Original Article
Pediatric supratentorial glioblastoma: clinico-pathological features and influencing factors of the outcome

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Abstract: Objective: pediatric glioblastoma is a rare brain tumor of childhood. The aim of this study was to evaluate the long-term survivals of the pediatric supratentorial glioblastoma patients and the clinical-pathological factors affecting their outcomes. Methods: A series of 31 consecutive patients (age range 2-18 years) who underwent primary tumor resection for pediatric supratentorial glioblastoma during the years 2007-2013 were included in this retrospective study. Their medical records and radiological data were analyzed, retrospectively. Immunohistochemistry of IDH1, Ki-67, EGFR, p53, NF1, MGMT and PTEN analyses were performed in all cases. Kaplan-Meier analysis was used to assess progression-free survival (PFS) and overall survival (OS), with the log-rank test being used to evaluate differences between survival curves. Results: Mean follow-up was 19.45 months (range 9-68 months). The median PFS was 10 months and the median OS was 16 months. Multivariate analysis revealed that the median OS was significantly associated with extent of resection (17 months for complete resected tumors vs. 12 months for incompletely resected tumors; P=0.030) and PTEN protein expression (23 months for positive vs. 15 months for negative; P=0.046). Extent of resection was also found to be significant prognostic factor of PFS. The rate of positive NF1 protein expression among children was higher, and these children experienced a better outcome. Conclusion: Pediatric supratentorial glioblastoma has a relatively better outcome compared with the adult patient. PTEN expression and complete resection are significant favorable factors in children with supratentorial glioblastoma.

Keywords: Pediatric, glioblastoma, outcome, immunohistochemistry

Introduction

Pediatric brain tumors, comprising approximately 24.5% of pediatric malignant tumors, are now the leading cause of death from childhood cancers [1]. Among them, glioblastoma is relatively rare (accounting for 3-8.5% of all CNS tumors in children) and highly invasive, poorly responsive to conventional treatments [2, 3]. The median survival for pediatric glioblastoma via conventional-dose chemotherapy and radiotherapy is currently around 11-24 months. At 5 years, the overall survival rate is 5-20% [3-6]. Therefore, considering the relatively better prognosis compared to their adult counterpart, debate exists as to whether pediatric glioblastoma have a different clinicohistopathological features and molecular pathways of tumorigenesis [7-9].

However, clinicohistopathological studies of pediatric glioblastoma are relatively rare and histopathological features of IDH1, Ki67, EGFR, p53, NF1, MGMT and PTEN using immunohistochemistry have not yet been reported. This retrospective study consisting 31 patients of pediatric supratentorial glioblastoma is one of the largest single institution series to be reported to date. Here, we report clinical features and pathological characteristics from an immunohistochemical study of IDH1, Ki67, EGFR, p53, NF1, MGMT and PTEN protein expression in pediatric glioblastoma. We have also tried to evaluate the clinico-pathological factors affecting their outcomes.
Material and methods

Patient population

We searched our departmental database retrospectively from January 2007 and September 2013 and identified 37 children with cerebral glioblastoma. In these 37 cases, 4 patients who were brainstem glioblastoma were excluded. 1 cerebellum and 1 in the fourth ventricle glioblastoma were also excluded from the further analysis. Thus, a total of 31 patients with pediatric supratentorial glioblastoma were included, and their medical records were reviewed. Diagnoses were based on histological and immunohistochemical features according to the 2007 WHO classification system and were reviewed by two pathologists (JQY and XH). Clinical outcome was assessed via the out-patient and telephone interview. All patients underwent follow-up imaging studies.

The starting point of PFS and OS was the day of surgery. Tumor progressions at last follow-up examination or death were the end points for PFS and OS, respectively. All patients or their relatives signed the informed consent for the publication of their clinical details. The trial was approved by the Biological and Medical Ethics Committee of West China Hospital.

Immunohistochemistry

Tumor samples obtained during surgical treatment were fixed in formalin and embedded in paraffin for histological studies. We reviewed the hematoxylin-and-eosin-stained slides of the tumor specimens and selected representative tissue blocks during review. Antibodies for IDH1 (dilution, 1:100; Dianova), Ki-67 (dilution, 1:100; Dako Cytomation), EGFR (dilution, 1:100; Zymed), P53 (dilution, 1:50; Dako Cytomation), NF1 (dilution, 1:100; Abcam), MGMT (dilution 1:50, Neomarkers) and PTEN (dilution, 1:50; Dako Cytomation) were used for immunohistochemical analysis according to the manufacturers’ instructions.

All immunostained slides were independently evaluated twice by two of the authors (JQY and XH) without knowing the outcomes of the patients. The presence of marker-positive tumor cells was the chief criterion used to distinguish positive cases from other negative cases. The Ki-67 labeling index was established by determining the percentage of positive nuclei among 1000 tumor cells. IDH1, P53, NF1, and MGMT staining was divided into two groups: negative (< 5% of cells stained) and positive (> 5% of cells stained). Stains for EGFR and PTEN were graded as follows: negative (< 10% of cells stained) and positive (> 10% of cells stained).

Statistical analysis

The effect of single variables on progression-free survival (PFS) and overall survival (OS) was estimated using the Kaplan-Meier method, and survival curves were compared using the log-rank method. The single variables included sex, age, initial KPS score (≥ 80 vs. < 80), tumor location (superficial lesion vs. deep lesion), extent of resection, IDH1, Ki67 index (> 20 vs. ≤ 20), EGFR, P53, NF1, MGMT and PTEN. Variables achieving P < 0.10 in univariate analysis, were subsequently introduced in a multivariate analysis (Cox model) as independent predictors of survival. All tests were two-tailed, and the significance level was set at 0.05. Statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical profile

During the study period, 256 children with cerebral glioma underwent surgery, 31 of which
Figure 2. A-C. Brain tumor of case 2 exhibited. A. Evidence of mitosis, vascular proliferation or necrosis. The entrapped neurons appear normal (H&E, ×200). B. Ki67 proliferative index is high (40%), ×400. C. PTEN immunostains, ×400 negative. D. Case 17 exhibited PTEN immunostains, ×400 positive.

Figure 3. Kaplan-Meier analysis of (A) progression-free survival and (B) overall survival of the entire population of 31 patients. Hash marks represent censored subjects.
were supratentorial glioblastoma (Figure 1). The mean age of the patients was 11.6 (range, 2-18) years, and the male to female ratio was 22:9. The number of patients younger than 3 years and older than 3 years was three (9.7%) and twenty-eight (90.3%), respectively. The majority of the tumors were located in the superficial cortical regions (n=21, 67.7%). Ten patients presented with lesions in the deep subcortical structures including basal ganglia and the thalamus.

Headache was the most common presenting symptom with nausea/vomiting in 20 patients (64.5%), followed by limb numbness/weakness in 7 patients (22.6%), and seizure in 6 patients (19.4%). Visual complaints included visual blurring and field defect. These were observed in 4 (12.9%) patients.

Complete and safe resection of the primary tumor was attempted in all patients. Complete resection was achieved in 23 patients (74.2%). Incomplete resection including the subtotal and biopsy excision was noted in 8 patients (25.8%). After surgery, six patients received three-dimensional conformational radiotherapy with a total dose of 60 Gy in 30 fractions. Five patients was performed Gamma knife surgery after the operation. The PCV (included the prednisone, CCNU, and vincristine) regimen was given in three patients and the temozolomide regimens was administered in seven patients.

Immunohistochemistry results

Only one of the 31 patients (3.2%) showed EGFR positive. All 31 cases were IDH1 negative. Ki-67 index was from 10 to 60% and 16 patients (64.5%) showed Ki-67 index over 20%.

Table 1. Univariate analysis of PFS and OS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median PFS (months)</th>
<th>Log rank, P</th>
<th>Median OS (months)</th>
<th>Log rank, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>8.0</td>
<td>0.681</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10.0</td>
<td>0.176</td>
<td>14</td>
</tr>
<tr>
<td>Age</td>
<td>&gt; 11 years</td>
<td>8.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 11 years</td>
<td>10.0</td>
<td>0.763</td>
<td>17</td>
</tr>
<tr>
<td>KPS score</td>
<td>≥ 80</td>
<td>10.0</td>
<td>0.514</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>&lt; 80</td>
<td>8.0</td>
<td>0.648</td>
<td>13</td>
</tr>
<tr>
<td>Location</td>
<td>Deep</td>
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<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>superficial</td>
<td>10.0</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Extent of resection</td>
<td>Complete resection</td>
<td>11.0</td>
<td>0.007</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Incomplete resection</td>
<td>7.0</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>KI-67 index</td>
<td>≤ 20</td>
<td>10.0</td>
<td>0.219</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>&gt; 20</td>
<td>8.0</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>EGFR</td>
<td>Positive</td>
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<td>0.327</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>10</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>P53</td>
<td>Positive</td>
<td>7</td>
<td>0.175</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>10</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>NF1</td>
<td>Positive</td>
<td>13</td>
<td>0.148</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>MGMT</td>
<td>Positive</td>
<td>7</td>
<td>0.107</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
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<td></td>
<td>16</td>
</tr>
<tr>
<td>PTEN</td>
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<td>11</td>
<td>0.099</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8</td>
<td></td>
<td>15</td>
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Table 2. Multivariate analysis of PFS and OS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>P value</th>
<th>95% CI</th>
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<tr>
<td>PFS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>1.530</td>
<td>0.060</td>
<td>0.983-2.380</td>
</tr>
<tr>
<td>Extent of resection</td>
<td>0.264</td>
<td>0.007</td>
<td>0.101-0.693</td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>1.723</td>
<td>0.026</td>
<td>1.066-2.786</td>
</tr>
<tr>
<td>Extent of resection</td>
<td>0.289</td>
<td>0.015</td>
<td>0.106-0.787</td>
</tr>
</tbody>
</table>

were supratentorial glioblastoma (Figure 1). The mean age of the patients was 11.6 (range, 2-18) years, and the male to female ratio was 22:9. The number of patients younger than 3 years and older than 3 years was three (9.7%) and twenty-eight (90.3%), respectively. The majority of the tumors were located in the superficial cortical regions (n=21, 67.7%). Ten patients presented with lesions in the deep subcortical structures including basal ganglia and the thalamus.

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Immunohistochemistry results

Only one of the 31 patients (3.2%) showed EGFR positive. All 31 cases were IDH1 negative. Ki-67 index was from 10 to 60% and 16 patients (64.5%) showed Ki-67 index over 20%.
P53 was positive for 87.1% of 31 tumors and MGMT positive was in 4 patients (12.9%). NF1 was negative for 26 patients (83.9%). 25.8% patients showed PTEN positive. Sex, age, location, preoperative seizure and KPS scores did not find any significant correlation with the immunohistochemical results. Figure 2 showed the result of the immunohistochemical findings.

**Outcome analysis**

The mean follow-up time was 19.45 months (ranging from 9 to 68 months). 4 patients (12.9%) remained alive to the last follow-up. The median PFS and OS among children with supratentorial glioblastoma were 10 months (95% CI, 8.228-11.772) and 16 months (95% CI, 14.021-17.979), respectively (Figure 3). The 1-, 2- and 5-year survival rates in the current series were 61.3%, 22.6% and 3.2%, respectively.

Univariate analysis showed that sex, age, preoperative KPS, deep location, Ki-67 index, IDH1, MGMT, P53, NF1 and EGFR were not associated with the PFS or the OS. There was a slight, not significant, trend to longer PFS in PTEN positive patients (P=0.099). While PTEN positive patients showed significant longer OS than PTEN negative patients in the univariate analysis (P=0.046). Complete resections of the tumor were significant with the longer PFS and OS than incomplete resection (P=0.007 and P=0.030, respectively). The PFS and OS based on univariate analysis are listed in Table 1.

In the multivariate analysis, extent of resection was the only independent prognostic factor both for PFS and OS (P=0.007 and P=0.015, respectively). Children with PTEN immunohistochemical positive showed longer median OS (23 months) than the children with PTEN negative (15 months). The PFS and OS based on multivariate analysis are listed in Table 2 and Kaplan-Meier curves of OS according to extent of resection and PTEN IHC are showed in the Figure 4.

**Discussion**

In this study, we reviewed a series of 37 consecutive cases of pediatric glioblastoma that underwent surgical treatment at our department in the past six years. While preliminary study have estimated pediatric glioblastoma to account for about 12% of all pediatric gliomas, our analysis of 256 such cases has suggested it may account for 14.5% of pediatric gliomas [10]. The median PFS and OS time were 10 months and 16 months, respectively. These results correlated well with previous studies that showed that glioblastoma in children exhibits long-term survival and a better prognosis than adult glioblastoma [3, 4, 11].

In the present series, the mean age at the time of diagnosis was 11.6±4.53 years (range 2-18...
years). Interestingly, there were two peaks in the occurrence of pediatric supratentorial glioblastoma, 9-11 years and 15-17 years. They accounted for 32.3% (n=10) and 35.5% (n=11) of all pediatric supratentorial glioblastoma in our series, respectively. When we divided the children into two groups (age > 11 years vs. age ≤ 11 years), we found no statistical significance between them. Though, younger age may be considered a good prognostic factor in the adult glioblastoma, this may not be applicable in the children [2, 10]. There was also a clear male predominance, with a male to female ratio of 2.5:1. Similar findings were also noted by other authors and the female sex has been stated to be a good prognostic factor in adult glioblastoma [12, 13]. Our study, however, did not show any such survival difference in pediatric patients.

Although the molecular genetics of adult glioblastoma has been intensely studied over the past years, studies about the molecular profile in pediatric glioblastoma remains rare. In contrast to adult glioblastoma, pediatric glioblastoma generally lack EGFR amplification and EGFR over expression is rare in pediatric glioblastoma [14]. Over expression rate of protein p53 in pediatric glioblastoma was reported approximately 53.7~63% [7, 8]. The rate of MGMT protein expression by immunohistochemistry may occur in 12%-70% of the pediatric high-grade gliomas according to the previous studies [15]. Such a large disparity in MGMT immunohistochemistry could be due to variation in types of antibodies used, antigen retrieval timings, protocols followed, and methods of fixation. In this study, we evaluated EGFR, P53 and MGMT status by immunohistochemistry in 31 pediatric glioblastomas. We found that the EGFR, P53 and MGMT protein expression was presented in 6.5%, 80% and 12.9% of the cases, respectively. Though these molecular expression rates were different from the adult glioblastoma, we did not find either of them had influence in the prognosis of the pediatric glioblastoma patients. Additionally, While IDH1 mutation is selective markers for secondary glioblastoma and deemed as an independent prognostic factor for patients with glioblastoma and associated with improved overall survival [16]. In our cohort, all 31 cases were IDH1 negative, similar with the observations made by Byeon et al., suggesting that the pediatric glioblastoma may be primary glioblastoma [8].

Ki-67 index are indicative of proliferative activity of tumors and its expression predicts the grade of astrocytic tumors. A study by Pollack et al. of 98 patients with pediatric high-grade gliomas, showed that a significant association was noted between Ki-67 index and progression-free survival, even after the analysis had been stratified by histology. Tumors with Ki-67 indices higher than 36% had an almost uniformly poor outcome [17]. In our study, the Ki-67 index varied from 10% to 60%, and the median index was 30%, higher than others reports, but we did not find a significant association between the Ki-67 index and the survival [17, 18].

NF1 gene mutations and absent neurofibromin expression could have significant consequences for astrocyte differentiation and astrocytoma formation in children. The loss of neurofibromin expression leads to high levels of activated RAS and increased cell proliferation and possible tumor formation. So far, the clinical and pathologic features of glioblastoma in patients with NF1 were only reported in several case reports [19]. Huttner et al. reviewed the published reports of NF1-associated glioblastoma and concluded that children with NF1 might be at risk of glioblastoma and that the prognosis of glioblastoma in children with NF1 might be better than those without NF1 [20]. Though we did not find a significant association between NF1 expression and the outcome of pediatric glioblastoma patients, we found that the overall survival time with positive NF1 expression was 27.8 months, obviously longer than 21.1 months in patients with negative NF1 expression. Meanwhile, positive NF1 expression accounts for 16.1% of all the pediatric glioblastoma, which is higher than the adult glioblastoma. Further investigation for the glioblastoma patients with NF1 in children would be necessary to discover the difference.

The prognostic significance of PTEN expression in patients with glioblastoma is still controversial. It is worthy to note that PTEN gene mutation may contribute to the loss of PTEN protein expression and can negatively modulate the PI3K/AKT pathway, thereby potentially having a crucial effect on the control of cell cycle and cell survival [21]. Hence, PTEN gene mutation
Pediatric glioblastoma could be important and valuable prognostic factor for glioma. In a prospective phase II trial, LV et al. detected a positive PTEN expression in 21% (n=6) of high-grade glioma (n=35) and found all the six positive PTEN expression were glioblastomas (5/6 de novo glioblastomas and 1/6 secondary glioblastomas) [22]. Das et al. performed a histomorphological analysis of long-term survivors of glioblastoma and found that PTEN expression was noted in six of seven (85.7%) patients [23]. While Kraus et al. reported that there was no prognostic significance in PTEN mutation when comparing 21 long-term (more than 24 months) and short-term (less than 6 months) survivors of glioblastoma [24]. In our study, PTEN expression was noted in six of 31 (20.7%) patients. Our results showed that positive PTEN expression was significantly related to a longer OS in pediatric glioblastoma patients, suggesting that PTEN gene mutation may be regarded as an important and valuable factor in predicting clinical outcome of pediatric glioblastoma.

It is widely accepted that the first-line therapy for glioblastoma is surgery. Maximal tumor resection followed by radiotherapy and chemotherapy shows the most favorable outcomes [3-5, 14, 18]. Yang et al. reported that gross total resection was significantly associated with long-term survival in pediatric patients with glioblastoma not of the brainstem. The median OS was 45.1 months in patients with gross total resection versus less than 1 year in patients who had subtotal resection or biopsy [4]. Even in the recurrent glioblastoma, reoperation can lead to a modest increase in survival when performed in patients with a KPS of at least 60 [25]. Additionally, by removing a portion of the mass, symptoms associated with mass effect can be partially or totally relieved [4]. However, the extent of resection depends on the location of the tumor, as superficially located tumors enable a more complete surgical removal of the mass [2]. In our series, although superficial location had a relatively higher rate of complete resection to the deep located tumor (85.7% vs. 50%), there was no significant association between locations of the lesion (superficial vs. deep) in the survival. Only Patients with complete resection had a significantly longer OS and PFS compared to patients with incomplete resection.

Conclusion
This study has analyzed the clinical pathology and prognosis factor of 31 pediatric glioblastoma patients. Pediatric glioblastomas have a relatively better outcome compared with the adult patient. PTEN expression and extent of resection are independent prognostic factor with overall survival. Nevertheless, the small sample size and the retrospective nature of the analysis limits firm statistical results. In our study, the NF1 and PTEN expression rates in children were higher than the adult patients compared with the previous studies. Although the number of patients is limited, these data suggested that the pathogenesis of pediatric glioblastoma may be different from the adult glioblastoma. Further investigation is needed to identify potential genetic differences between pediatric and adult GBM.

Acknowledgements
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Disclosure of conflict of interest
None.

Abbreviations
KPS, Karnofsky Performance Scale; IDH1, isocitrate dehydrogenase 1; EGFR, epidermal growth factor receptor; NF1, neurofibromin 1; PTEN, phosphatase and tensin homolog; MGMT, O-6-methylguanine-methyltransferase; PFS, progression-free survival; OS, overall survival.

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