Factors predicting progression of low-grade diffusely infiltrating astrocytoma

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Abstract

Background: Low-grade diffuse astrocytoma (DA) is considered benign tumor (World Health Organization [WHO] grade II), but it has an inherent tendency for malignant progression, which is quite variable. Aim: To identify malignant progression in an individual case of DA, we studied the clinico-radiological and immunohistochemical factors and correlated with progression of DA at a dedicated tertiary level neurosciences centre NIMHANS, Bangalore, India. Patients and Methods: Consecutive adult patients who had undergone tumor decompression for lobar supratentorial DA at our institute from 1994 to 1998 were retrospectively selected and followed up for clinico-radiological progression. The clinico-radiological and histomorphological features were studied. With the use of immunohistochemistry, proliferation index [MIB-1 labeling index (LI)], p53 protein expression, microvessel density (MVD) count [assessed using anti-CD34 antibody] were analyzed and correlated with progression-free survival (PFS) Results: There were 13 patients. Mean age was 34 years. The most common presenting symptom was seizures. The median follow-up was 54 months. There were four recurrences, with median interval of 75 months. Eight patients received radiotherapy. Younger patients (<40 years), seizure as the presenting symptom and postoperative radiotherapy were associated with longer PFS, while gemistocytic morphology (>20% gemistocytic cells), MVD value >20 correlated with shorter PFS, albeit statistically insignificant. MIB1 LI did not correlate with recurrence pattern. Moreover, p53 LI > 10% correlated with early progression (P = 0.04). Conclusion: Our study highlights some of the clinical, histological and immunohistochemical parameters that predict progression on DA. Validation on a larger sample may be useful to plan appropriate treatment in an individual case.

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Introduction

Low-grade diffuse astrocytoma (World Health Organization [WHO] grade II) constitutes a minor group among
Diffusely infiltrating astrocytomas. These tumors have an inherent tendency of malignant transformation to a higher grade (WHO grade III/IV), which is highly variable. [1] Some clinical factors are known to determine prognosis of these patients, [2],[3],[4],[5],[6],[7],[8] but to date, no biological marker has been identified that unambiguously stratifies the individual patient at risk for malignant progression.

One of the most frequent and earliest genetic alterations recognized to occur in these tumors is the p53 mutation. [9],[10] However, the prognostic relevance of p53 mutation is still debated. [9],[11],[12],[13],[14] Some studies have shown that the frequency of p53 mutation is higher in tumors that progress to anaplastic astrocytoma and glioblastoma. [11],[15],[16] Further, with respect to growth and progression of tumor, angiogenesis plays an important role. The extent of angiogenesis as reflected by the microvascular density count in low-grade astrocytomas has predicted the prognosis and, importantly, subsequent malignant transformation of the tumor. [17]

In order to identify prognostic markers that govern early recurrence and, in turn, the progression in low-grade diffuse astrocytoma, we have assessed some clinical and histopathological features of diffuse astrocytoma (DA) along with the tumor's proliferative activity (reflected by MIB-1 labeling) and p53 immunoreactivity in the present study. We have also evaluated microvascular density counts in these tumors. The results were correlated with the progression of these tumors.

**Patients and Methods**

Consecutive adult patients (>18 years) with lobar supratentorial, DA (WHO grade II), who underwent surgical decompression at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India, between 1994 and 1998 were retrospectively selected and followed up for clinico-radiological progression. Clinico-radiological progression was defined as worsening of symptoms with corroborative evidence of radiological recurrence in these patients. All the cases diagnosed as low-grade DA (n = 38) during this period were collected. The formalin-fixed, paraffin-embedded blocks of histologically diagnosed cases were retrieved and fresh sections were prepared and reviewed by a single neuropathologist (VS). Twenty-five of these cases noted to have smaller foci of hypercellularity and increased mitosis suggesting progression within the tumor were excluded from the study and hence the study was conducted on 13 patients who satisfied stringent criteria of WHO grade II pure DA. The clinical data, imaging, operative findings, and patient outcome, including the incidence of recurrence and patient management were documented from the hospital records. Follow-up data were obtained by personal evaluation at the outpatient clinic or by postal communication through a designed questionnaire.

The specific histological features were presence of gemistocytes, stromal microcystic change (MCC), and tumor infiltrating lymphocytes (TIL).

**Immunohistochemistry**

Paraffin sections of thickness 5 µ were collected on silane-coated slides and subjected to immunohistochemistry (IHC) using monoclonal antibodies: MIB-1 (Ki-67-specific monoclonal antibody; DAKO, Denmark, Dilution-1:50), p53 (Dako, Denmark; Clone-DO7 Dilution-1:200), CD34 (Biogenex, USA; Clone-QBEND/10, Dilution-1:40). After preliminary standard processing steps, the sections were incubated with the primary antibody for 2 hours followed by secondary antibody; super-sensitive non-biotin HRP Detection Kit (Biogenex) for 1 hour. Chromogenic substrate used was 3,3’-diaminobenzidine (Sigma, Germany). Sections were counterstained with hematoxylin. Standard positive controls were used for MIB-1 and CD34 stainings. For p53 staining, glioblastoma sections that previously showed strong immunoreactivity were used as positive controls. A negative control slide in which the primary antibody is excluded was incorporated with each batch of slides. The staining pattern was nuclear for MIB-1 and p53 and cytoplasmic for CD34. MIB-1 and p53 immunolabeling were scored by a visual semiquantitative method and the labeling index (LI) was calculated as the percentage positivity after counting 1,000 tumor cells. Microvascular density (MVD) counting was done on CD34-stained
slides. MVD was defined as the number of single-layered microvessels (venules and capillaries) counted under 200X magnification (20 objective lens and 10 eye piece). Within each tumor specimen, four separate randomly selected sites were counted and then averaged for the individual case as described earlier. [18]

Statistical analysis

Data was analyzed with SPSS 15 software. Data appear as mean ±SD for quantitative variables. Kaplan-Meier survival curves with log-rank statistical test was used to obtain median survival rates and correlate the histological features, MIB-I LI and p53 LI and MVD count with progression. When applicable, independent t test was applied to compare between the two groups. P < 0.05 was considered significant.

**Results**

The study group included 13 adult patients. The mean age of patients was 34.07±8.27 years. Three-fourths of the patients were male. Median follow-up was 54 months. There were four recurrences [Figure 1]c, with median recurrence period being 75 months. Younger patients (under the age of 40 years) had a longer progression-free interval (PFS) [Table 1].

The mean duration of symptoms was 22 months and seizure (62%) was the most common symptom. This was associated with longer PFS [Table 1]. The most common locations were frontal and temporal lobes (31% each) and a majority of tumors were hypodense and nonenhancing on cranial computed tomography (CT) scan [Figure 1]a. However, minimal contrast enhancement was evident in about 23% of the cases. However, the latter feature had no association with PFS. Almost 70% patients underwent total/gross total decompression [Figure 1]b. The extent of resection did not correlate with PFS. Eight patients received postoperative radiotherapy, which was associated with longer PFS. Taken together, we noted that the age of patients below 40 years, presence of seizures, and administration of radiotherapy were clinical factors associated with longer PFS in diffuse astrocytoma, albeit statistically insignificant [Table 1].

Among histological features that were considered for analysis, patients whose tumors revealed predominantly gemistocytic cells (39%) had a shorter PFS (P = 0.94), albeit statistically insignificant [Figure 2]a [Table 2]. The presence of MCC (77%) and TIL (38%) in the tumor did not correlate with PFS.

[Table 3] summarizes the immunohistochemical profile. MIB-1 labeling index (MIB 1 LI) was generally low [Figure 2]b. The means that MIB1 LI was 2.52±0.57. There was no prognostic cut-off value for MIB-1 LI in our series. Immunoreactivity for p53 protein was seen in nine cases [Figure 2]c. The mean p53 LI was 11.69±9.64. There was only one recurrence in p53-negative tumors, with a recurrence interval of 125 months, while there were three recurrences in p53-positive tumors, with a significantly shorter median recurrence interval of 67 months. We noted that a p53 LI>10% was associated with shorter PFS (P = 0.04) [Figure 3] [Table 4].

The mean MVD count was 35.24±17.11 in our series [Figure 2]d. An MVD count in the tumor of more than 20 was associated with early recurrence in patients. However, this did not reach statistical significance (P=0.55) [Table 4]. The mean value of MVD in the enhancing and non-enhancing tumor was 28.05±20.14 and 37.27±16.75, respectively. However, there was no statistically significant correlation of the MVD values between enhancing and non-enhancing tumors (P = 0.46; independent t test).

**Discussion**

Diffuse astrocytomas comprise 10-15% of all astrocytic brain tumors and have a longer survival when compared to anaplastic astrocytoma and glioblastoma. However, the biological behavior of each tumor is unpredictable and prognostication of an individual case is one of the most difficult tasks in neuro-oncology. A number of factors have been implicated as early prognostic indicators of malignant progression, but have
shown inconsistent results.

Clinical variables

Among the clinical variables, age is a well-established prognostic factor for survival in low-grade gliomas, including diffuse astrocytoma. [2],[3],[4],[5],[6],[7] A definite association between age and survival has been established, with the prognosis being worse in older patients (>40 years). [6] In keeping with this observation, we also noted patients below the age of 40 years to have a better, PFS even though the difference did not reach statistical significance, which could be explained by the small number of cases in our study. Similarly, presence of seizures was associated with longer PFS, a feature noted in earlier study also. [2] While Pignatti et al. have noted seizures to be associated with longer survival only in univariate, but not in multivariate analysis. [6]

Diffuse astrocytomas are generally non-enhancing tumors or show minimal contrast enhancement on CT scan. [19] In our series, contrast enhancement was evident in one-fourth of cases; however, this feature had no effect on PFS.

Maximum surgical excision of DA tumors is an established favorable prognostic factor. [20] It has been noted earlier that the risk of recurrence in low-grade gliomas minimizes, and PFS increases when the residual tumor volume postoperatively is small (<10 cm³), [21] though this feature failed to show conclusive results in another study. [22] We were also unable to correlate the extent of tumor resection with patient survival (P = 0.55). The major drawback in assessing the prognostic value of extent of resection in various earlier studies is the non-quantitative assessment and the retrospective design of the study, similar to ours.

With respect to adjuvant therapy, we have noted that administration of radiotherapy is associated with longer PFS, consistent with EORTC Trial 22845, [23] though it was not statistically significant. Another study confirms postoperative radiotherapy to have an independent prognostic value for PFS. These authors even recommend postoperative radiotherapy for all patients with low-grade gliomas regardless of the extent of surgery and the histological subtypes. [24]

Histopathological variables

In earlier studies, prognosis in DA has been correlated with various histological features. The gemistocytic variant has been associated with rapid progression to higher grade (WHO grade III/IV), when compared to fibrillary or protoplasmic variants. [25] The biological basis of this is not clear since gemistocytes by themselves are terminally differentiated astrocytes and have a low proliferative activity. However, these gemistocytes have been noted to carry a higher p53 mutation, and this has been suggested to be the underlying cause for rapid progression of gemistocytic astrocytomas. [26] Presence of a higher percentage (>20%) of gemistocytes in DA has been associated with shorter survival. Some authors even suggest gemistocytic astrocytoma to be considered as a variant of anaplastic astrocytoma (WHO grade III) [27] In the present study, when gemistocytes comprised more than 20% cells in the tumor, we noted shorter PFS, although it was not statistically significant, which could be influenced by the small number of cases.

Other histological features that we correlated with patient survival were microcystic change (MCC) and tumor infiltrating lymphocytes (TIL). MCC has shown to correlate with better survival in DAs. [28] Even though MCC was noted in a large number of tumors in our series, it did not influence PFS. Similarly, we could not find any prognostic correlation between TIL and patient survival. TIL has been observed in 28-52% of gliomas as determined on surgical biopsy material. [29] T-Lymphocytes constitute a large portion of these cells and studies have shown that these T-cells are small, nonblastic and negative for activation antigens. [30] The significance of TIL in astrocytomas with respect to tumor behavior is unclear. Earlier studies have shown that astrocytoma growth does not seem to be dramatically modified by these cells, implying that either the infiltration is insufficient, or that tumor cells are resistant, or that TIL are functionally inactive. [31]

Immunohistochemical variables

MIB 1 LI
Analysis of a wide range of astrocytic tumors of different grades among various studies has shown an overall correlation of tumor cell proliferation with clinical outcome. Several studies have given a cutoff value for MIB-1 (Ki 67 monoclonal antibody) labeling index (LI) that helps prognosticate DA (WHO II), which is quite variable with a range of 2-8%. However, this correlation was not replicated in all studies. In their study of 96 grade II diffuse astrocytomas, Hilton et al. did not observe any prognostic significance. We have also not observed this correlation. This lack of correlation of MIB1-LI and patient survival is probably due to the fact that the range of MIB-1 labeling in DA tumors is so low and uniform throughout the tumor that it fails to differentiate individual tumors in terms of behavior and prognosis.

p53

The effect of alteration of the p53 gene on patient prognosis in low-grade astrocytomas has revealed ambiguous results. While some studies highlight the association with poor prognosis in diffuse astrocytoma, others have not shown any correlation between p53 mutation or immunoreactivity with survival. To perplex the issue, one study has established a better survival in p53-positive astrocytomas. Watanabe et al., in 1997, noted significant concordance between p53 mutations and p53 protein accumulation. They found that a higher p53 LI reflects more aggressive behavior of the tumor and noted progressive increase in the p53 LI with the increasing grade of the tumor.

We have noted p53 immunoreactivity in 69% of cases. The number of immunolabeled nuclei in individual tumors is in the range of 10-30%. Interestingly, we found that tumors with p53 LI of more than 10% correlated with shorter recurrence interval (P = 0.04). We have also observed the significant difference in the recurrence period between p53-negative and p53-positive tumors (125 vs 67 months). This underscores the importance of p53 immunoreactivity in low-grade astrocytomas.

Microvascular density

Microvascular density (MVD) represents the degree of tumor neovascularization, and it has been found to be an important indicator of malignant behavior in many human neoplasms. The relationship of tumor microvascular density to patient prognosis has been shown in diverse brain tumors of varied grades. Abdulrauf et al. have also shown MVD to be an independent prognostic marker of survival in fibrillary low-grade astrocytomas. In the present study, we observed that MVD count of more than 20 are associated with shorter PFS, although the value was not statistically significant, probably because of the fewer number of cases.

Conclusion

Taken together, our study shows that in patients aged below 40 years, seizures as the presenting symptom and administration of postoperative radiotherapy are favorable prognostic factors. In contrast, gemistocytic morphology, p53 overexpression and microvascular density count of more than 20 are unfavorable factors, which are indicative of early recurrence and aggressive behavior of the tumor. Since this information is essential in predicting the behavior of the tumor, validation of these results on larger sample sets would be useful, and thus may influence the clinical decision-making process.

References


