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The Importance of Proliferative Activity and Angiogenesis in Meningioma and Glioma

Menenjiom ve Gliomalarda Proliferatif Aktivite ve Damarlanmanın Önemi

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ABSTRACT

OBJECTIVE: The aim of this study was to investigate the usefulness of a mean vascular density (MVD) and proliferative activity labeling index (LI) in gliomas and meningiomas as biological markers in histological grading.

MATERIALS AND METHODS: Our study included 18 cases of glioma and 36 cases of meningioma. These cases were graded according to the WHO classification. CD-34 and Ki-67 immunohistochemical methods were used on paraffin-embedded tissues. Distribution of CD-34 and Ki-67 LI values were determined for different histologic grades of gliomas and meningiomas. The results were analyzed with statistical methods.

RESULTS: In gliomas, the values of Ki-67 were detected as 3.33±2.50 in grade I, 5.01±2.28 in grade III and 10.33±4.85 in grade IV. The mean values of CD-34 were found as 11.80± 9.71 in grade III glioma and 8.40±5.45 in grade IV glioma.

The values of Ki-67 were detected as 4.40±0.40 in atypical, 4.26±4.05 in psammomatous and 3.69±3.24 in meningothelial meningioma. CD-34 expression was 19.60±0.20 in angiomatous meningioma and 6.14±1.91 in malignant meningioma.

CONCLUSION: Our results show that Ki-67 LI and angiogenesis can be useful in tumor grading of gliomas. However, the score of angiogenesis and Ki-67 LI adds little information to predict tumor grade in meningiomas, in contrast to gliomas.

KEY WORDS: Meningioma, Glioma, Proliferative activity, Angiogenesis

ÖZ

AMAÇ: Bu çalışmanın amacı damarlanma faktörünü ve proliferasyon aktivite indeksini, glioma ve meninjiomaların histolojik evrelenmesinde biyolojik bir belirleyici olarak kullanılabilirliğinin araştırmaktı.

GEREÇ VE YÖNTEM: Çalışmamız 18 gliom ve 36 meninjiom vakası içermekteydi. Bu olgular Dünya sağlık Örgütünün sınıflamasına göre evrelendi. Parafin blok halindeki dokulara CD-34 ve Ki-67 immünhistokimyasal yöntemleri uygulandı. Farklı histolojik evredeki gliom ve meninjiomlarda CD-34 ve Ki-67 dağılımı belirlendi. Bulgular istatistiksel yöntemler ile değerlendirildi.

BULGULAR: Gliomalarda Ki-67 evre I'de 3.33±2.50, evre III'te 5.01±2.28 ve evre IV'te 10.33±4.85 olarak belirlendi. CD-34 ün ortalama değerleri evre II gliomada 11.80± 9.71 ve evre IV gliomada 8.40±5.45 olarak bulundu.

Ki-67 değerleri atipik meninjiomda 4.40±0.40, psammomatözde 4.26±4.05 ve meningotelyalde 3.69±3.24 olarak belirlendi. CD-34 ise anjiomatöz ve malign meninjiomda 19.60±0.20 ve 19.60±0.20 olarak saptandı.

SONUÇ: Bizim sonuçlarımız gliomaların evrelenmesinde Ki-67 işaretleme indeksinin (LI) ve damarlanmanın faydalı olabileceğini göstermektedir. Fakat meninjiomların evrelenmesinde gliomaların aksine damarlanmanın da, Ki-67 LI'nın da fazla bir katkısı olmamaktadır.

ANAHTAR SÖZCÜKLER: Meninjiom, Gliom, Proliferatif aktivite, Damarlanma

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INTRODUCTION

Cell kinetics play an important role in tumor behavior. Determination of proteins in the control of proliferation in normal cells enables a better understanding of cellular transformation and proliferation mechanisms. Measurement of proliferative activity is important in determining the tumor grade, recurrence span and malignancy (5).

Subjective methods such as mitotic index and necrosis are still used in determining the grade of central nervous system (CNS) tumors but more informative techniques like Ki -67 have been used recently (5).

Ki-67 is a nuclear antigen expressed in all phases of the cell cycle excluding the G0 phase (5,11).

Neovascularization is a neuropathological hallmark in gliomas and angiogenetic factors and may play an important role in malignant tumor progression (9).

A number of studies have assessed the importance of vascular proliferation in astrocytic tumors (14).

MATERIALS AND METHODS

Fifty-four CNS tumors (18 gliomas, 36 meningiomas) were diagnosed and graded according to the WHO grading system. Hematoxylin-eosin slides of the cases were reviewed and the immunoperoxidase technique was performed for Ki-67 and CD-34 monoclonal antibodies to selected sections of each case. Antigen staining was carried out with Ki-67 and CD 34 proteins and the Ultravision Polyvalent, Rabbit, HRP-AEC kit (Neomarkers- Biogen USA).

CD-34 positive tumor areas with a high density of vascularization were chosen for evaluation. Positive-stained vessels were counted in 5 different areas at X40 magnification using an Olympus BX51 microscope in each case. The mean vascular density was calculated.

For the evaluation of Ki-67 labeling index (LI), at least 1000 cells from each tumor were counted in different microscopic fields at a magnification X40 using an Olympus BX51 microscope. The percentage of positive stained cells was calculated.

The MVD and LI were independently recorded by two pathologists and the results were averaged.

Results were statistically analyzed with the SPSS 11.5 PC package program. The Mann-Whitney U test, Kruskal-Wallis test and Sperman's correlation analysis tests were used to compare the results.

RESULTS

There were 7 women and 11 men with glial tumors with a mean age of 50.67±8.33.

There were 19 female, 17 male meningioma patients with a mean age of 54.86±9.05

Ki-67 immunoreaction yielded positive results in 14 cases of gliomas (total 18 gliomas) and 24 cases of meningiomas (total 36 meningiomas). Although control slides stained positive, unstained cases could be due to the quality of the paraffin blocks.

CD-34 immunostaining was positive in 18 glioma cases and in 36 meningioma cases.

The mean Ki-67 values in glioma and meningioma cases were 6.80±4.68 and 3.10±2.80 respectively ($p<0.05$), whereas the mean CD-34 values were 8.38±6.48 and 6.89±4.17 respectively ($p>0.05$) (Table I).

There were 11 men and 7 women with mean positive values of CD-34 in gliomas of 6.20±2.94 and 11.80±9.06 respectively. Ki-67 staining also yielded positive results in 9 men and 5 women with mean values in gliomas of 5.71±4.42 and 8.76±4.96 respectively.

In meningiomas, the mean values of CD-34 were 6.87±4.21 in men and 6.91±4.25 in women whereas the mean values of Ki-67 were 3.85±3.32 in men and 2.47±2.20 in women.

Table I. Immunopositivity of gliomas and meningiomas with Ki-67 and CD-34

	n	Ki-67	p*	n	CD34	p*
Glioma	14	6.80±4.68		18	8.38±6.48	
Meningioma	24	3.10±2.80	**0.007**	36	6.89±4.17	0.446
Total	38			54		

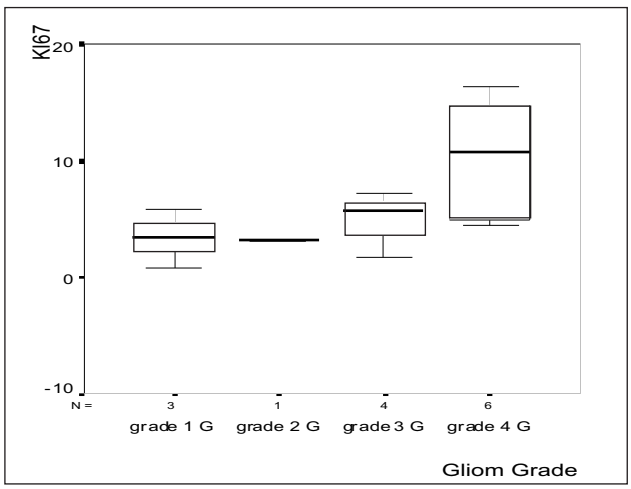
*Mann-Whitney U

The mean values of CD-34 were 11.80± 9.71 in grade III glioma and 8.40± 5.45 in grade IV glioma. The staining of CD-34 was not statistically significant in different grades of gliomas (p=0.161).

In gliomas, the values of Ki-67 were detected as 3.33± 2.50 in grade I, 5.01± 2.28 in grade III and 10.33± 4.85 in grade IV. The evaluation of Ki-67 staining according to glioma grading was statistically significant (p=0.007) (Graphic I).

The values of Ki-67 were detected as 4.40± 0.40 and 2.80± 0.30 respectively in atypical and malignant meningiomas. Otherwise the values of Ki-67 were 4.26± 4.05 in psammomatous and 3.69± 3.24 in meningothelial meningioma (Figure 1).

CD-34 was expressed as 19.60±0.20 in angiomatous meningioma, 7.00±0.70 in atypical meningioma and 6.14±1.91 in malignant meningioma (Figure 4).



Graphic I: Ki-67 immunoreactivity according to glioma grading

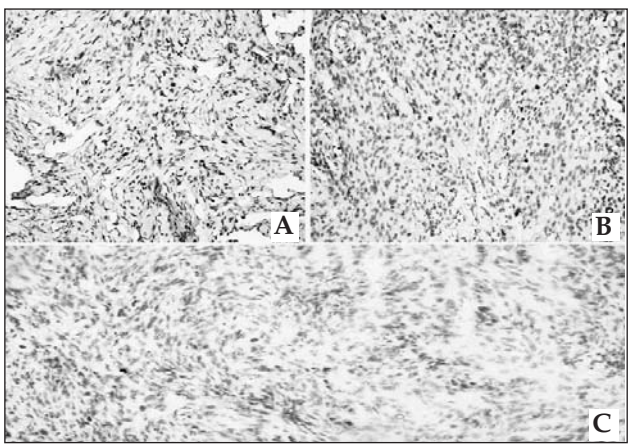


Figure 1: Ki-67 immunoreaction in meningiomas: **A:** atypical, **B:** meningotheliomatous, **C:** transitional meningiomas (Ki-67X 20)

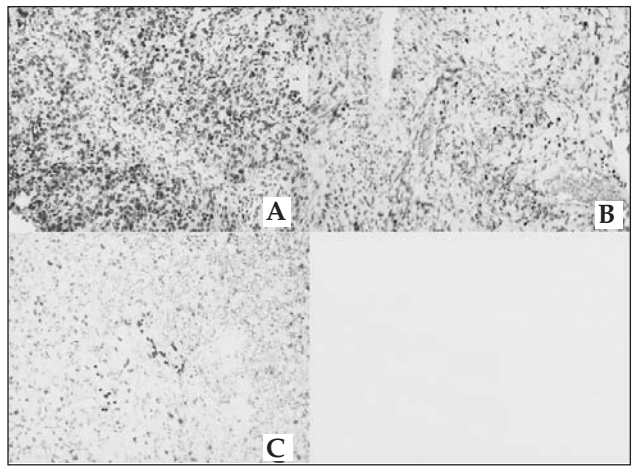


Figure 2: Immunopositivity of gliomas with Ki-67. A grade IV, B grade III and C grade II gliomas (Ki-67X 20)

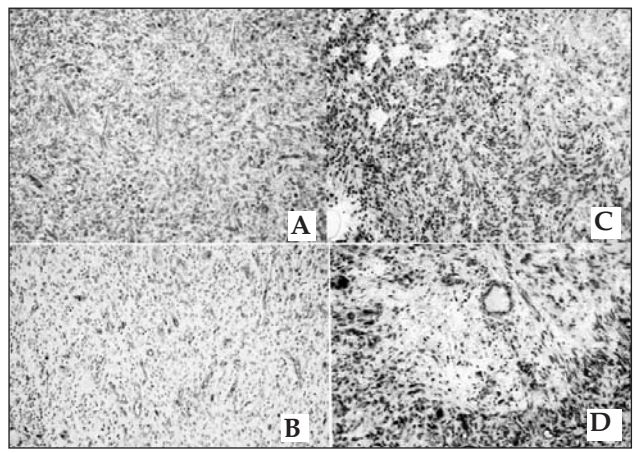


Figure 3: CD-34 expression in gliomas. A grade III, B grade II, C grade I and D grade IV gliomas (CD-34X20)

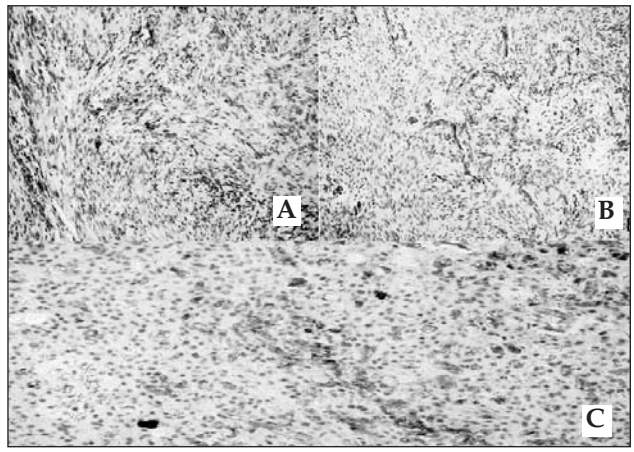


Figure 4: CD-34 positive reaction in meningiomas. A atypical, B transitional and C angiomatous meningiomas (CD-34X20)

DISCUSSION

We detected a positive correlation with histological grade and Ki-67 expression in gliomas. Grade-dependent increase of Ki-67 LI values were more prominent than CD-34 values and this is consistent with the literature (Figure 2 and Figure 3).

Giannini et al. revealed that MIB-I LI was a very significant prognostic criterion for 50 patients with anaplastic astrocytomas treated predominantly with gross resection. However the cutoff values for MIB-I count were broadly scattered (6).

The cutoff values of Ki-67 differ from one study to another, thus making comparisons of values between laboratories difficult. These discrepancies can be explained by the regional tumor heterogenity, immunohistochemicalprocedures and interpretation of immunoreactivity (12).

Kayaselcuk et al. have reported that Ki-67 LI values ranged from 5.6+23.29 to 15.0+1.8 in a glioblastoma multiforme group (5).

Montine et al. found a cutoff point for Ki-67 of 7.5% in astrocytoma (6,7). They suggested that a Ki-67 index of ≥7.5% in astrocytomas was associated with a higher histologic grade and poorer survival rate (2). Tihan et al. also showed a figure of around 15% in stereotactic biopsies (13).

Abdulrauf et al. reported that microvessel density and vascular endothelial growth factor (VEGF) level are prognostic markers of survival in patients with fibrillary low-grade asrocytomas (1, 3). However, Vaquero et al suggested that in contrast to low-grade astrocytomas the angiogenesis score of low-grade olidenrogliomas adds little information to predict tumor behavior (15).

The importance of angiogenesis as a prognostic factor influencing survival has been studied in brain tumors in a number of studies and a relationship between high microvessel density, age, higher histological grade and survival has been reported in astrocytic tumors. It has been shown that MVD production is stimulated by low oxygen concentration in cultured cells and this is the presumed mechanism of the increased expression of MVD in tumors with significant ischemia/necrosis such as glioblastoma multiforme (GBM) (1).

Although grade-dependent increase of vascularization is not statistically important, the CD-34 values of high-grade gliomas were higher in our study.

The mean Ki-67 values were detected as 6.80±4.68 in glioma and 3.10±2.80 in meningioma at this study. This may support a lower histologic grade and a better prognosis of meningioma rather than glioma. The mean values of CD-34 were 8.38±6.48 in gliomas and 6.89±4.17 in meningiomas.

The values of Ki-67 were detected as 4.44±0.40 and 2.80±0.30 respectively in atypical and malignant meningioma. Otherwise it is interesting that the Ki-67 values were 4.26±4.05 in psammomatous and 3.69±3.24 in meningothelial meningioma. There is variation in positivity ranges of Ki-67 among the low-grade meningiomas and this may indicate that there is no relation between histologic grade and Ki-67 in meningiomas.

Roggendorf et al found a correlation between Ki-67 values and the recurrence and clinical behavior in meningiomas (10).

The origin of the Ki-67 antigen is not known yet and false positive results cannot be excluded. At the same time, Ki-67 immunoreactivity is highly dependent on fixation time, specimen storage and antigen retrieval technique.

Kayaselcuk et al. found a Ki-67 LI value of 0.99+1.15 and 33.12+29.39 respectively for low grade and malignant meningiomas (5).

CD-34 yield was strongest in angiomatous meningioma because of its angiomatous supply, although it has neither a poor prognosis nor a high histologic grade.

Meningioma does not reveal vascular changes like glioma (7). They arise from the arachnoidal cap cells and grow within the highly vascularized meningial compartment. In this location, meningiomas are able to provide their blood supply and do not need neovascularization (7). However, gliomas usually arise within the white matter where they find a sparse vascular network that is in contrast to the situation for meningiomas (7).

Lamzsuz et al. indicated that MVD immunoreactivity did not correlate with WHO grade in meningiomas (7). Our results are also consistent with the literature. Despite the increase of vascularity with grade, similar differences were detected within one grade among the various histological subtypes of meningiomas in our study.

Vaquero Ju et al. found that scores of Ki-67 LI and MVD do not correlate in GBM (7). Tumor angiogenesis is a better histological factor than MIB-I LI for estimation of behavior but MIB-I LI may be more useful as an independent factor influencing survival (4).

Table II. Immunopositivity of tumors with Ki-67 and CD-34 according to histopathological subtypes

Type of tumor	n	Ki-67	p*	CD34	p*
GLIOMA					
Grade I	4	3.33±2.50		6.30±3.69	
Grade II	2	3.10±0.00	**0.007**	3.88±0.82	
Grade III	5	5.01±2.28		11.80±9.71	0.161
Grade IV	7	10.33±4.85		8.40±5.45	
MENINGIOMA					
Psammomatous	5	4.26±4.05		6.44±4.15	
Meningothelial	7	3.69±3.24	0.858	5.94±2.07	
Transitional	13	2.83±2.70		7.77±4.61	
Fibroblastic	6	2.56±2.69		4.70±2.93	0.583
Atypical	1	4.40±0.40		7.00±0.70	
Angiomatous	1	0.60±0.00		19.60±0.20	
Malignant	3	2.80±0.30		6.14±1.91	
TOTAL	**54**				

*Kruskal-Wallis

CONCLUSION

Tumor angiogenesis plays a critical role in the malignant nature of gliomas to supply nutrition of the invaded cells, expanding to the adjacent normal tissues.

Microvessel density is not meaningful for meningiomas as well as gliomas in grading.

Ki-67 is a very popular laboratory technique but this method has some serious deficiencies.

Our results reveal the advantage of a combined approach including evaluation of routine histological parameters and immunohistochemical techniques.

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Abbreviations
vascular endothelial surface score (VESS)
proliferative activity index (MIB-I LI)
labeling index (LI)
mean vascular density (MVD)
central nervous system (CNS)
vascular endothelial growth factor (VEGF)
glioblastoma multiforme (GBM)