landscape of tumor immunity. PD-L1 exerts its immunomodulatory effects by binding to its cognate receptor, Programmed Death-1 (PD-1), located on T cells. This interaction triggers a cascade of signaling events that culminate in the suppression of T-cell activation and proliferation. The PD-1/PD-L1 axis constitutes a critical immune checkpoint, a regulatory mechanism that serves to fine-tune immune responses and safeguard against autoimmunity. However, tumor cells have evolved sophisticated strategies to exploit this checkpoint, effectively evading immune surveillance and thwarting immune-mediated destruction.[5-7]

PD-L1 expression has been extensively studied in various cancers, including melanoma, lung cancer, and renal cell carcinoma, where it has been shown to possess both prognostic and predictive significance. In the realm of meningiomas, several studies have embarked on elucidating the association between PD-L1 expression and tumor grade, recurrence, and patient outcomes. However, the results of these investigations have been far from conclusive, with some studies reporting a positive correlation between PD-L1 expression and aggressive tumor behavior, while others have failed to demonstrate such an association. Ki-67, a nuclear protein intricately linked to cellular proliferation, has also garnered attention as a potential prognostic marker in meningiomas. The Ki-67 labeling index, a quantitative measure of the proportion of cells actively engaged in the cell cycle, serves as a widely employed indicator of tumor cell proliferation. In meningiomas, a higher Ki-67 labeling index has been associated with a more aggressive tumor phenotype, characterized by a higher tumor grade and a less favorable prognosis.[8-10] This study aimed to delve into the intricate relationship between PD-L1 expression, Ki-67 labeling index, histopathological grade, and recurrence in meningiomas.

2. Methods

This retrospective study encompassed a cohort of 139 patients diagnosed with meningioma at the Department of Anatomical Pathology, Dr. M. Djamil General Hospital, Padang, Indonesia, between January 2020 and December 2023. The study meticulously adhered to the ethical guidelines stipulated by the Research Ethics Committee of Dr. M. Djamil General Hospital, Padang, ensuring the protection of patient rights and confidentiality. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks from the archived pathology specimens were retrieved for each patient. The FFPE blocks represent the gold standard for preserving tissue architecture and cellular details, allowing for retrospective histopathological and immunohistochemical analyses. For each case, representative sections were cut from the FFPE blocks at a thickness of 3 µm using a microtome. These sections were then carefully mounted onto glass slides coated with silane, a compound that enhances tissue adhesion and prevents detachment during subsequent staining procedures. The slides were then meticulously stained with hematoxylin and eosin (H&E), a widely employed staining technique in histopathology that provides excellent visualization of cellular morphology and tissue architecture. H&E staining involves the sequential application of hematoxylin, a basic dye that stains acidic structures such as nuclei blue-purple, and eosin, an acidic dye that stains basic structures

such as cytoplasm pink-red. This differential staining allows for the clear delineation of cellular components and the assessment of tissue morphology. The H&E-stained slides were then systematically examined under a light microscope by experienced pathologists. The pathologists meticulously evaluated the histopathological features of each meningioma, including cellular morphology, growth patterns, and the presence of specific diagnostic features. The tumors were then classified into distinct subtypes and assigned WHO grades (I, II, or III) based on the 2021 WHO Classification of Tumors of the Central Nervous System, the internationally recognized gold standard for meningioma classification.

Immunohistochemistry (IHC) is a powerful technique that allows for the visualization and localization of specific proteins within tissue sections. It leverages the exquisite specificity of antibodies to bind to their corresponding antigens, providing valuable insights into protein expression patterns and their potential clinical significance. For IHC staining, additional sections were cut from the FFPE blocks at a thickness of 3 µm and mounted onto silane-coated glass slides. These sections underwent a series of preparatory steps to ensure optimal staining quality. First, the slides were heated to 60°C on a slide warmer to firmly adhere the tissue sections to the slides, preventing detachment during subsequent processing. Next, the slides were immersed in xylene, an organic solvent that removes paraffin, rendering the tissue sections permeable to antibodies. The slides were then rehydrated through a graded series of alcohols (100%, 90%, 80%, and 70%) to gradually introduce water into the tissue, preventing abrupt changes that could disrupt tissue morphology. Antigen retrieval is a critical step in IHC that aims to unmask antigenic epitopes that may have been masked during formalin fixation. This process involves subjecting the tissue sections to heat-induced epitope retrieval (HIER) using a sodium citrate buffer (pH 6.0). The slides were immersed in the buffer solution and heated in a microwave oven or a pressure cooker, breaking the cross-links formed during fixation and restoring the accessibility of antigenic sites for antibody binding. After antigen retrieval, the tissue sections were treated with hydrogen peroxide to quench endogenous peroxidase activity, preventing non-specific background staining. The sections were then incubated with a normal blocking serum to block non-specific binding sites, further reducing background staining and enhancing the signal-to-noise ratio. The slides were then incubated with primary antibodies specific for PD-L1 (PD-L1/CD274 clone 22C3, Dako) and Ki-67 (Rabbit monoclonal Antibody). The antibodies were diluted in a phosphate-buffered saline (PBS) solution to the appropriate concentrations and applied to the tissue sections. The slides were incubated in a humidified chamber to prevent antibody drying and ensure even staining. After primary antibody incubation, the slides were washed with PBS to remove unbound antibodies. The slides were then incubated with secondary antibodies conjugated to biotin, a small molecule that binds with high affinity to streptavidin. This biotin-streptavidin interaction forms the basis of the streptavidin-biotin complex (SBC) method, a widely used detection system in IHC. The slides were then incubated with a streptavidin-horseradish peroxidase (HRP) conjugate. HRP is an enzyme that catalyzes the oxidation of a chromogenic substrate, 3,3'-diaminobenzidine (DAB), producing a brown precipitate at the site of antibody binding. This brown precipitate serves as a visual marker for the presence and localization of the target proteins, PD-L1 and Ki-67. After DAB visualization, the slides were counterstained with hematoxylin to provide nuclear contrast and enhance the visualization of tissue morphology. The slides were then dehydrated through a graded series of alcohols, cleared in xylene, and permanently mounted with a coverslip using a mounting medium.

The immunostained slides were meticulously evaluated under a light microscope by experienced pathologists. PD-L1 expression was assessed using the Combined Positive Score (CPS), which takes into account the number of PD-L1 stained cells (tumor cells, lymphocytes, and macrophages) relative to the total number of viable tumor cells. A CPS score of ≥10% was considered positive, indicating clinically significant PD-L1 expression. The Ki-67 labeling index was determined as the percentage of tumor cells

exhibiting positive nuclear staining for Ki-67. This index reflects the proportion of cells actively engaged in the cell cycle, providing a quantitative measure of tumor cell proliferation.

The data collected from the study were systematically organized and analyzed using SPSS software, a powerful statistical analysis tool. Descriptive statistics were employed to summarize the clinicopathological characteristics of the study population, including age, gender, tumor location, recurrence status, histopathological subtype, and WHO grade. The association of PD-L1 expression and Ki-67 labeling index with histopathological grade and recurrence was rigorously assessed using the Chi-square test, a statistical test used to determine if there is a significant association between two categorical variables. A p-value of <0.05 was considered statistically significant, indicating that the observed association was unlikely to have occurred by chance alone.

3. Results

Table 1 presents the patient and tumor characteristics of the 45 meningioma cases included in the study. The most common age group is 31-40 years (33.3%), followed closely by 41-50 years (51.1%). This indicates that your study population predominantly consists of middle-aged adults. Notably, there are no patients in the youngest (0-10 years) or oldest (71-80 years) age categories. There is a strong skew towards female patients (88.9%), which aligns with the general epidemiology of meningiomas, where they are more common in women. The most common tumor locations are the frontal lobe (22.2%) and, interestingly, the sphenoid wings (22.2%). This might be worth investigating further to see if there are any specific characteristics associated with meningiomas in this location within your dataset. Nearly half of the tumors (48.9%) are recurrent, highlighting the importance of understanding factors associated with recurrence in meningioma. Atypical meningioma is the most frequent subtype (33.3%), followed by meningothelial and transitional (both 13.3%). The presence of chordoid meningiomas (8.9%) is notable. The majority of tumors are WHO Grade I (51.1%), indicating mostly benign tumors. However, a substantial proportion are Grade II (44.4%), suggesting a need to identify factors that distinguish between grades and predict aggressive behavior. A majority of tumors (66.7%) show positive PD-L1 expression. This is a significant finding, given the growing interest in PD-L1 as a therapeutic target in various cancers. Most cases (68.9%) have a high Ki-67 labeling index, indicating a high proliferative rate and potentially more aggressive behavior. Among PD-L1 positive tumors, a higher proportion also have high Ki-67 expression (53.3%). This suggests a potential link between PD-L1 expression and proliferative activity in meningiomas. In contrast, high Ki-67 expression is less common (15.6%) in PD-L1 negative tumors.

Table 2 explores the relationship between PD-L1 expression, histopathological grade (risk level), and recurrence in your meningioma cases. A slightly higher proportion of positive PD-L1 cases are found in the high-risk group (WHO Grade II and III - 37.8%) compared to the low-risk group (WHO Grade I - 28.9%). However, this difference is not statistically significant (p-value = 0.14). This suggests that PD-L1 expression alone may not be a strong predictor of higher-grade meningiomas in your dataset. Similar to positive PD-L1, there's a slightly higher proportion of negative PD-L1 cases in the low-risk group (22.2%) compared to the high-risk group (11.1%). Again, this difference is not statistically significant. A significantly higher proportion of positive PD-L1 cases are associated with recurrence (42.2%) compared to those without recurrence (24.4%). This difference is statistically significant (p-value = 0.006), indicating that positive PD-L1 expression may be a potential marker for predicting recurrence in meningioma. Conversely, negative PD-L1 expression is more frequently observed in cases without recurrence (26.7%) compared to those with recurrence (6.7%).

Table 1. Patient and tumor characteristics (n=45).

Characteristic	Category	Frequency (n)	Percentage (%)
Age (years)	0-10	0	0
	11-20	0	0
	21-30	2	4.4
	31-40	15	33.3
	41-50	23	51.1
	51-60	3	6.7
	61-70	2	4.4
	71-80	0	0
Gender	Male	5	11.1
	Female	40	88.9
Tumor location	Frontal	10	22.2
	Temporal	8	17.8
	Parietal	5	11.1
	Occipital	1	2.2
	Fronto-temporal	2	4.4
	Fronto-parietal	1	2.2
	Parieto-occipital	1	2.2
	Falx cerebri	1	2.2
	Cerebellopontine angle (CPA)	2	4.4
	Sellar	4	8.9
	Spheno-orbital	7	15.6
	Sphenoid wings	1	2.2
	Spinal	2	4.4
Recurrence	Yes	22	48.9
	No	23	51.1
Histopathological subtype	Meningothelial	6	13.3
	Fibrous	2	4.4
	Transitional	6	13.3
	Psammomatous	2	4.4
	Angiomatous	2	4.4
	Microcystic	3	6.7
	Secretory	1	2.2
	Lymphoplasmacyte-rich	1	2.2
	Metaplastic	0	0
	Chordoid	4	8.9
	Clear cell	1	2.2
	Rhabdoid	0	0
	Papillary	0	0
	Atypical	15	33.3
	Anaplastic	2	4.4
WHO grade	Grade I	23	51.1
	Grade II	20	44.4
	Grade III	2	4.4
PD-L1 expression	Positive	30	66.7
	Negative	15	33.3
Ki-67 expression	High	31	68.9
	Low	14	31.1
PD-L1 positive & Ki-67	High	24	53.3
	Low	6	13.3
PD-L1 negative & Ki-67	High	7	15.6
	Low	8	17.8

Table 2. Association of PD-L1 expression with histopathological grade and recurrence in meningioma.

PD-L1 expression	Histopathological grade	Frequency (n=45)	Percentage (%)	Recurrence	Frequency (n=45)	Percentage (%)
Positive	High risk (WHO Grade II and III)	17	37.8	Yes	19	42.2
	Low risk (WHO Grade I)	13	28.9	No	11	24.4
Negative	High risk (WHO Grade II and III)	5	11.1	Yes	3	6.7
	Low risk (WHO Grade I)	10	22.2	No	12	26.7
Total	45	100				
p-value vs histopathological	0.14					
p-value vs recurrence	6					

Figure 1 illustrates the varying levels of PD-L1 expression in meningioma samples as detected by immunohistochemical staining (IHC) at 400x magnification. (A) PD-L1 expression in positive control (placenta) (IHC, 400x) panel shows a positive control for PD-L1 staining using placental tissue. The placenta is known to express PD-L1, and this image confirms the successful staining procedure. The brown staining indicates the presence of PD-L1, validating the antibody and staining technique used in the study. (B) High expression of PD-L1 on the tumor cell membrane as well as the membrane and cytoplasm of surrounding inflammatory cells (IHC, 400x) panel demonstrates high PD-L1 expression in a meningioma sample. The strong brown staining is observed on the membrane of tumor cells, as well as in the membrane and cytoplasm of inflammatory cells surrounding the tumor. This high expression suggests that PD-L1 may be playing a role in immune evasion in this particular tumor, potentially by suppressing the activity of anti-tumor immune cells. (C) Moderate expression of PD-L1 on tumor cell membranes (IHC, 400x) panel shows moderate PD-L1 expression in another meningioma sample. The brown staining is less intense compared to panel (B) and is primarily localized to the membrane of tumor cells. This moderate expression level could still have implications for tumor progression and response to immunotherapy, although its effect might be less pronounced than in cases with high expression. (D) Tumor cells did not stain for PD-L1 (IHC, 400x) panel shows a meningioma sample with no detectable PD-L1 expression. The absence of brown staining indicates that PD-L1 is not expressed in this tumor, or its expression is below the detection limit of the IHC assay. This finding suggests that PD-L1 may not be involved in immune evasion in this particular tumor and that other mechanisms might be at play.

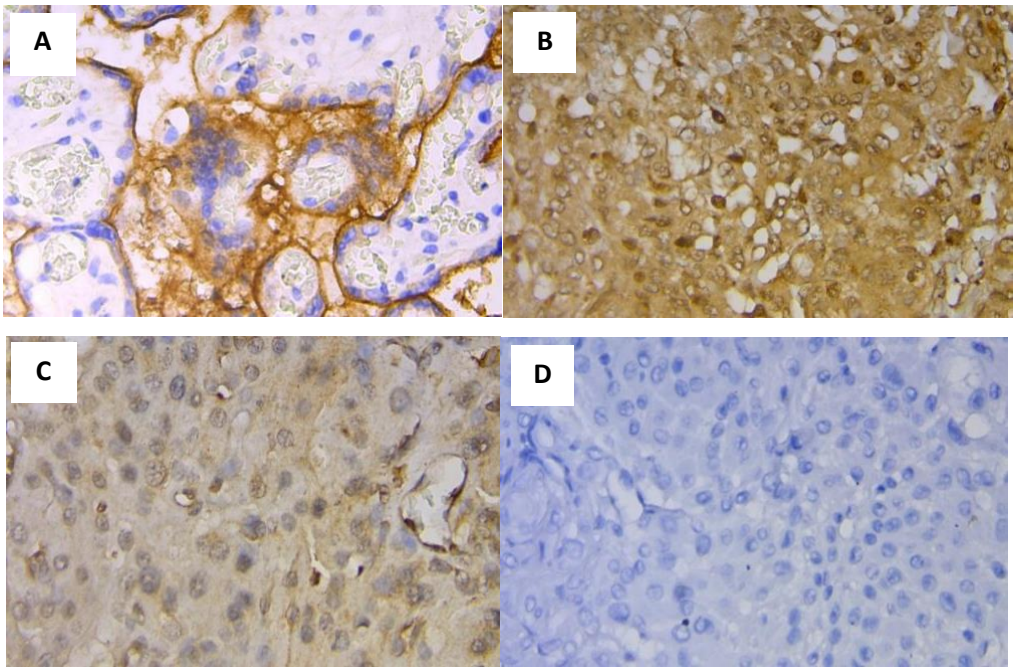

Figure 1. (A) PD-L1 expression in the positive control (placenta) (IHC, 400x). (B) High expression of PD-L1 on the tumor cell membrane as well as the membrane and cytoplasm of surrounding inflammatory cells (IHC, 400x). (C) Moderate expression of PD-L1 on tumor cell membranes (IHC, 400x). (D) Tumor cells did not stain for PD-L1 (IHC, 400x).

Table 3 examines the relationship between Ki-67 expression, histopathological grade (risk level), and recurrence in your meningioma cases. A slightly higher proportion of high Ki-67 expression cases are in the high-risk group (WHO Grade II and III - 40%) compared to the low-risk group (WHO Grade I - 28.9%). However, this difference is not statistically significant (p-value = 0.067). This suggests that Ki-67 expression alone might not be a strong predictor of higher-grade meningiomas in your dataset, although the trend is worth noting. Similarly, there's a slightly higher proportion of low Ki-67 expression cases in the low-risk group (22.2%) compared to the high-risk group (8.9%). Again, this difference isn't statistically significant. A notably higher proportion of high Ki-67 expression cases are associated with recurrence (42.2%) compared to those without recurrence (26.7%). This difference is statistically significant (p-value = 0.013), indicating that high Ki-67 expression may be a valuable marker for predicting recurrence in meningioma. Conversely, low Ki-67 expression is more frequently observed in cases without recurrence (24.4%) compared to those with recurrence (6.7%).

Table 3. Association of Ki-67 expression with histopathological grade and recurrence in meningioma.

Ki-67 expression	**Histopathological grade**	**Frequency (n=45)**	**Percentage (%)**	**Recurrence**	**Frequency (n=45)**	**Percentage (%)**
High	High risk (WHO Grade II and III)	18	40	Yes	19	42.2
	Low risk (WHO Grade I)	13	28.9	No	12	26.7
Low	High risk (WHO Grade II and III)	4	8.9	Yes	3	6.7
	Low risk (WHO Grade I)	10	22.2	No	11	24.4
Total		45	100		45	100
p-value vs histopathological	67					
p-value vs recurrence	13					

Figure 2 showcases the varying degrees of Ki-67 expression in meningioma samples, visualized through immunohistochemical staining (IHC) at 400x magnification. (A) Ki67 expression in the positive control (Skin) (IHC, 400x) panel serves as a positive control for Ki-67 staining using skin tissue, which is known to have a high proliferation rate and thus expresses Ki-67. The brown staining within the nuclei of skin cells confirms the effectiveness of the staining procedure and validates the antibody used. (B) High expression of Ki67 in tumor cell nuclei (IHC, 400x) panel demonstrates high Ki-67 expression in a meningioma sample. The intense brown staining is concentrated within the nuclei of tumor cells, indicating a high proportion of cells actively engaged in the cell cycle. This high expression suggests a rapid proliferation rate, which is often associated with more aggressive tumor behavior and a higher likelihood of recurrence. (C) Moderate expression of Ki67 in tumor cell nuclei (IHC, 400x) panel shows moderate Ki-67 expression in another meningioma sample. The brown nuclear staining is less intense compared to panel (B), indicating a lower proportion of proliferating cells. While still indicative of active cell division, this moderate expression level might suggest less aggressive behavior compared to the high expression observed in panel (B). (D) Tumor cells did not stain for Ki67 (IHC, 400x) panel displays a meningioma sample with no detectable Ki-67 expression. The absence of brown staining in the tumor cell nuclei suggests a very low proliferation rate or a state of cellular quiescence. This might indicate a less aggressive tumor with a lower chance of recurrence.

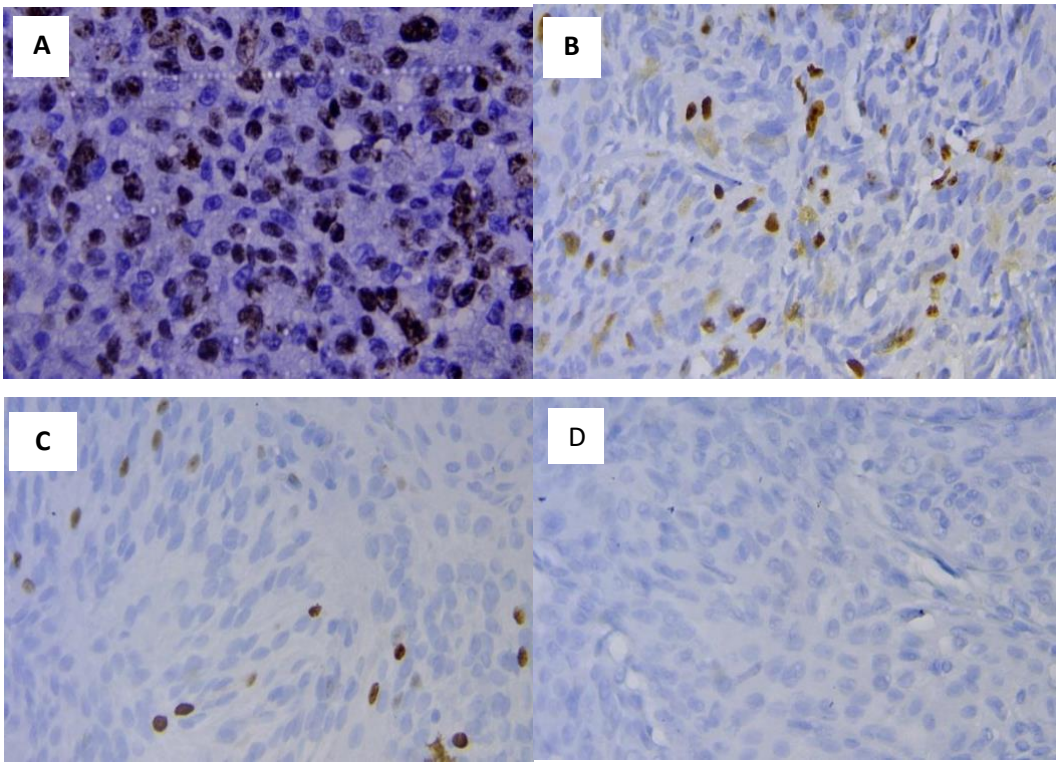

Figure 2. (A) Ki67 expression in positive control (Skin) (IHC, 400x). (B) High expression of Ki67 in tumor cell nuclei (IHC, 400x). (C) Moderate expression of Ki67 in tumor cell nuclei (IHC, 400x). (D) Tumor cells did not stain for Ki67 (IHC, 400x).

Table 4 delves into the combined effect of PD-L1 and Ki-67 expression on the histopathological grade of meningioma. PD-L1 Positive & Ki-67 High group shows a higher proportion of cases in the high-risk group (WHO Grade II and III - 33.3%) compared to the low-risk group (WHO Grade I - 20%). However, this

difference is not statistically significant (p-value = 0.232). PD-L1 Positive & Ki-67 Low a higher proportion of cases are in the high-risk group (4.4%) compared to the low-risk group (8.9%), but the difference is not statistically significant. PD-L1 Negative & Ki-67 High a higher proportion falls in the high-risk group (6.7%) compared to the low-risk group (8.9%), but without statistical significance. The PD-L1 Negative & Ki-67 Low group also shows a higher proportion in the low-risk group (13.3%) compared to the high-risk group (4.4%), but the difference is not statistically significant.

Table 4. Association of combined PD-L1 and Ki-67 expression with histopathological grade in meningioma.

PD-L1 and Ki-67 expression	Histopathological grade	Frequency (n=45)	Percentage (%)	p-value
PD-L1 Positive & Ki-67 High	High risk (WHO Grade II and III)	15	33.3	232
	Low risk (WHO Grade I)	9	20	
PD-L1 Positive & Ki-67 Low	High risk (WHO Grade II and III)	2	4.4	
	Low risk (WHO Grade I)	4	8.9	
PD-L1 Negative & Ki-67 High	High risk (WHO Grade II and III)	3	6.7	
	Low risk (WHO Grade I)	4	8.9	
PD-L1 Negative & Ki-67 Low	High risk (WHO Grade II and III)	2	4.4	
	Low risk (WHO Grade I)	6	13.3	
Total		45	100	

Table 5 analyzes the relationship between the combined expression of PD-L1 and Ki-67 and the recurrence of meningioma. PD-L1 Positive & Ki-67 High group shows the highest association with recurrence (35.6%), and this difference is statistically significant (p-value = 0.013). This indicates that patients with tumors expressing both PD-L1 and high Ki-67 have a significantly increased risk of recurrence. PD-L1 Positive & Ki-67 Low the recurrence rate in this group is lower (6.7%) and not statistically significant. PD-L1 Negative & Ki-67 High group also shows a relatively low recurrence rate (6.7%) without statistical significance. PD-L1 Negative & Ki-67 Low notably, this group has no recorded recurrences (0%).

Table 5. Association of combined PD-L1 and Ki-67 expression with recurrence in meningioma.

PD-L1 and Ki-67 expression	Recurrence	Frequency (n=45)	Percentage (%)	p-value
PD-L1 Positive & Ki-67 High	Yes	16	35.6	13
	No	8	17.8	
PD-L1 Positive & Ki-67 Low	Yes	3	6.7	
	No	3	6.7	
PD-L1 Negative & Ki-67 High	Yes	3	6.7	
	No	4	8.9	
PD-L1 Negative & Ki-67 Low	Yes	0	0	
	No	8	17.8	
Total		45	100	

4. Discussion

The study revealed that 66.7% of the examined meningioma cases displayed positive PD-L1 expression. This means that over half of the tumor samples showed evidence of PD-L1 protein, primarily located on the cell membrane of the tumor cells. In some instances, PD-L1 was also found in the membrane and cytoplasm of the inflammatory cells

surrounding the tumor. This observation aligns with existing research indicating that PD-L1 expression is not exclusive to tumor cells and can also be found in immune cells infiltrating the tumor microenvironment. While the study's findings are consistent with the broader observation that PD-L1 is expressed in meningiomas, the exact prevalence (66.7% in this case) may differ from those reported in other studies. Studies with smaller sample sizes may have less representative results compared to larger studies. The specific subtypes of meningioma included in a study can influence the overall PD-L1 positivity rate, as different subtypes may have varying expression patterns. The specific antibodies used to detect PD-L1 and the scoring systems employed to interpret the immunohistochemical staining can lead to variations in reported prevalence. PD-L1, or Programmed Death-Ligand 1, is a transmembrane protein that plays a critical role in regulating immune responses. It functions as an immune checkpoint, a mechanism that helps to prevent excessive immune activation and autoimmunity. PD-L1 achieves this by binding to its receptor, PD-1 (Programmed Death-1), which is found on T cells, a type of white blood cell crucial for immune defense. When PD-L1 binds to PD-1, it sends a signal that inhibits T cell activation and proliferation. This dampening effect on the immune response is essential for preventing the immune system from attacking the body's own healthy cells and tissues. While PD-L1 plays a vital role in maintaining immune balance, tumor cells can exploit this mechanism to their advantage. By expressing PD-L1 on their surface, tumor cells can essentially "switch off" the anti-tumor activity of T cells, allowing them to evade immune destruction. This process is known as immune evasion and is a significant factor in tumor progression and survival. The study found a significant association between positive PD-L1 expression and recurrence in meningiomas. This means that tumors expressing PD-L1 were more likely to recur after initial treatment. This finding is crucial because it suggests that PD-L1 may play a role in the aggressive behavior of meningiomas and their ability to resist treatment. The link between PD-L1 and recurrence is supported by previous research in other cancer types. Studies in melanoma, lung cancer, and renal cell carcinoma have also shown that PD-L1 expression is associated with a worse prognosis, including a higher risk of recurrence and metastasis. The overexpression of PD-L1 on tumor cells can create an "immune-privileged" site, effectively shielding the tumor from the body's natural defenses. This allows the tumor to grow and spread unchecked, leading to a higher likelihood of recurrence and a poorer overall prognosis. The association between PD-L1 expression and recurrence in meningiomas has important implications for treatment strategies. It suggests that targeting PD-L1 could be a promising therapeutic approach for meningiomas, especially those with high PD-L1 expression. Immune checkpoint inhibitors, a class of drugs that block the interaction between PD-L1 and PD-1, have shown remarkable success in treating various cancers. These drugs work by "releasing the brakes" on the immune system, allowing T cells to recognize and attack tumor cells more effectively. Several clinical trials are currently investigating the use of immune checkpoint inhibitors in meningiomas, and the preliminary results are encouraging. If these trials continue to show positive outcomes, PD-L1-targeted therapies could become an important part of the treatment landscape for meningiomas, particularly for those at high risk of recurrence.[11-14]

Ki-67 is a nuclear protein that serves as a reliable indicator of cellular proliferation. It is expressed during the active phases of the cell cycle (G1, S, G2, and M phases), when cells are actively dividing and replicating their DNA. Ki-67 is absent in resting cells (G0 phase), which are not actively dividing. This characteristic makes Ki-67 a valuable tool for assessing the growth rate and aggressiveness of tumors, including meningiomas. The Ki-67 labeling index is a quantitative measure of the proportion of cells within a tumor that are expressing Ki-67. It is typically expressed as a percentage, reflecting the number of Ki-67 positive cells out of the total number of tumor cells. A higher Ki-67 labeling index indicates a greater proportion of actively dividing cells, suggesting a faster growth rate and potentially more aggressive tumor behavior. In this study, 68.9% of the

meningioma cases exhibited a high Ki-67 labeling index, defined as a value greater than 4%. This finding signifies that a majority of the examined tumors had a high proportion of actively proliferating cells. This observation aligns with previous research that has demonstrated a correlation between Ki-67 expression and various clinical parameters in meningiomas, including tumor grade, recurrence, and overall prognosis. A higher Ki-67 labeling index is often associated with a more aggressive tumor phenotype. This is because tumors with a high proliferative rate tend to grow more rapidly, invade surrounding tissues more readily, and have a greater propensity for recurrence after treatment. In meningiomas, a high Ki-67 labeling index has been linked to an increased risk of recurrence, even in tumors that are histologically classified as low-grade. The significant association observed between high Ki-67 expression and recurrence in this study further strengthens its role as a prognostic marker in meningiomas. By identifying tumors with a high proliferative drive, Ki-67 can help clinicians stratify patients into different risk categories and tailor treatment strategies accordingly. Patients with meningiomas exhibiting high Ki-67 expression may benefit from closer monitoring, more aggressive surgical resection, or adjuvant therapies to reduce the risk of recurrence. Conversely, patients with low Ki-67 expression may be candidates for less intensive treatment approaches. The Ki-67 labeling index can be a valuable tool in guiding treatment decisions for meningioma patients. While histopathological grading remains the cornerstone of meningioma classification, Ki-67 can provide additional information about tumor behavior and help refine treatment strategies. For example, in cases where the histopathological diagnosis is uncertain or borderline between grades, Ki-67 can help to clarify the risk of recurrence and guide the decision between observation, surgery, or radiotherapy. Additionally, Ki-67 can be used to monitor treatment response and detect early signs of recurrence. While Ki-67 is a valuable prognostic marker on its own, its utility can be further enhanced when used in conjunction with other markers, such as PD-L1. As demonstrated in this study, the combination of high Ki-67 expression and positive PD-L1 expression was associated with the highest risk of recurrence. This suggests that a multi-marker approach could provide a more comprehensive assessment of tumor behavior and guide more personalized treatment decisions.[15,16]

The combined analysis of PD-L1 and Ki-67 expression revealed a striking pattern: patients whose tumors exhibited both positive PD-L1 and high Ki-67 expression faced the highest risk of recurrence. This finding points to a potential synergistic, or cooperative, effect between these two markers in driving tumor aggressiveness and the likelihood of the tumor returning after treatment. To grasp the significance of this synergy, it's essential to consider the roles of PD-L1 and Ki-67. As discussed earlier, PD-L1 helps tumor cells evade the immune system by suppressing the activity of T cells, which are crucial for fighting cancer. Ki-67 indicates how rapidly tumor cells are dividing. A high Ki-67 level suggests a fast-growing tumor. The interplay between these two factors could create a microenvironment that favors tumor growth and dissemination. Imagine a scenario where a tumor not only evades the immune system (thanks to PD-L1) but also proliferates rapidly (due to high Ki-67). This combination creates a dangerous situation where the tumor can grow unchecked and potentially spread to other areas, increasing the chances of recurrence. This combined marker analysis holds significant potential for refining the prognosis of meningioma patients. By assessing both PD-L1 and Ki-67, clinicians can identify patients at the highest risk of recurrence. This knowledge allows for more personalized treatment planning and follow-up care. The identification of this high-risk group opens avenues for exploring more aggressive treatment strategies. More frequent imaging scans or check-ups to detect any signs of recurrence early. Additional treatments like radiation therapy or chemotherapy after surgery to eliminate any remaining tumor cells and reduce the risk of recurrence. Enrolling in clinical trials evaluating novel therapies, such as immune checkpoint inhibitors that target PD-L1, to enhance the immune response against the tumor. While the study's findings are promising, further research is

needed to validate these results and explore their full clinical implications. Larger, prospective studies with longer follow-up periods can confirm the prognostic value of combined PD-L1 and Ki-67 expression in meningiomas. In addition to validation, future research should delve into the molecular mechanisms that underpin the synergistic effect of PD-L1 and Ki-67 in promoting recurrence. Understanding these mechanisms could lead to the development of new therapeutic strategies that target these pathways and disrupt the tumor's ability to evade the immune system and proliferate uncontrollably. The combined analysis of PD-L1 and Ki-67 has the potential to revolutionize the clinical management of meningiomas. By providing a more accurate assessment of recurrence risk, this approach can guide treatment decisions, improve patient outcomes, and potentially pave the way for more personalized and effective therapies.[17,18]

This study revealed an intriguing observation: while both PD-L1 and Ki-67 expression showed significant associations with recurrence in meningiomas, neither marker individually nor their combined expression significantly correlated with histopathological grade. This finding presents a noteworthy contrast to some previous reports that have suggested a link between PD-L1 or Ki-67 expression and tumor grade in meningiomas. Histopathological grade is a crucial factor in determining the prognosis and treatment of meningiomas. It is assigned based on microscopic examination of the tumor tissue and reflects the degree of abnormality in the tumor cells and their growth pattern. The World Health Organization (WHO) grading system for meningiomas categorizes tumors into three grades. Grade I (Benign) tumors have a slow growth rate and are typically associated with a favorable prognosis. Grade II (Atypical) tumors exhibit increased cellularity, mitotic activity, and other features suggestive of more aggressive behavior. Grade III (Anaplastic/Malignant) tumors are highly aggressive, with rapid growth and a tendency to invade surrounding tissues and metastasize. Several studies have investigated the relationship between PD-L1 or Ki-67 expression and histopathological grade in meningiomas. Some studies have reported a positive correlation, suggesting that higher expression of these markers is associated with higher-grade tumors. However, other studies have failed to find a significant association, similar to the findings of this study. The study included a relatively small sample size of 45 meningioma cases. With a larger sample size, the statistical power to detect subtle associations between these markers and tumor grade would be increased. Meningiomas are a diverse group of tumors with varying histopathological subtypes and molecular profiles. This heterogeneity could mask any potential correlation between PD-L1/Ki-67 expression and grade. Tumor grade is determined by a complex interplay of various factors, including genetic mutations, epigenetic alterations, and microenvironmental influences. PD-L1 and Ki-67 may be just two pieces of the puzzle, and their individual contributions to tumor grade might be overshadowed by other factors. To gain a more comprehensive understanding of the relationship between PD-L1/Ki-67 expression and tumor grade, future studies should incorporate comprehensive molecular profiling. This would involve analyzing the expression of a wide range of genes and proteins involved in tumor development and progression. By integrating this information with PD-L1/Ki-67 expression data, researchers could identify more precise molecular signatures that predict tumor grade and guide treatment decisions. While the lack of a significant correlation with grade in this study might seem counterintuitive, it is important to emphasize that PD-L1 and Ki-67 still hold significant prognostic value in meningiomas. Their strong association with recurrence, independent of tumor grade, highlights their potential as valuable tools for risk stratification and treatment planning.[19,20]

5. Conclusion

This study investigated the association of PD-L1 expression and Ki-67 labeling index with histopathological grade and recurrence in meningioma. The key findings indicate that while neither PD-L1 nor Ki-67 significantly correlated with tumor grade, both markers individually, and

especially in combination, were significantly associated with recurrence. Specifically, positive PD-L1 expression and a high Ki-67 labeling index (>4%) were identified as potential prognostic markers for predicting recurrence in meningioma. These findings suggest that the interplay between immune evasion and cell proliferation plays a crucial role in meningioma recurrence. Further large-scale prospective studies are needed to validate these findings and explore their potential therapeutic implications, particularly concerning the use of PD-L1 inhibitors in high-risk meningioma patients.

6. References

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