RESEARCH ARTICLE

Prognostic Factors in Oligodendrogliomas: a Clinical Study of Twenty-Five Consecutive Patients

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Abstract

Background: The purpose of this study was to evaluate the prognostic significance of Ki-67 and subjective microvascular density (SMVD) indexes together with other factors in patients with oligodendroglioma. Materials and Methods: In this retrospective study, oligodendroglioma specimens obtained from twenty-five consecutive patients were evaluated for Ki-67 and SMVD indices to help determine histological grading and investigate the fidelity of these markers in clinical prognosis. Other potentially prognostic factors were Karnofsky performance scale, tumor histological grade, and adjuvant radiotherapy. Results: The Ki-67 proliferation index appeared to have a strong correlation with the grade of the tumor and the survival. Age, gender, adjuvant radiotherapy, surgical resection type (complete versus incomplete) did not have any influence on recurrence. The SMVD index correlated significantly with the 3 to 5-year survival. Conclusions: Ki-67 and MVD indexes are important and useful markers in estimating the prognosis of oligodendrogliomas.

Keywords: Oligodendroglioma - ki-67 index, microvascular density - karnofsky performance scale - CD34 staining

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Introduction

The common supratentorial brain tumors in children are oligodendroglioma, astrocytoma, primitive neuroectodermal tumor; ependymoma and teratoma while the common infratentorial pediatric brain tumors are astrocytoma, primitive neuroectodermal tumor, choroids plexus papilloma and ependymoma (Ahmed et al., 2007). Oligodendroglial tumors are classified into two groups; grade II (low grade) and grade III (high grade) (Louis et al., 2007). Although surgery has been accepted as the initial treatment, the optimal treatment is still under debate (Shaw et al., 1992; Cairncross et al., 1994; Scerrati et al., 1996; Allam et al., 2000; Engelhard et al., 2003). Overall survival rates of malignant central nervous system tumors decreased over time and this was in relationship with tumor grade (Zahir et al., 2014). In different studies various prognostic factors have been identified (Allam et al., 2000). Studies showing an association between proliferation markers like Ki-67 and prognosis have supported the benefits of Ki-67 evaluation in determining malign progression (Ocal et al., 2014). Vascular endothelial growth factor along with Ki-67 can serve as a valuable marker for the prognosis of brain tumors such as neuroblastoma (Gheytanchi et al., 2014). Haroon's study indicates that with high grade breast tumors, clinical utility of Ki-67 is greater in combination with other prognostic markers (Haroon et al., 2013). Some of good prognostic factors are considered as younger age, frontal location, absence of neurologic deficit at diagnosis, initial presentation with seizure, higher performance status, more extensive surgical resection, radiation therapy (if complete resection was achieved), WHO grade II histology, lower proliferative activity, and chromosomal loss at 1p and 19q (Engelhard et al., 2003).

The aim of the present study was to evaluate the effects of Ki-67 and microvacular density indexes on recurrence and prognosis in oligodendrogliomas.

Materials and Methods

The present study included a retrospective analysis of 34 patients with the pathological diagnosis of oligodendroglial tumors that was been treated at Dışkapı Yıldırım Beyazıt Teaching and Research Hospital. Four cases were excluded from the study because of having astrocytic component. Hospital folders of 30 patients were retrospectively searched for demographic data of patients such as age gender, presenting symptom, presence of neurologic deficit at presentation, performance status,

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radiological findings, extent of surgery, application of radiotherapy, and histopathological diagnosis. Patients or their relatives were inquired to be informed regarding the current status of the patients. Five patients with inadequate data or those who couldn't be reached were also excluded from the study thereby 25 patients were enrolled into the study.

Karnofsky Performance Status Scale (KPS) was used to evaluate the daily life performance. Patients were classified according to their KPS as; 10-20 points group IV, 30-40 points group III, 50-70 points group II, and 80-100 points group I. Patients were divided into two groups according to their KPS scores as group I (good performance level, KPS≥50), and group II (KPS≤50, poor performance level). Cut off level of KPS were accepted as 50 because scores below 50 indicate considerable assistance and frequent medical care as well as poor prognosis (Yates et al., 1980).

Patients were grouped according to their treatment modality; surgery alone and surgery plus radiotherapy. In our routine clinical practice, every patient undergoes brain MRG (contrast enhanced) on postoperative first day to determine the amount of resection. This classification was as follows: *i)* Total resection: There was no tumor tissue visible on the contrast enhanced MRI. *ii)* Near-complete resection: There was residual tumor tissue less than 10% of preoperative tumor tissue). *iii)* Biopsy: Less than 90% tumor removal.

Sixteen (11 patients with grade II tumor and 5 patients with grade III tumors) of 25 patients underwent radiotherapy (RT). The decision for radiotherapy was made according to the histopathological grade of the tumor, ki-67 proliferation index, and general status of the patient.

Histopathological evaluation

Haematoxylin-Eosin (HE) stained slides were re-evaluated by a senior neuropathologist. Based on the World Health Organization (WHO) classification criteria, 18 patients were classified as grade II and seven patients were classified as grade III oligodendroglioma (Allam et al., 2000). Specimens of all of 25 patients were stained with Ki-67 and 21 of them were stained with CD34 antibody.

Evaluation of the Ki-67 immunohistochemical staining

On each section, the most intense three areas stained with Ki-67 were identified and 500 cells were counted randomly on these areas. A ratio of the number of nuclei stained positively with Ki-67 antigen was determined in each area and the average of these ratios was considered as Ki-67 index. (Figure 1)

Evaluation of the CD-34 immunohistochemical staining

The most intense regions of vascularity were determined and the microvessel count was performed under microscope magnified 200x first and x400 subsequently. While counting, areas showing continuity with each other were considered as a single vascular structure. Also, individually dispersed positively staining cells without any lumen and clusters of cells were accepted as isolated vascular structure. In this way, microvascular density (MVD) values were determined. Additionally, a subjective vascularization score was determined by looking over the sections of the site of the highest vascularization area and these areas were scored from 1 to 4 subjectively as Weidner et al recommended(Weidner et al., 1993). In this way, subjective MVD (SMVD) values were determined and vascular endothelial proliferation areas were scored as 3 or 4.

Degree of mitotic activity

Mitotic activity was assessed analyzing the routine HE stained slides without using a separate immunohistochemical marker. The number of mitotic figures was counted under light microscopy on HE stained slides at 50 high power field. (Figure 2).

Statistical analysis

Data analysis was performed using SPSS 11.5 software. Descriptive statistics were expressed as mean ± standard deviation for continuous variables and percentage for categorical variables. Variables about the survival of patients were assessed with one way ANOVA. Mann-Whitney U test was used in post-hoc evaluation. To evaluate if there is any statistically significant difference between categorical variables and recurrence Chi-square or Fisher's exact test was performed. Additionally, Kaplan-Meier survival analysis with Log-rank was used in order to detect the contribution of categorical variables on survival. Three and 5-year survival for all groups were also calculated.

The odds ratio, 95% confidence interval, and the level of significance for each variable were calculated. Risk factors with the greatest impact on mortality were detected by multivariate Cox regression. P value less than 0.05 was considered statistically significant.

Results

This study included 17 male and 8 female patients. The mean age of patients was 47.7 years (16-73 years). The mean age of patients with grade II tumors was 47.1 years and 49.1 in patients with grade III tumors. There was no statistically significant difference between the mean ages of the groups (p>0.05).

Presenting symptoms were headache (80%), epileptic seizure (60%), motor deficit (44%), vomiting (32%), visual disturbance (32%), and behavioral change (16%). Mean duration of symptoms was 11 months (1-58 months). Initial Karnofsky performance scale (KPS) was good (KPS I and II) in 80% of patients, however, KPS was poor in 20% (KPS III and IV).

In 16% (4) of patients, complete tumor removal was achieved. In 84% (21) of them, near- complete resection was performed. There were no biopsies. Seventy-two percent (18) of patients had grade II tumors and the remaining were harboring grade III tumor. Mean follow-up time was 50,6 months (6-120 months). Seven patients died during follow-up. Average survival time was 40,4 months (6-89 months). The mean age of patients who died during follow-up was 57 ± 15 years. The mean age was 44 ± 13

years in survivors and there was no statistically significant survival difference between these two groups (p> 0,05).

Mean Ki-67 proliferation index in patients with grade II tumors was 4.2±3.6 (0-15) and 16.3±7.4 in patients with grade III tumor and the difference in Ki-67 index was highly significant (p<0,0001). Patients were divided into two groups according to their Ki-67 proliferation index; group I: Ki-67 proliferation index ≤4, Group II Ki-67 proliferation index ≥5. Ki-67 proliferation index was ≥5 in four of the seven patients who died due to tumor recurrence and the mean Ki-67 proliferation index in these four patients was 13.1. We detected a statistically significant correlation between Ki-67 proliferation index and grade of the tumor (r=0.615, p<0.001). Notably, a similar statistically significant correlation between Ki-67 proliferation index and the survival was observed (CI:1.0-1.4 p<0.05). Additionally overall survival and the grade of the tumor was correlated significantly (p<0.05).

However, gender, age, adjuvant radiotherapy appeared to have no statistically significant influence on survival (p > 0,05 for each parameter). Likewise, age and gender did not have any significant influence in recurrence (p=0,084 and p=0.0661, respectively).

In comparison to complete resection, incomplete resection appeared to increase the recurrence rate; However, this increase didn't reach a statistically

Table 1. Single Variable Cox Regression Analysis for The Risk Factors Of Mortality

Variables	Odds Ratio	95% Ci	P Valu
Age	1.1	0.99-1.22	0.070
Gender			
Female	1		
Male	1.58	0.2-8.84	0.600
Excision			
Subtotal	1		
Total	3.97	0.66-23.90	0.132
Grade			
Grade 2	1		
Grade 3	5.07	0.90-28.66	0.066
Radiotherapy			
-	1		
+	33.91	0.01-134615.8	0.405
Ki-67Group	1.17	1.04-1.32	0.012
≤4			
>4	1		
MVD(X200)	1.66	0.27-10.17	0.582
MVD(X400)	1.01	0.99-1.03	0.396
Variables	1.01	0.98-1.05	0.466
Mitosis	1.17	1.00-1.36	0.044

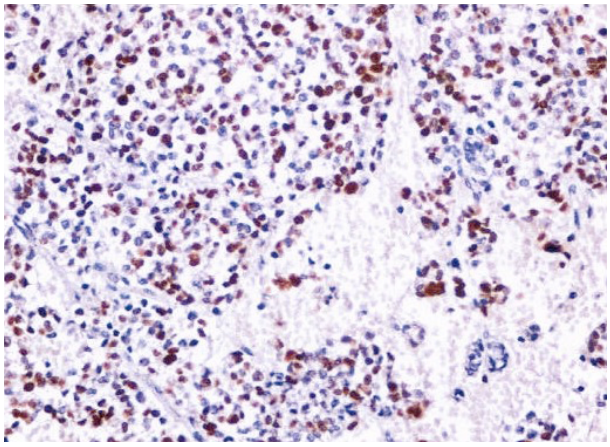

Figure 1. Oligodendroglioma with High Index Ki-67 (X200, Streptavidin Biotin Peroxidase Stain)

Table 2. Single Variable Statistical Work-Up for The Risk Factors Of Recurrency

Variables	No Recurrence (N=16)	Recurrence (N=9)	P Value	Odds Ratio(95% Ci)
Age	43.7±13.33	54.8±15.50	0.084	1.06(0.99-1.14)
Gender				
Female	6(%37.5)	2(%22.2)	-	1
Male	10(%62.5)	7(%77.8)	0.661	2.10(0.32-13.61)
Excision				
Subtotal	14(%87.5)	7(%77.8)	-	1
Total	2(%12.5)	2(%22.2)	0.602	2.00(0.23-17.34)
Grade				
Grade 2	13(%81.3)	5(%55.6)	-	1
Grade 3	3(%18.8)	4(%44.4)	0.205	3.47(0.56-21.35)
Radiotherapy				
-	6(%37.5)	1(%11.1)	-	1
+	10(%62.5)	8(%88.9)	0.355	4.80(0.47-48.46)
Ki-67Group	5.6±5.20	11.2±9.31	0.089	1.13,(0.98-1.29)
≤4	10(%62.5	3(%33.3)	-	1
>4	6(%37.5)	6(%66.7)	0.226	3.33(0.60-18.54)
MVD(X200)	106.1±51.18	108.2±51.33	0.933	1.00(0.98-1.02)
MVD(X400)	50.4±31.49	52.5±25.15	0.880	1.00(0.97-1.04)
Smvd				
Score 1	7(%63.6)	2(%33.3)	-	1
Score 2	3(%27.3)	2(%33.3)	0.486	2.33(0.22-25.24)
Score 3	1(%9.1)	2(%33.3)	0.184	7.00(0.40-123.35)
Mitosis	1.4±2.97	4.8±7.44	0.258	1.17(0.89-1.55)
M=0	7(%63.6)	-	-	1
M>0	4(%36.4)	6(%100.0)	0.035	-

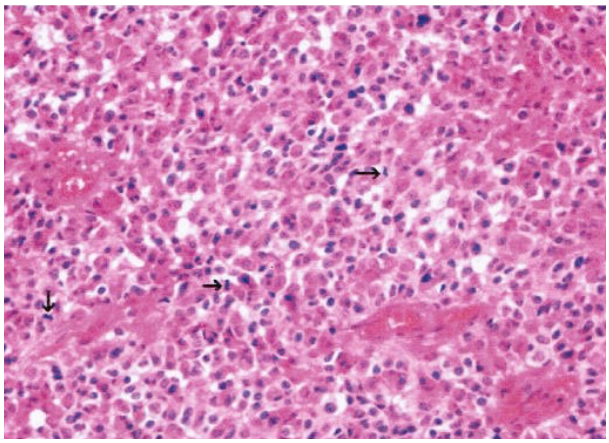

Figure 2. Anaplastic Oligodendroglioma with High Mitotic Activity (X200, Hematoxylin Eosin Stain)

significant level (p=0,602). Moreover, there was no statistically significant difference between the grades of the tumor (II or III) and the recurrence (p=0.205). The recurrence rate was higher in patients who did not undergo radiotherapy but this difference was not statistically significant (p=0.355).

Recurrence rate was found to increase with the increase in Ki-67 index, However, this correlation was not statistically significant (p=0.089). Similarly, there was no correlation between MVD levels on the x200 and x400 magnifications and recurrence (p=0.933). However, the degree of mitosis revealed a non-statistically significant effect on the recurrence risk (p=0,258).

While age and the extent of resection did not exert any significant effect on the three and five year survival times (p=0.597 and 0.104, respectively), the grade of the tumor was a significant parameter on 3 and 5-year survival (p=0.044). Survival was shorter in patients with grade III tumors.

Adjuvant radiotherapy and Ki-67 index were not statistically significant factors on 3 and 5-year survival (p=0.164 and p= 0.578, respectively). However, there was a statistically significant correlation between SMVD score and 3 and 5 year survival (p=0.049). Patients having more than 2 mitosis experienced shorter survival times compared to those patients having less than two mitosis (p=0.021).

The sensitivity of SMVD in predicting the grade of tumor was 83.3% and specificity was 72.7%. Cut off point for SMVD was found as 2. SMVD value over two was statistically significant for predicting tumor grade as III.

When Ki-67 index compared to SMVD at x200 and x400 magnification, it was found that SMVD score was directly correlated with the increase in Ki-67 staining (rho=0,643 and p=0,005). However, we didn't detect a similar correlation for MVD. In addition, there was not a statistically significant correlation between the count of mitosis and the MVD and SMVD value at x200 and x400 magnification.

Discussion

Our study demonstrated that there is a strong relationship between the Ki-67 index and the overall survival. A similar relationship was observed between the tumor grade and survival. As a novel contribution, we detected a significant inverse correlation between the survival and the subjective microvascular density (SMVD).

Deletions of 1p/19q are well known prognosticators in oligodendrogliomas(Engelhard et al., 2003). However, analyzing these deletions cannot be routinely executed in every hospital thus there is a need for other less sophisticated indicators in order to determine the prognosis in patients with oligodendrogliomas. In our study, we unfortunately couldn't test the deletions of these genes.

Poor prognostic factors for oligodendrogliomas are as follows; older age, contrast enhancement, presence of neurologic deficit at the time diagnosis, poor Karnofsky performance status, inability to resect safely, WHO grade III histology, higher proliferative activity, increased angiogenesis, p16/CDKN2A deletion, 10q loss, EGFr amplification, and increased p53 (Engelhard et al., 2003).

In the differentiation between the grade II and grade III tumors, anaplasia is the main criteria according to the Current WHO classification system1. Necrosis, increased mitotic activity, cellular pleomorphism, and vascular proliferation are also associated with poor prognosis (Coons and Pearl, 1998; Coleman et al., 2006; Miller et al., 2006). Although classification scheme does not contain the frequency of mitosis, previous studies using Ki-67 immunohistochemistry reported that low grade oligodendroglioma with high Ki-67 index might show more aggressive course (Heegaard et al., 1995; Heesters et al., 1999; Kayaselcuk et al., 2002; Wharton et al., 2004; Coleman et al., 2006). Therefore, histological grade and Ki-67 proliferation index have become microscopic variables predicting a poorer prognosis in oligodendrogliomas. However, a cut off value waits to be defined for Ki-67. Variations in staining procedures for Ki-67 in different laboratories also stand as a major drawback. In the present study, we clearly detected a statistically significant correlation between the Ki-67 index and the grade of the tumor. We also found that higher Ki-67 index was correlated with decreased survival. This finding was consistent with the literature that suggests Ki-67 proliferation index may be an independent prognostic factor in oligodendroglioma (Schiffer et al., 1997; Reis-Filho et al., 2000).

As well-know, WHO classification classifies mitotic activity together with microvascular proliferation and necrosis as criteria of anaplasia1. Likewise, we also found that mitosis ≥2 seemed to be a factor increasing the recurrence of the tumor. According to our findings, extent of resection and adjuvant radiotherapy prolonged the time of tumor recurrence, but they didn't reach a statistically significant level. This result may be due limited number of patients and retrospective nature of our study. However, we found that higher Ki-67 index correlated with increased mortality significantly.

The impact of increased microvascular density (MVD) on prognosis is controversial in oligodendroglioma. Vaquero et al suggested that the presence of microvessel more than 100 at x200 magnification didn't affect the

prognosis (Vaquero et al., 2000). In our study we found that there was no statistically significant impact of SMVD score at 3 and 5-year survival times. However, SMVD was a reliable indicator in predicting the grade of the tumor.

We performed complete resection in (16%, 4) of our cases. In remaining cases (84%, 21) incomplete resection was performed. In a study of Quon et al., authors reported complete resection in 17,5%, incomplete resection in 69,3% and biopsy in 8% of cases. In this study authors reported a significant correlation between grade of the tumor and resection type (Quon et al., 2010). Similarly, other studies in the literature reported similar resection rates (Fortin et al., 1999; Allam et al., 2000; Olson et al., 2000). Our resection rates well correlated the other studies in the literature except that we didn't perform any biopsy procedures because of the lack of stereotactic set up.

Ki-67 proliferation index and SMVD are important immunohistochemical indicators on prognosis and in the management of patients after surgery. These two indicators have a significant relationship with grade of the tumor as well as the overall survival time. However, multicenter prospective studies with greater number of patients will further enlighten the prognostic factors and treatment strategies of oligodendrogliomas.

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