Background: p53 is a tumor suppressor gene implicated in the genesis of a variety of malignancies including brain tumors. Overexpression of the p53 protein is often used as a surrogate indicator of alterations in the p53 gene. Aims: In this study, data is presented on p53 protein expression in adult cases (>15 years of age) of astrocytic (n=152) and oligodendroglial (n=28) tumors of all grades. Of the astrocytic tumors, 86% were supratentorial in location while remaining 14% were located infratentorially - 8 in the cerebellum and 13 in the brainstem. All the oligodendrogliomas were supratentorial. Materials and Methods: p53 protein expression was evaluated on formalin-fixed paraffin-embedded sections using streptavidin biotin immunoperoxidase technique after high temperature antigen retrieval. Results: Overall 52% of supratentorial astrocytic tumors showed p53 immunopositivity with no correlation to the histological grade. Thus, 58.8% of diffuse astrocytomas (WHO Grade II), 53.8% of anaplastic astrocytomas (WHO Grade III) and 50% of glioblastomas (WHO Grade IV) were p53 protein positive. In contrast, all the infratentorial tumors were p53 negative except for one brainstem glioblastoma. Similarly, pilocytic astrocytomas were uniformly p53 negative irrespective of the location. Among oligodendrogliarial tumors, the overall frequency of p53 immunopositivity was lower (only 28%), though a trend of positive correlation with the tumor grade was noted - 25% in Grade II and 31.5% in grade III (anaplastic oligodendroglioma). Interestingly, p53 labeling index (p53 LI) did not correlate with the histopathological grade in both astrocytic and oligodendrogliarial tumors. Conclusions: Thus, this study gives an insight into the genetic and hence biological heterogeneity of gliomas, not only between astrocytic tumors vs. oligodendrogliomas but also within astrocytic tumors with regard to their grade and location. With p53 gene therapy trials in progress, this will possibly have future therapeutic implications.

Key Words: p53, immunohistochemistry, astrocytoma, oligodendroglioma
expression in adult astrocytic tumors and oligodendrogliomas of all grades. The objective was to establish any differences/nexus in p53 protein expression and hence in p53 gene alterations over this wide histopathological spectrum and thus gain an insight into the biological relationship between these tumors.

Materials and Methods

Surgically obtained specimens of 152 astrocytic tumors and 28 oligodendrogliomas were selected from the records of the Neuropathology Laboratory, Department of Pathology, All India Institute of Medical Sciences, New Delhi. All these tumors were from adults (>15 years of age). The case selection was random, with no bias, the only criteria being the availability of adequate tissue in the paraffin block for performing immunohistochemical staining. Clinical details viz. age and site were recorded in each case.

The haematoxylin and eosin (H&E) stained sections of all 180 cases were reviewed by two pathologists (CS & MCS). Diagnosis and grading were confirmed in all cases using WHO criteria after concomitant agreement.

One or two representative blocks of formalin-fixed paraffin-embedded tissue in these 180 cases were selected and serial 5 micron thick sections were cut and taken on poly-L-lysine coated slides. Immunohistochemical staining was performed using the streptavidin-biotin immunoperoxidase technique (Large volume universal DAKO LSAB kit, M/s DakoPatts, Denmark).

Firstly, high temperature antigen retrieval using microwave was done by immersing the sections in 10 mM citrate buffer (pH 6.0) and heating inside a 600-watt microwave oven in full power for 30 minutes. The slides were then allowed to cool to room temperature and subsequently washed briefly with 0.05 M Tris—hydrochloric acid (Tris-HCl) buffer, pH 7.4.

The DO-1 monoclonal antibody to p53 (M/s Santacruz, USA) was used in a dilution (1:100) followed by overnight incubation at 4°C. This was followed by incubation for 30 minutes at room temperature with biotinylated secondary antibody of anti-mouse immunoglobulins. Subsequently, peroxidase conjugated streptavidin was applied and sections were incubated at room temperature for 30 minutes. Antigen antibody complexes were visualized with substrate chromogen Harris Haematoxylin.

After rinsing in distilled water, the sections were counterstained with 0.05 M Tris-HCl buffer (pH 7.4) containing 1 micro liter of H₂O₂. During each batch of staining, appropriate positive and negative controls were used. Positive controls were used by omitting the primary antibody. Sections from a case of glioblastoma multiforme overexpressing the p53 protein were used as positive control. All incubations were done inside the humid chamber. Between each step, sections were washed in Tris-HCl buffer for 3X5 mts.

The complete absence of nuclear stain or very occasional positively stained cells were regarded as negative. For p53 immunopositive cases, 1000 to 5000 tumor cells were counted in each case from at least 5 representative microscopical fields at high power magnification (400 X). An eyepiece pinhole was used to facilitate counting. In cases where there was uneven distribution of immunolabeling it was evident, fields from areas of maximal labeling were chosen for counting. Otherwise fields were chosen at random, taking care to avoid areas of necrosis (in cases of glioblastoma) as well as infiltrative edges of the tumor wherein tumor cells surrounded normal neurons and glia. The counting was done by two pathologists without knowledge of the histological grade. The results were calculated as a percentage of labeled nuclei.

The grading of the 152 astrocytic tumors according to WHO criteria was as follows:

- Pilocytic astrocytoma (WHO Grade I) : 15
- Diffuse (low grade) astrocytoma (WHO Grade II) : 38
- Anaplastic astrocytoma (WHO Grade III) : 29
- Glioblastoma multiforme (GBM-WHO Grade IV) : 70

Among the 28 oligodendrogial tumors, 12 were oligodendroglias (WHO Grade II) and 16 were anaplastic oligodendroglias (WHO Grade III).

The majority of the astrocytic tumors (131/152 or 86%) were supratentorial in location. Only 21 tumors were infratentorial – 8 in the cerebellum and 13 in the brainstem. It is to be noted here that all the 8 cerebellar tumors and 4 of the brainstem tumors were pilocytic astrocytomas. All the 28 oligodendroglial tumors were supratentorial in location. The age and sex distribution of the astrocytic and oligodendroglial tumors is shown in Table 1.

p53 protein immunoreactivity was distinctly and uniformly absent in all pilocytic astrocytomas both in supratentorial and infratentorial locations. However, a distinct difference was noted between supratentorial diffuse astrocytomas, anaplastic astrocytomas and GBMs vs. their infratentorial counterparts. Thus, amongst 131 such supratentorial tumors, p53 immunopositivity was noted in 68 (51%) and distributed over all grades - 58.8% of diffuse astrocytomas, 53.8% of anaplastic astrocytomas and 50% of GBMs (Figure 1). There was no correlation between the frequency of p53 immunopositivity with the tumor grade and thus approximately 50% of the tumors in all grades were immunopositive (Table 2).

Table 1: Age Distribution of Astrocytic Tumors and Oligodendroglias

<table>
<thead>
<tr>
<th>Grade (Total No.)</th>
<th>Supratentorial No. Range (Yrs.) Mean (Yrs.)</th>
<th>Infratentorial No. Range (Yrs.) Mean (Yrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilocytic astrocytoma (Gr.I) (15)</td>
<td>3 25-43 26.3</td>
<td>12 17-50 29.6</td>
</tr>
<tr>
<td>Diffuse astrocytoma (Gr.II) (38)</td>
<td>34 16-48 31.9</td>
<td>4 24-51 37.8</td>
</tr>
<tr>
<td>Anaplastic astrocytoma (Gr.III) (29)</td>
<td>26 21-50 38.8</td>
<td>3 18-42 26.3</td>
</tr>
<tr>
<td>Glioblastoma multiforme (Gr.IV) (70)</td>
<td>68 22-78 42</td>
<td>2 20-45 32.5</td>
</tr>
<tr>
<td>Oligodendroglioma (Gr.I) (12)</td>
<td>12 21-71 42.2</td>
<td>— —</td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma (Gr.III) (16)</td>
<td>16 20-71 41.1</td>
<td>— —</td>
</tr>
</tbody>
</table>
Table 2: p53 protein immunoreactivity in astrocytic tumors

<table>
<thead>
<tr>
<th>Grade (Total No.)</th>
<th>Supratentorial tumors</th>
<th>Infratentorial tumors</th>
<th>Mean±S.D</th>
<th>(Range)</th>
<th>Mean±S.D</th>
<th>(Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilocytic astrocytoma (Gr.I) (15)</td>
<td>0/3</td>
<td>--</td>
<td>0/12</td>
<td>--</td>
<td>(8 cerebellum+4 brainstem)</td>
<td></td>
</tr>
<tr>
<td>Diffuse astrocytoma (Gr.II) (39)</td>
<td>20/34</td>
<td>18.8±14.3</td>
<td>0/4</td>
<td>--</td>
<td>(2.2 - 47.1) (Brainstem)</td>
<td></td>
</tr>
<tr>
<td>Anaplastic astrocytoma (Gr.III) (29)</td>
<td>7/26</td>
<td>16.9±13.5</td>
<td>0/3</td>
<td>--</td>
<td>(3.5 - 46) (Brainstem)</td>
<td></td>
</tr>
<tr>
<td>Glioblastoma (Gr.IV) (70)</td>
<td>34/68</td>
<td>16.5±12.7</td>
<td>1/2 (50%)</td>
<td>10.8</td>
<td>(2.5 - 40.2) (Brainstem)</td>
<td></td>
</tr>
<tr>
<td>Total (152)</td>
<td>68/131</td>
<td>--</td>
<td>1/21</td>
<td>--</td>
<td>(51.9%)</td>
<td>(5%)</td>
</tr>
</tbody>
</table>

*p53 LI between the various grades: NS

Table 3: p53 protein immunoreactivity in oligodendrogliomas

<table>
<thead>
<tr>
<th>Grade (Total No.)</th>
<th>p53 protein positive</th>
<th>Mean±S.D</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligodendroglioma (Gr.II) (12)</td>
<td>3/12 (25%)</td>
<td>6.6±2.85</td>
<td>2.1-10</td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma (Gr.III) (16)</td>
<td>5/16 (31.25%)</td>
<td>8.5±4.81</td>
<td>2.2-14.8</td>
</tr>
<tr>
<td>Total (28)</td>
<td>8/28 (28.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p53 LI between Grade II vs. Grade III: NS

Discussion

Overexpression of the p53 protein is often used as a surrogate indicator of alterations in the p53 gene status, although it is now well documented that p53 protein overexpression may occur for reasons other than mutation in the p53 gene.4-6,11

The present study revealed differences in p53 protein expression between astrocytic tumors and oligodendrogliomas. Further, heterogeneity in p53 protein immunoreactivity amongst astrocytic tumors was observed with regard to their histological grading and location. This is the first Indian study from a single center documenting such heterogeneity.

Thus, approximately 50% of supratentorial diffuse astrocytomas, anaplastic astrocytomas and GBM showed p53 protein immunoreactivity. Similar observations have also been reported in earlier studies3-6 which have detected p53 protein immunohistochemically in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs. Further confirmation has been made available from genetic studies which have revealed allelic loss of chromosome 17p and p53 mutations in approximately 15-40% of diffuse astrocytomas, 35-60% of anaplastic astrocytomas and 35-70% of GBMs.

In contrast, infratentorial (brainstem) astrocytic tumors were found to be rarely p53 protein positive in this study (5%). In contrast, infratentorial (brainstem) astrocytic tumors were found to be rarely p53 protein positive in this study (5%). It is now well established that GBMs develop by either one of the two pathways – one that requires p53 inactivation (secondary GBM) and the one that requires epidermal growth
factor receptor (EGFR) amplification (primary or de novo GBM). Thus, p53 mutation and p53 protein accumulations have been noted in 10% and 35% of primary GBMs respectively, while in secondary GBMs the incidence has been >65% and >90% respectively. Brainstem GBMs in adults are rare and possibly most are primary/de novo, while in the supratentorial location a considerable percentage of adult GBMs are secondary. This may account for the differences in p53 protein expression between supratentorial astrocytic tumors of grades II to IV versus their brainstem counterparts. Studies on EGFR expression however need to be done to confirm this.

Another interesting observation in this study was that pilocytic (grade I) astrocytomas were all uniformly p53 protein negative irrespective of their location. Earlier reports have also shown that p53 mutations are either absent or rare in pilocytic astrocytomas, indicating that inactivation of the p53 possibly does not play a role in their evolution. No distinct pattern of loss of any particular tumor suppressor gene has been reported in these tumors till date, though the role of neurofibromatosis type 1 (NF 1) gene has been extensively studied. Thus, based on available evidence, pilocytic astrocytomas appear to be genetically different from other grades of astrocytic tumors and need further molecular investigations.

Based on these differences observed in p53 protein expression, it may be suggested that p53 immunostaining can have potential diagnostic utility. Especially in small biopsies/stereotactic biopsies wherein there may be problems in deciding between pilocytic vs. diffuse astrocytoma, p53 immunopositivity if present will tilt the diagnosis in favor of the latter. However, negativity for p53 protein in such a situation will not be conclusive.

This study also revealed that p53 protein expression was much lower in oligodendrogliomas (28.5%) as compared to that seen in supratentorial astrocytic tumors (52%). This is in keeping with genetic studies which have demonstrated p53 gene mutations only in 10-15% of these tumors, though immunoreactivity for p53 protein has been reported in a much higher percentage of cases.

In astrocytic tumors, no obvious relationship could be demonstrated between the frequency of p53 immunopositivity and the tumor grade which is similar to the results of previous studies. In contrast, p53 positivity increased with the histological grade in oligodendrogliomas. Barbireschi et al reported p53 protein expression only in high grade oligodendrogliomas (3/11). Pavlek et al. in a study of 36 cases noted 100% p53 positivity in malignant oligodendrogliomas whereas only 64% of Type II and 28% of Type I low-grade oligodendrogliomas showed positivity for p53, thus suggesting its correlation with tumor malignancy. Pal et al. noted p53 protein overexpression in 58.3% of high-grade oligodendrogliomas in contrast to only 8.3% of low-grade ones. Thus, similar to our study their overall frequency of p53 immunoreactivity in oligodendrogliomas was 34.5%. In contrast, Kros et al. in a large study of 84 oligodendrogliomas noted p53 immunoreactivity in 75% of their cases irrespective of grade.

With regard to p53 LI, our results are in agreement with the reports of Saito et al. who observed that the mean p53 staining indices were not statistically significantly different between low and high-grade oligodendrogliomas. However, Hagel et al. proposed a cut-off p53 LI value of 2% which correlated well with the tumor grade and could be used as an independent predictor of survival.

Thus, the present study revealed dissimilarities in p53 protein expression between astrocytic tumors with respect to their grade and location. Thus, p53 does not seem to play a major role in pilocytic astrocytomas unlike astrocytic tumors of Grades II to IV. More cases need to be studied to establish its role in adult brainstem gliomas as well as in the determination of the clinical outcome.

The present study also revealed differences between astrocytic tumors and oligodendrogliomas, thus reflecting basic differences in the biology of their initiation and progression. p53 alterations do not seem to be an early genetic event in astrocytic tumors of Grades II to IV. Studies to look at other genetic pathways in oligodendrogliomas are therefore required.

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