Original Article

Immunohistochemical Study of IDH1, p53, and Ki67 in Gliomas at a Tertiary Care Centre in India

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Abstract

Background: Gliomas are the most common primary CNS tumor, accounting for 81% of all malignant intracranial tumors. The incidence rate of gliomas varies considerably according to sex, race, and nationality. The use of biomarkers such as IDH1/2 and MDM2 status is becoming increasingly common in the West. Most of the information regarding these molecular alterations in gliomas is derived from Western literature. Minimal data is available involving these molecules in glial tumors among the Indian population. This study will help estimate the frequency of these molecular alterations in gliomas in the Indian population.

Materials and Methods: In this prospective study, 60 histopathologically proven cases of gliomas were taken. Representative sections were taken from the tumor area, wax blocks were prepared, and IHC (IDH1, p53, Ki67) was performed.

Results: Out of 60 cases, there were 35 (58.3%) males and 25 (41.7%) females. The maximum number of cases was observed in the 6th decade. Glioblastoma multiforme was the most common histological subtype with 23 cases (38.3%), followed by Diffuse Astrocytoma with 16 cases (26.7%). A correlation between various histological subtypes and the grade of glial tumors with IDH1 immunoexpression was found to be highly significant [$p<0.001$]. A correlation between various histological subtypes and the grade of glial tumors with the Ki67 score was found to be highly significant [$p<0.001$]. A correlation between various histological subtypes and p53/IDH1 expression, as well as various histological grades and p53/IDH1 expression, was found to be significant [$p=0.006$,$p<0.001$].

Conclusion: Our study showed a statistically significant correlation between various histological subtypes and grades of gliomas with p53/IDH1.

Keywords:

gliomas, IDH1, p53, Ki67

Introduction

Gliomas are the most common primary malignant brain tumors in adults, arising from glial cells that correspond to the cells which gave them birth. They can originate from astrocytes, oligodendrocytes (or a mix of these two cell types), and ependymal cells. While most of these tumors are typically malignant, some types do not consistently behave in a malignant fashion [1].

In the last few decades, our understanding of neuropathology and neuro-oncology has changed enormously. Still, gliomas remain one of the most difficult lesions to treat. The current treatment approach, starting from microsurgical resection to fluorescence-guided surgery, still has difficulty achieving good results. Thus, the biological treatment approach has become a burning topic of research [2]. As far as diagnosis is concerned, histological analysis remains the mainstay. Difficulties may arise in cases of tumor heterogeneity, morphological overlap with other gliomas, and partial sampling of the lesion. Therefore, many studies have used molecular techniques aiming to find biomarkers with diagnostic, prognostic, or therapeutic relevance. The important biomarkers identified are overexpression of EGFR, 1p/19q deletion, TERT promoter region mutation, p53 mutation, IDH1 mutation, and altered expression of MDM2 [2,3].

The use of biomarkers such as IDH1/2 and MDM2 status is becoming increasingly common in the West. However, most information regarding these molecular alterations in gliomas is available from Western literature only. There is insufficient data involving these molecules in glial tumors among the Indian population. This study was undertaken to determine the immunohistochemical expression of IDH1 and p53 as surrogate markers of genetic mutations in different grades of gliomas. This will eventually help estimate the frequency of these molecular alterations in gliomas in the Indian population, as well as understand their correlations with clinical parameters, histomorphological features, and demographic data.

Materials and Methods

In this prospective study, 60 histopathologically proven near-total or gross-total excision cases of gliomas were included from May 2019 to November 2020. Their clinical details in the form of age, sex, clinical presentation, and site of the tumor were recorded. All the specimens were subjected to careful and detailed gross examination, fixed in 10% buffered formalin, processed, and paraffin-embedded. Sections were stained with hematoxylin and eosin stain. Immunohistochemistry was assessed by subjecting one section each from a representative block to R132H (IDH1), p53, and Ki67 markers. IHC stains were performed using the standard streptavidin-biotin peroxidase technique.

Cases showing diffuse and strong cytoplasmic staining for IDH1 in >10% of tumor cells were considered positive for IDH1. Cases showing nuclear positivity for p53 in >50% of tumor cells were considered positive for p53. A negative control slide in which the primary antibody was excluded was considered negative. For Ki67, a hot spot (area with the highest density of immunostained nuclei) was selected, and adjacent fields were counted to include 1000 nuclei. Distinct nuclear staining of the tumor cells was recorded as positive. Ki67 LI was recorded as a percentage of positively stained tumor nuclei in 1000 tumor cells [4].

All procedures performed in the current study were approved by the institutional ethical committee of the University of Health Sciences (Letter No. BREC/Th/19/Patho/02 dated 23.12.19) in accordance with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all participants included in the study after an explanation of the study. The representative blocks will be preserved for up to 5 years in the Department of Pathology. After histopathological examination and immunohistochemistry, the biomedical waste generated during the procedure will be discarded as per the institutional policy of biomedical waste (management and handling rules), 2016 [5].

All the data enlisted in the case proforma was collected and analyzed with the help of a software package (SPSS version 24.0). Frequency distribution and cross-tabulation were used to create summary tables and compare items within and across various categories. Chi-square test and Student t-test were used for qualitative and quantitative variables, respectively. Correlations were assessed using the Spearman test. Differences between groups were considered significant only when the p-value was <0.05.

Results

Grading and classification of histopathologically confirmed 60 glioma cases were carried out using the WHO 2016 classification and grading system. Our study group comprised 19 cases (31.7%) (maximum number of cases) of glioblastoma multiforme, IDH wild type [Figure 1], followed by 13 cases (21.7%) of diffuse astrocytoma, IDH mutant type [Figure 2]. There were 5 (8.3%) cases of oligodendroglioma, IDH mutant type [Figure 3]; 4 (6.7%) cases each of anaplastic astrocytoma, IDH mutant type [Figure 4], and glioblastoma multiforme, IDH mutant type [Figure 5]; 3 (5.0%) cases each of diffuse astrocytoma, IDH wild type, and anaplastic oligodendroglioma, IDH mutant type; 2 (3.3%) cases each of oligodendroglioma, NOS type, anaplastic astrocytoma, IDH wild type, and anaplastic oligodendroglioma, NOS type. There was 1 (1.7%) case each of pilocytic astrocytoma [Figure 6], ependymoma, and anaplastic ependymoma [Table 1].

The total study group was classified according to WHO grading criteria. There was 1 (1.7%) case of grade I, 24 (40%) cases of grade II, 12 (20%) cases of grade III, and 23 (38.3%) cases of grade IV. The maximum number of cases, i.e., 40%, were found to be of grade II, followed by grade IV (38.3%).

The majority of glioma patients (23.3%) in our study were in the sixth decade of their life, followed equally by the third, fourth, and fifth decades. The mean age at diagnosis was 43.58 years, with a median age of 45.0 years. The minimum age observed was 12 years (a male child with pilocytic astrocytoma), while the maximum age was 75 years (a male with glioblastoma multiforme, IDH wild type). There was a male predominance compared to females. Males constituted 58.3% of total cases, whereas 41.7% were female. Overall, the male-to-female ratio in our study was 1.2:1.

Patients presented with various complaints. Headache was the most common symptom, followed by seizures, accounting for 42 cases (70%) and 22 cases (36.7%), respectively. Other presenting complaints included slurring of speech, weakness of limbs, loss of consciousness, memory loss, urinary incontinence, vertigo, and loss of sleep.

The most common location of glioma involvement in our study was the frontal lobe, with 25 cases (41.7%), followed by the temporal lobe with 11 cases (18.3%). Fronto-temporal, occipital, insular, and parieto-occipital were the least common sites, with two cases (3.3%) each. Glioblastoma multiforme was the most common histological subtype, with 23 cases (38.3%), followed by diffuse astrocytoma, with 16 cases (26.7%). Anaplastic ependymoma, ependymoma, and pilocytic astrocytoma were the least common histological subtypes, with one case (1.7%) each.

Immunoexpression of IDH1 in all 60 cases was taken into consideration. Fifty percent of the cases demonstrated IDH1 positivity, while the remaining 50% showed no IDH1 expression. All positive cases showed diffuse cytoplasmic staining. The association between various histological subtypes and the grade of glial tumors with IDH1 immunoexpression was found to be highly significant ($p < 0.001$) [Table 2].

On immunohistochemical analysis of gliomas for p53, 26.7% of cases were positive, while 73.3% of cases were negative. The association between various histological subtypes and the grade of glial tumors with p53 immunoexpression was found to be statistically non-significant ($p = 0.851$, $p = 0.592$) [Table 3]. Cases were also categorized according to their Ki67 labeling index. Twenty-eight cases (46.7%) had Ki67 LI < 5%, 8 cases (13.3%) had Ki67 LI 5–20%, and 24 cases (40.0%) had Ki67 LI > 20%. The maximum cases (46.7%) had Ki67 LI < 5%, followed by 40.0% of cases with Ki67 LI > 20%. The association between various histological subtypes and the grade of glial tumors with Ki67 expression was found to be highly significant ($p < 0.001$) [Table 4].

Table 1: Spectrum of intracranial gliomas according to WHO classification 2016

Gliomas	WHO classification	Percentage
Pilocytic Astrocytoma	1	1.7
Diffuse Astrocytoma, IDH mutant	13	21.7
Diffuse Astrocytoma, IDH wild	3	5
Oligodendroglioma, IDH mutant	5	8.3
Oligodendroglioma, NOS	2	3.3
Anaplastic Astrocytoma, IDH mutant	4	6.7
Anaplastic Astrocytoma , IDH wild	2	3.3
Anaplastic Oligodendroglioma, IDH mutant	3	5
Anaplastic Oligodendroglioma , NOS	2	3.3
Glioblastoma Multiforme, IDH wild	19	31.7
Glioblastoma Multiforme, IDH mutant	4	6.7
Ependymoma	1	1.7
Anaplastic Ependymoma	1	1.7
Total	60	100

Table 2: Association between WHO grade and IDH1

IDH1	WHO Grade					Fisher's Exact Test	
	Grade I	Grade II	Grade III	Grade IV	Total	$\chi 2$	P Value
Positive	0 (0.0%)	18 (75.0%)	8 (66.7%)	4 (17.4%)	30 (50.0%)	18.116	<0.001
Negative	1 (100.0%)	6 (25.0%)	4 (33.3%)	19 (82.6%)	30 (50.0%)		
Total	1 (100.0%)	24 (100.0%)	12 (100.0%)	23 (100.0%)	60 (100.0%)		

Table 3: Association between WHO grade and p53

p53	WHO Grade					Fisher's Exact Test	
	Grade I	Grade II	Grade III	Grade IV	Total	$\chi 2$	P Value
Positive	0 (0.0%)	5 (20.8%)	5 (41.7%)	6 (26.1%)	16 (26.7%)	2.166	0.592
Negative	1 (100.0%)	19 (79.2%)	7 (58.3%)	17 (73.9%)	44 (73.3%)		
Total	1 (100.0%)	24 (100.0%)	12 (100.0%)	23 (100.0%)	60 (100.0%)		

Table 4: Association between WHO grade and Ki67 Labelling Index

Ki67 LI	WHO Grade					Fisher's Exact Test	
	Grade I	Grade II	Grade III	Grade IV	Total	$\chi 2$	P Value
<5%	1 (100.0%)	22 (91.7%)	3 (25.0%)	2 (8.7%)	28 (46.7%)	37.908	<0.001
5-20%	0 (0.0%)	1 (4.2%)	1 (8.3%)	6 (26.1%)	8 (13.3%)		
>20%	0 (0.0%)	1 (4.2%)	8 (66.7%)	15 (65.2%)	24 (40.0%)		
Total	1 (100.0%)	24 (100.0%)	12 (100.0%)	23 (100.0%)	60 (100.0%)		

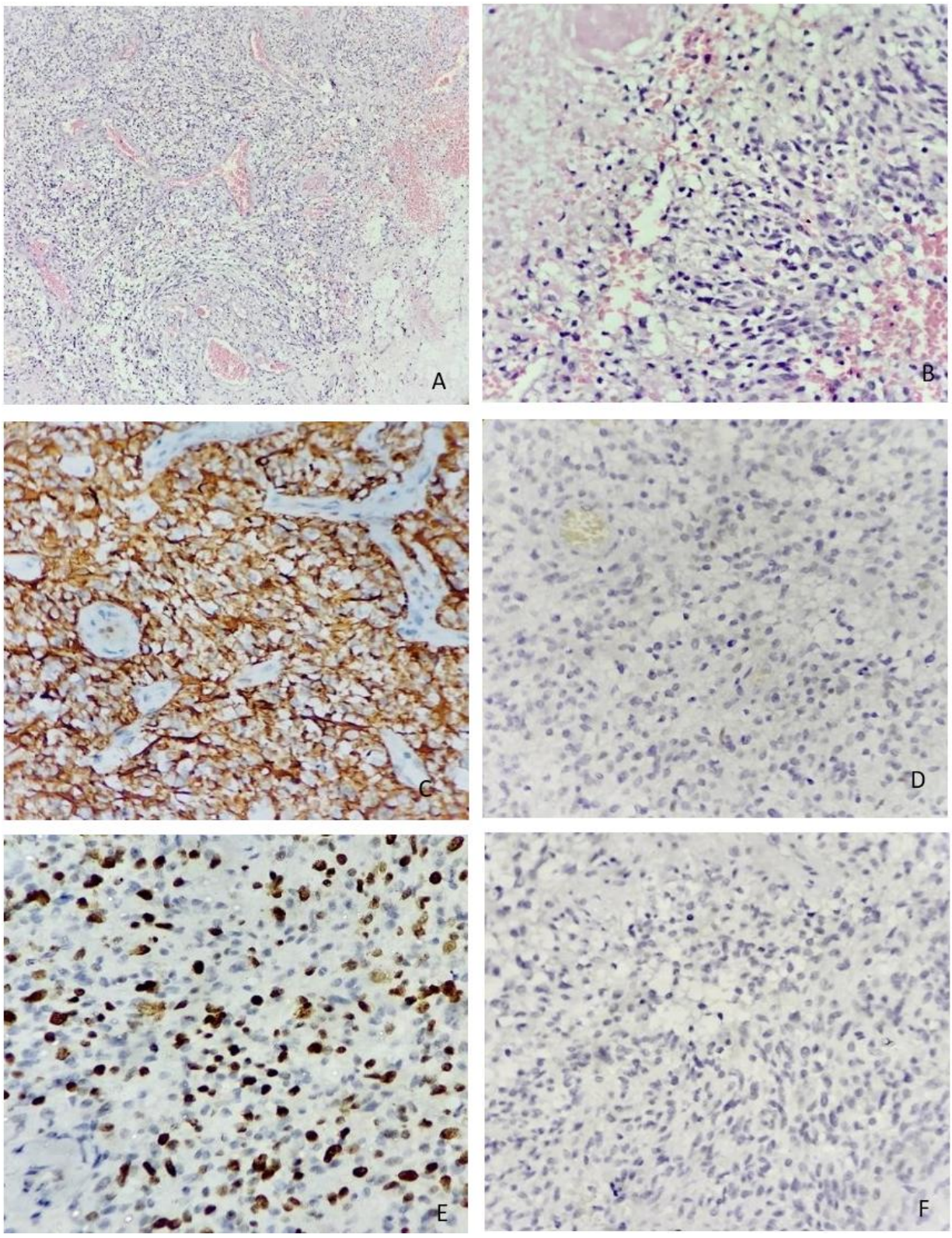

Figure 1: Photomicrograph of Glioblastoma Multiforme, IDH Wild (WHO Grade IV) – A. 100X (H&E) and B. 400X (H&E) showing dense cellularity, marked pleomorphism, extreme hyperchromasia, and coagulative necrosis; C. GFAP – positive in tumor cells; D. IDH1 – negative in tumor cells; E. Ki67 >20%; F. p53 – negative (<5%).

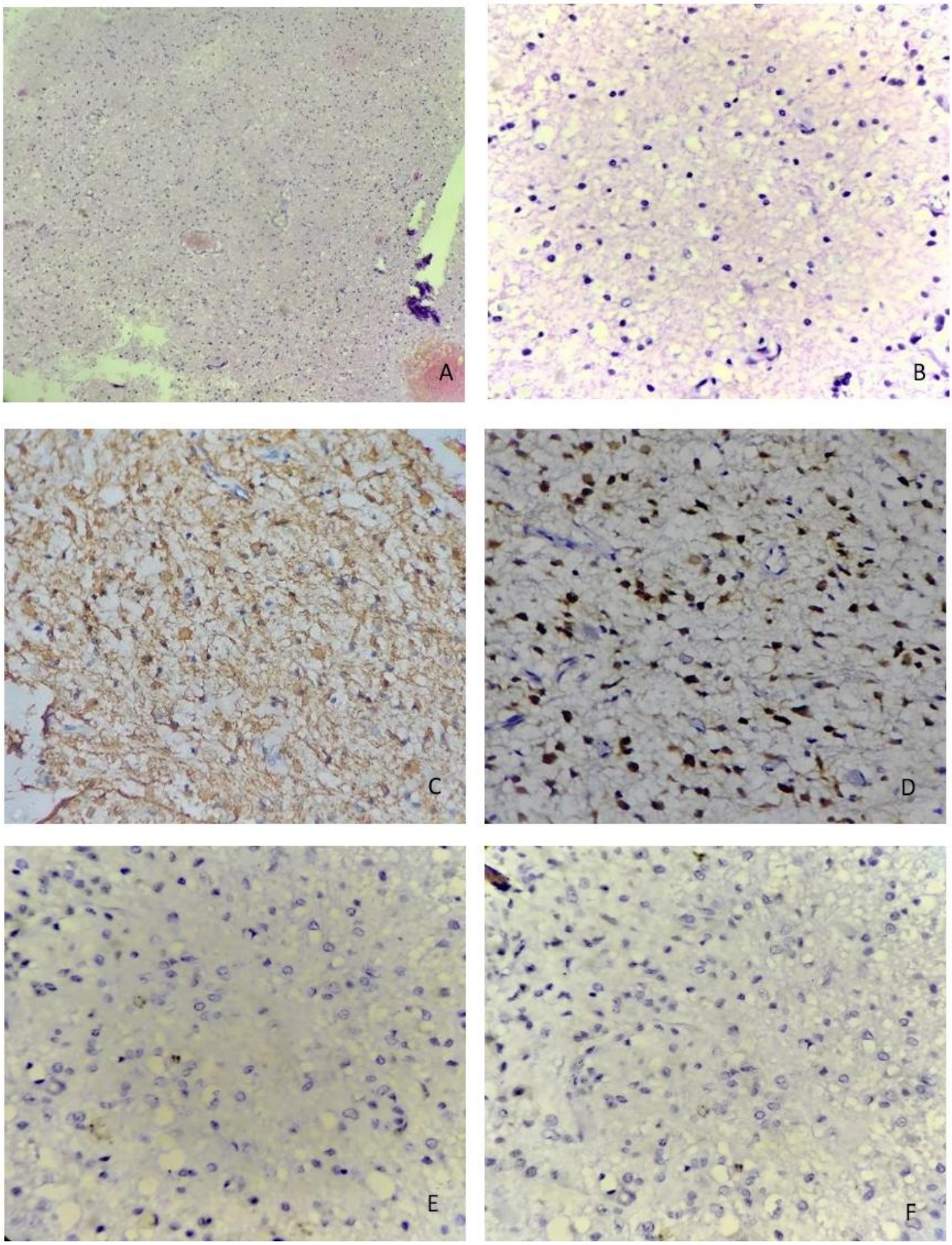

Figure 2: Photomicrograph of Diffuse Astrocytoma, IDH Mutant (WHO Grade II) – A. 100X (H&E) and B. 400X (H&E) showing stellate cell geometry with delicate processes forming a microcystic web; C. GFAP – diffuse positivity in tumor cells; D. IDH1 – cytoplasmic positivity in >10% of tumor cells; E. Ki67 <5%; F. p53 <5%.

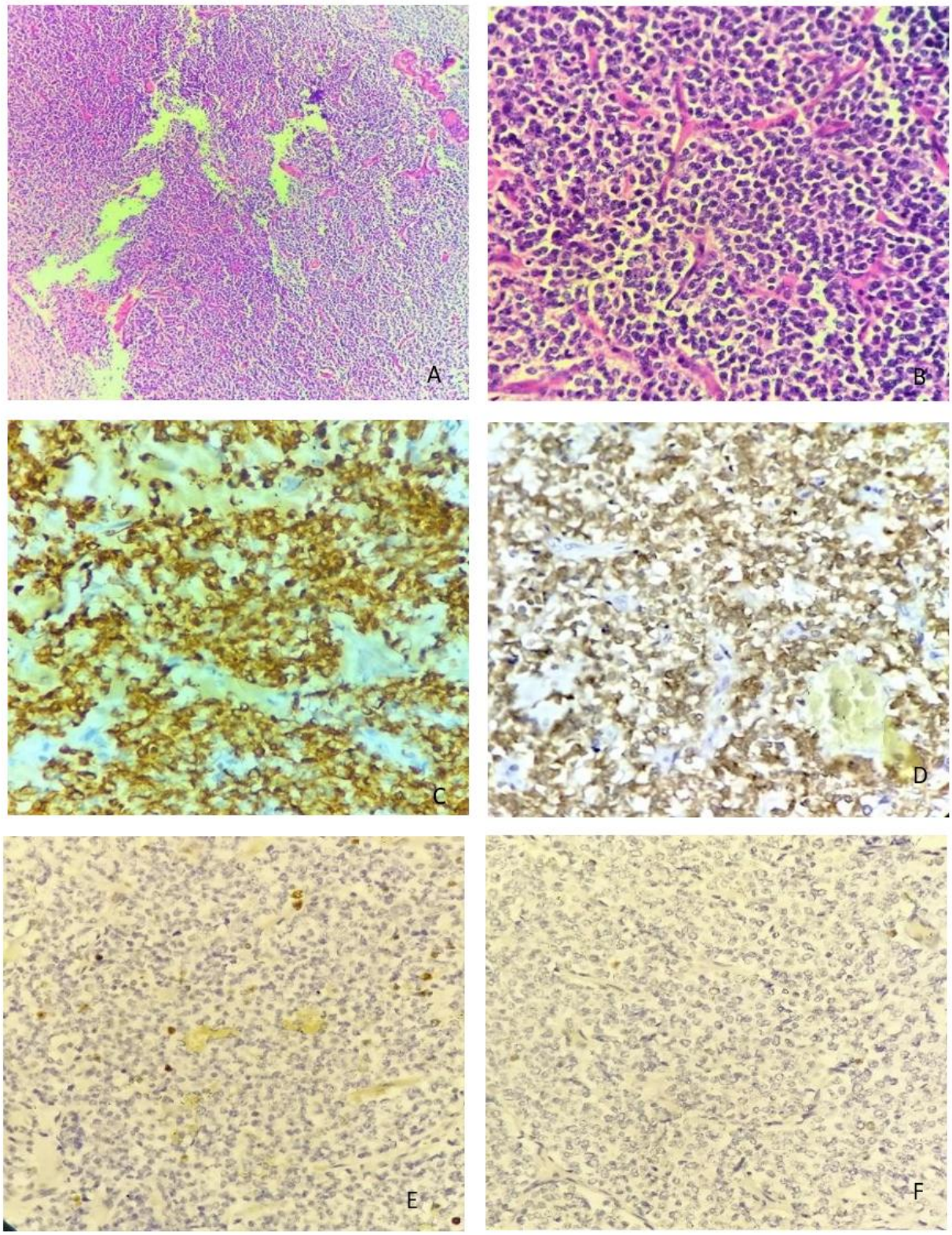

Figure 3: Photomicrograph of Oligodendroglioma, IDH Mutant (WHO Grade II) – A. 100X (H&E) and B. 400X (H&E) showing uniform cytoarchitecture, delicate vasculature, nuclear lobulation, coarse chromatin, cytoplasmic retraction, and ill-defined borders; C. GFAP – diffuse positivity in tumor cells; D. IDH1 – positive in tumor cells; E. Ki67 <5%; F. p53 <5%.

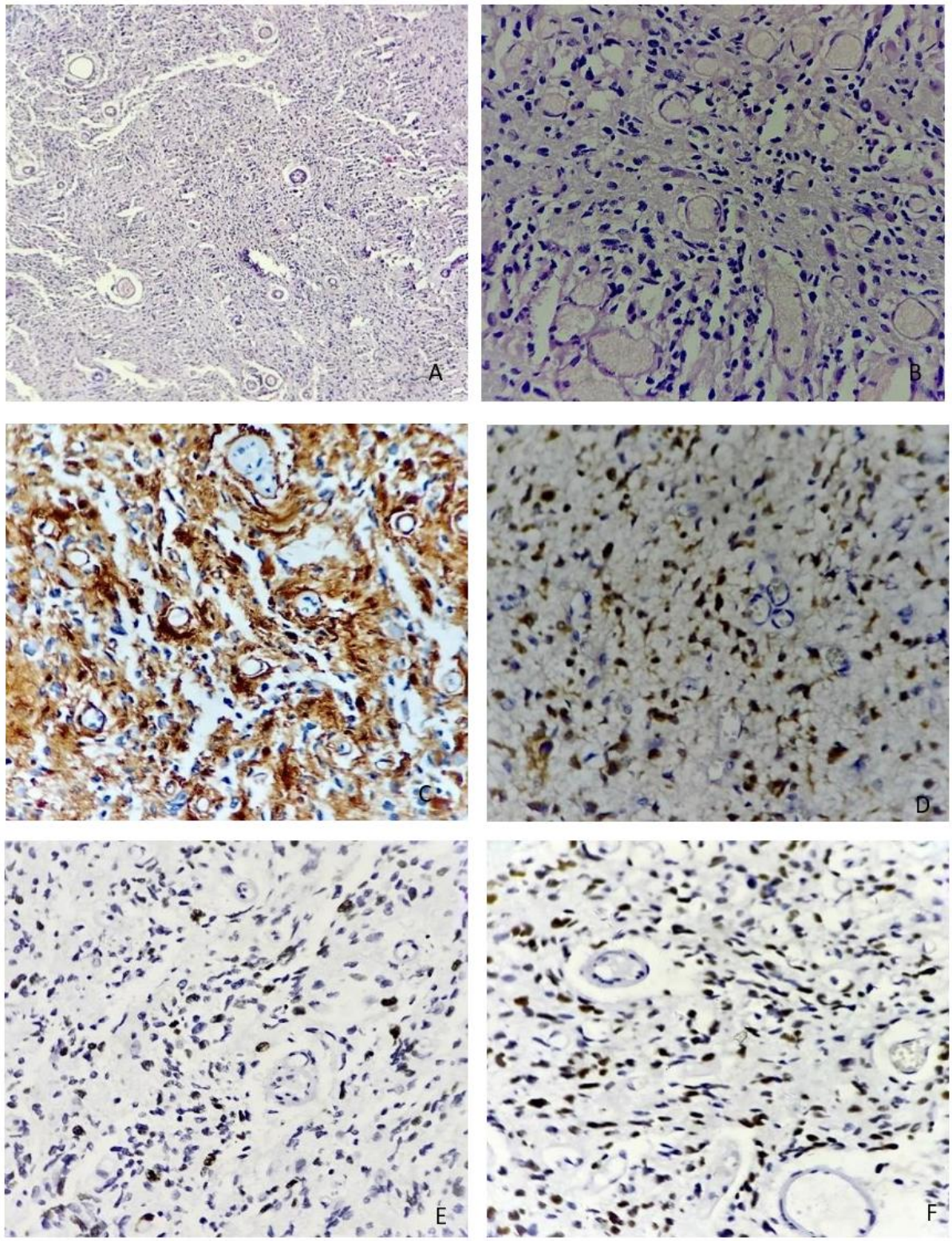

Figure 4: Photomicrograph of Anaplastic Astrocytoma, IDH Mutant (WHO Grade III) – A. 100X (H&E) and B. 400X (H&E) showing moderate to marked cellular pleomorphism, delicate vasculature, and multiple mitotic figures; C. GFAP – positive in tumor cells; D. IDH1 – positive in >10% of tumor cells; E. Ki67 >20%; F. p53 – positive in tumor cells (>50%).

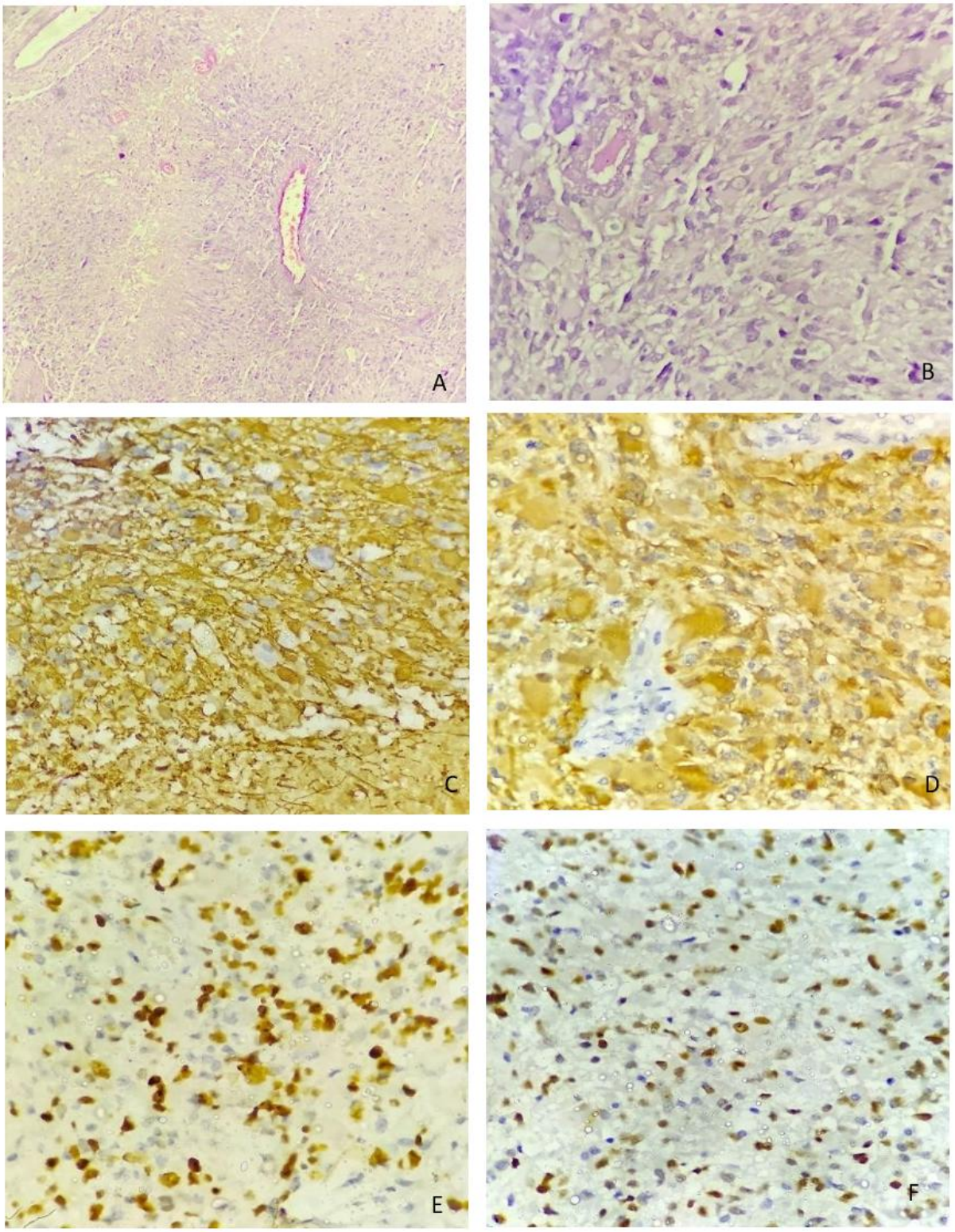

Figure 5: Photomicrograph of Glioblastoma Multiforme, IDH Mutant (WHO Grade IV) – A. 100X (H&E) and B. 400X (H&E) showing dense cellularity, marked pleomorphism, and coagulative necrosis with "palisading" tumor cells; C. GFAP – diffuse positivity in tumor cells; D. IDH1 – cytoplasmic positivity in >10% of tumor cells; E. Ki67 >20%; F. p53 – nuclear positivity in >50% of tumor cells.

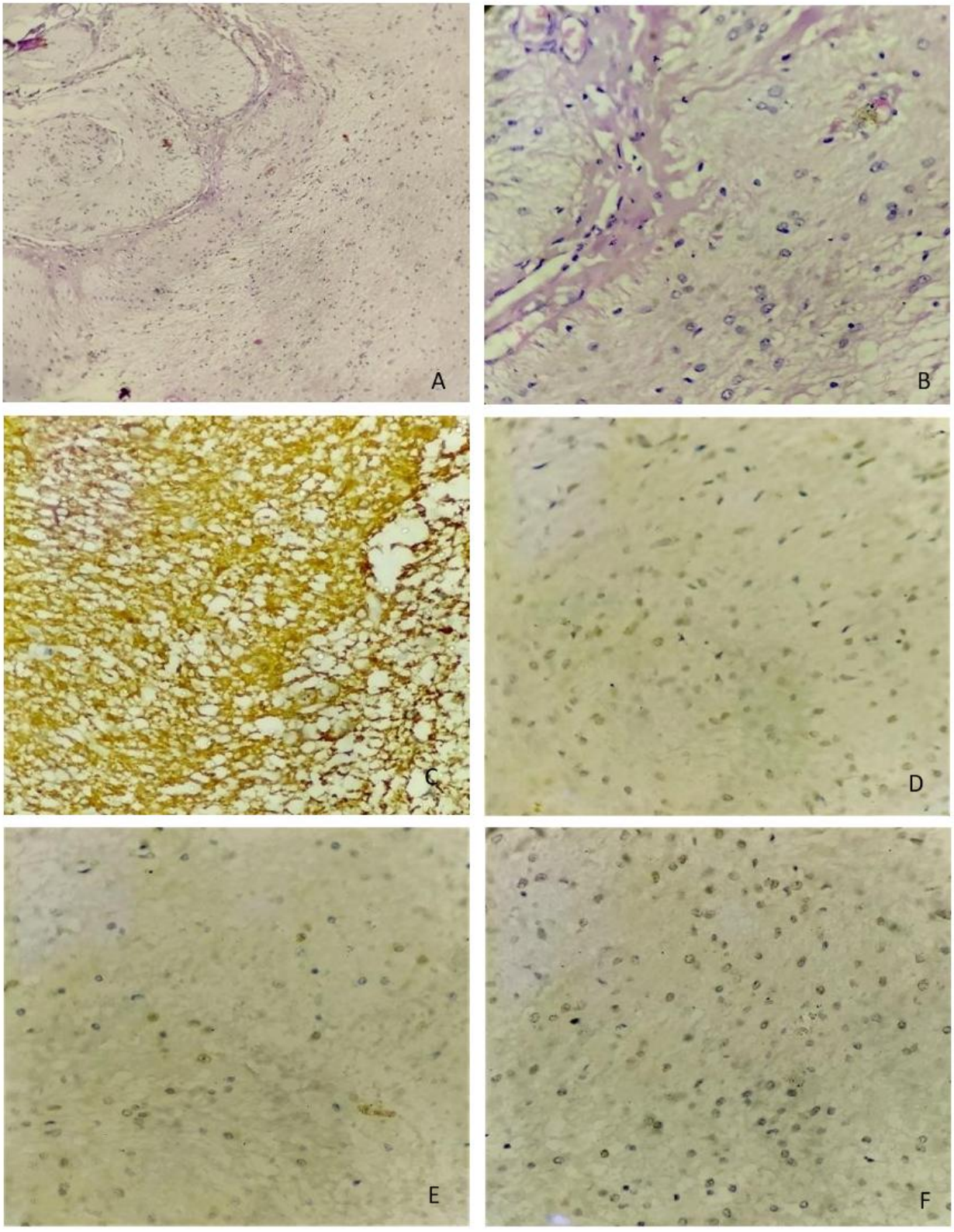

Figure 6: Photomicrograph of Pilocytic Astrocytoma (WHO Grade I) – A. 100X (H&E) and B. 400X (H&E) showing compact fibrillary areas; C. GFAP – diffuse positivity in tumor cells; D. IDH1 – negative in tumor cells (<10%); E. Ki67 <5%; F. p53 – negative (<5%).

The correlation between p53 and IDH1 immunoexpression was found to be statistically non-significant ($p = 0.080$), while the correlation between various histological subtypes and p53/IDH1 expression, and between various histological grades and p53/IDH1 expression, was found to be significant ($p = 0.006$, $p < 0.001$). The correlation between IDH1 expression and Ki67 expression was found to be non-significant in various groups ($p = 0.191$), while the correlation between p53 and Ki67 expression was found to be statistically significant ($p = 0.014$). The p53/IDH1 combination in terms of the distribution of Ki67 was statistically significant in various groups ($p = 0.020$).

Discussion

In the past decade, there has been a shift from the traditional principle of using neuropathological diagnoses, primarily based on microscopic features, to adopting molecularly oriented diagnoses. This change has been driven by genetic as well as epigenetic discoveries. The WHO 2016 classification has incorporated certain key molecular parameters (IDH and ATRX) into the classification of CNS tumors. These molecular subtypes play a very important role in clinical practice, as treatment strategies are now being planned in accordance with the molecular subtype.

The age and sex distribution of present and previous studies indicate that the incidence of gliomas is higher in the older age group and is more common in males [6–13]. The most common location of involvement by glioma in our study was found to be the frontal lobe with 25 cases (41.7%), followed by the temporal lobe with 11 cases (18.3%). Our findings are in concordance with Jaiswal et al. [13], which also had similar results with the frontal lobe (28%) being the most common site of involvement. This may be because the frontal lobe is the largest lobe.

We observed headache as the most common symptom, followed by seizures, accounting for 42 cases (70%) and 22 cases (36.7%) respectively. Our findings are consistent with Jaiswal et al. [13], who conducted a similar study and found seizures (62%) to be the most common symptom, followed by signs of raised intracranial pressure like headache (54%) and vomiting.

With respect to WHO grading, the maximum number of cases, i.e., 40%, were found to be of grade II, followed by grade IV in the present study. Our findings are in concordance with Shaban et al. [11] and Jaiswal et al. [13], who also found grade II as the most common grade of gliomas in their studies, while the majority of other studies found the maximum number of cases in the grade IV category [6,7,10,15].

In the present study, glioblastoma multiforme was found to be the most common histological subtype with 23 cases (38.3%), followed by diffuse astrocytoma with 16 cases (26.7%). Similar studies done by other investigators also found glioblastoma multiforme as the most common histological subtype [6,7,9,10,13–15]. However, Chatterjee et al. [12] observed both glioblastoma multiforme and diffuse astrocytoma as the major histological subtypes in their studies.

IDH1 is a key metabolic enzyme in the glycolytic pathway. Its mutation denotes a unique genotype with a favorable prognosis in gliomas, but the role of the mutated IDH1 enzyme and its metabolites in tumor initiation and maintenance largely remains unresolved. Mutations in this enzyme are consistent and frequent in low-grade gliomas and secondary glioblastoma multiforme. In our study, 50% of cases were IDH1 positive, while 50% were IDH1 negative. In studies done by Jaiswal et al. [13] and Hu et al. [16], IDH1 immunoexpression was found to be 51% and 68.70%, respectively, similar to our findings. Other studies reported a lower frequency of IDH1 positivity [7,9,10,12]. Gliomas expressing IDH1 positivity range from 34.0% to 68.70% in the literature worldwide [7,9–11,13,16]. The wide variation in the frequency of IDH1 mutation in different studies may be due to varied sample sizes, distinct proportions of histopathological subtypes, and ethnic variations.

We found a statistically significant association between the frequency of various histological types and the distribution of IDH1 (χ^2 = 21.185, p < 0.001). Anaplastic ependymoma (100%), low-grade diffuse astrocytomas (81.2%), followed by oligodendroglioma (71.4%) represent the largest group of IDH1 positivity. Saeed et al. [7] showed the highest number of IDH1 immunopositivity in secondary glioblastoma and low-grade diffuse astrocytoma in their observations. However, the p-value failed to reach statistical significance (0.056). Shaban et al. [11] had findings similar to Saeed et al. [5], with secondary glioblastoma, oligodendroglial tumours, and diffuse astrocytoma (87%, 80%, and 72.7%, respectively) representing the largest group of IDH1 positivity (p < 0.033). A study conducted by Jaiswal et al. [13] showed statistically significant immunopositivity in the subgroup of gemistocytic astrocytoma, oligoastrocytoma, and anaplastic astrocytomas (p = 0.001).

In line with previous studies [5,9,11] that reported a higher frequency of IDH1 in low-grade diffuse astrocytoma, oligodendroglial tumours, anaplastic astrocytoma, and secondary glioblastoma in comparison with primary glioblastoma and other pathological subtypes, the present study also showed a higher degree of IDH1 mutation in astrocytic tumours and oligodendroglial tumours, suggesting they probably have a common cell of origin.

Patients in the subgroup WHO grade II had the largest proportion of IDH1 positivity (75%), followed by subgroup grade III (66.7%), while subgroup grade I (100%) and grade IV (82.6%) represent the largest groups showing IDH1 negativity in our study. There was a statistically significant association between the grade of glial tumors and IDH1 expression (p < 0.001). Our findings are in concordance with Mishra et al. [10], who found a statistically significant association (p < 0.001) between IDH1 positivity and WHO grade (grade II and III), while other studies also reported grade II and grade III subgroups with the highest IDH1 positivity but failed to reach statistical significance [7,11,13]. Our findings are in agreement with the literature, indicating that IDH1 mutation is an early event in the pathogenesis of diffusely infiltrating gliomas.

Mutations of the p53 gene lead to an alteration of its protein, and this mutant protein is metabolically more stable with a longer half-life than the wild protein. This results in the accumulation of mutant protein in the nucleus, reaching a threshold of immunohistochemical detection. Based on these differences, immunohistochemistry has been used to screen glial tumors for the presence of detectable p53 protein, indicating the presence of p53 gene alterations. In the present study, out of 60 glioma cases, 16 cases (26.7%) were positive for p53, while 44 cases (73.3%) were negative for p53. Mishra and Chatterjee et al. show results in agreement with the present study, while a few studies reported higher positivity [14,16]. Overall, p53 expression in different grades of gliomas varies widely in various studies conducted worldwide, ranging from 13.6% to 58.7% [10,12,14,16,17]. The reasons for this wide range of variation may be attributed to the properties of different antibodies used, tissue fixation procedures, variation in techniques of incubation and antigen fixation, subjectivity in scoring, and different cut-off values for marking p53 immunopositivity.

No significant association was found between histological subtype and p53 expression (p value = 0.851) in our study. The same conclusion was made by Mohammad et al. [14], who also did not find any statistical association between histological grade and p53 positivity (p value = NA). The reason is the small sample size in both studies, which limits drawing a statistically significant conclusion. Our results showed that p53 mutation frequency is higher in grade III and grade IV gliomas. However, statistically, no significant association between the grade of the tumour and p53 expression was found (p value = 0.592).

The association between WHO grade of glioma and p53 positivity varied in different studies. Many studies found a statistically significant correlation between p53 immunopositivity and high-grade gliomas [14,16,18]. In our study, there is variable positivity in low-grade and high-grade gliomas, showing no clear demonstration of correlation between tumour grade and p53 expression.

Also, no clear role of p53 expression in pilocytic tumours could be demonstrated, as we had only one case of pilocytic astrocytoma in our study.

The proliferation marker Ki67 has been suggested as an ancillary marker in glioma diagnostics. The Ki67 protein is located in the cell nucleus and can be detected in the active phases of the cell cycle, whereas it cannot be detected in the quiescent phase (G0). By comparing the expression of Ki67 reported by other authors, it is evident that wide differences exist among Ki67 values of various studies [19–21]. The general Ki67 values reported by WHO for gliomas are below 4% for diffuse astrocytomas and between 5–10% for anaplastic astrocytomas, while the reported mean values for glioblastoma multiforme are between 15–20% [22]. However, a considerable overlap of the Ki67 labelling index exists between different histologic grades and Ki67 values. Such differences may be partially explained by technical issues, different sensitivities of detection methods, different fixation protocols, and antigen retrieval methods applied by different workers.

Besides, various approaches are used for estimating Ki67 scoring as no precise guidelines exist, and there is considerable variability in interobserver observations. Thus, Ki67 should be prudently used in combination with histopathological features.

In the present study, the association between Ki67 expression and histological subtype, as well as histological grade, was found to be statistically significant ($p < 0.001$). Our findings are in concordance with earlier studies in the literature [23–30]. All of them found a statistically significant difference between various grades of gliomas in terms of Ki67 expression. However, a few studies reported no clear correlation between the Ki67 labelling index of astrocytic tumours and histopathologically determined grade (p value > 0.05).

In studies done by Skjulsvik et al. [26] and Theresia et al. [29], they observed considerable overlap between the Ki67 proliferative index of different grades of gliomas, but no significant difference in Ki67 values was observed between glioma types of the same grade. Similar to the observations of Skjulsvik et al. [26] and Theresia et al. [29], our study showed overlapping values of Ki67 LI between grade III and grade IV. Therefore, Ki67 LI has a limitation in differentiating gliomas by grade, but it is useful in differentiating between low-grade and high-grade gliomas.

We also studied the relationship between IDH1, p53, and Ki67 in all the cases. Distribution of cases according to p53/IDH1 combination expression revealed the maximum number of cases, i.e., 25 cases (41.7%) were both p53/IDH1 negative, while 11 cases (18.3%) were both p53/IDH1 positive. The association between various histological subtypes in terms of distribution of p53/IDH1 was found to be statistically significant ($p = 0.006$).

On correlation between WHO grade and p53/IDH1 combination expression, a statistically significant difference was observed ($p < 0.001$). Grade III had the largest proportion of p53 positive/IDH1 positive cases, while Grade IV had the largest proportion of p53 positive/IDH1 negative cases. As p53 and IDH1 expression was found in Grade II and Grade III astrocytoma in our study, it suggests that these tumors acquire p53 mutations along with IDH1 in the early stage of the pathogenesis of gliomas.

Correlation between IDH1 and p53 expression was found to be non-significant ($p = 0.080$). Also, correlation between IDH1 and Ki67 expression was found to be non-significant ($p = 0.191$). Our findings were not in concordance with the literature [19,31]. The reason may be a small sample size, technical reasons, and variability in interobserver observations for reporting Ki67.

Our study shows that 68.8% of p53 positive glioma cases have high proliferative activity with Ki67 > 20%, while 52.3% of p53 negative glioma cases have low proliferative activity with Ki67 < 5%. Correlation between p53 and Ki67 expression was found to be significant ($p = 0.014$). Our findings were consistent with Ellison et al. [25], which showed similar findings ($p = 0.0377$).

These findings may suggest that largely, p53 positive gliomas are associated with high proliferative activity, which may lead to malignant progression and poorer prognosis, while p53 negative gliomas have lower proliferative activity and thus, better prognosis.

Our study involved a small number of cases, which was one of the main limiting factors. Moreover, expression of IDH1, p53, and Ki67 was carried out by immunohistochemistry only. Therefore, to validate the results at the genomic level, gene sequencing should be done, which was not performed in our study due to resource limitations. Also, owing to the time-bound nature of our study, we were not able to carry out long-term follow-up of patients to determine the prognostic significance of our findings.

Conclusion

In conclusion, our study sheds light on the histomorphological spectrum of gallbladder pathologies encountered in cholecystectomies performed for cholelithiasis in an Indian Archipelago. Chronic cholecystitis emerged as the most common condition in our study, followed by cholesterolosis. This higher incidence of cholesterolosis is akin to studies on the North Indian population. We identified a low incidence of neoplastic gallbladder pathology in this region, with only 0.9% of cases of dysplasia and 0.14% of cases of GBC, which align more closely with existing data from Southern India. We did not encounter any incidental GBC cases in our study and hence favour 'selective' over 'routine' histopathological examination strategy. Although the findings of this study cannot be generalised, it serves as the basis for future research endeavours in this direction. The adoption of the strategy of selective histopathological examination of resected gallbladder specimens necessitates comprehensive validation studies and cost-benefit analyses in order to frame evidence-based guidelines and optimize diagnostic practices in gallbladder pathology within the Indian healthcare context.

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Conflicts of interest: *There are no conflicts of interest.*

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