Expression of FLI1 in Astrocytoma and its Association with WHO Grade

*Sultana S,[1] Rahiduzzaman SM,[2] Yeamin MA,[3] Rashid HO,[4] Akter J,[5] Islam M,[6] Jeba R[7]

Abstract

Background: Astrocytoma is one of the most common glial tumors of the central nervous system. Prognosis and treatment modalities for astrocytoma vary according to their grades. Understanding the biological behavior of tumors and their sensitivity to chemotherapy or radiotherapy is difficult through histopathological grading alone. The transcription factor Friend leukemia virus integration 1 (FLI1) is abnormally expressed in astrocytoma and is associated with tumor grade.

Objective: To see FLI1 expression in astrocytoma and its association with WHO grade.

Methodology: It was a cross-sectional observational study conducted with 50 samples selected using purposive sampling technique at the Department of Pathology of Dhaka Medical College. The samples were drawn from histologically diagnosed astrocytoma cases within the period from March 2022 to February 2024. These samples were categorized into histopathological grades according to the WHO classification 2016. The collected 50 paraffin blocks were sectioned, stained with hematoxylin and eosin (H&E). Immunostaining with FLI1 was done in all cases. Relevant information was collected and recorded in a predesigned data sheet. Statistical analysis was carried out as required.

Results: Among total 50 cases, the patients' ages ranged from 4 to 92 years, with a mean age of 37.8 ± 19.9 years. The male-to-female ratio was 1.5:1. Among total 50 cases grade IV tumor was 36% followed by Grade I tumors (22.0%) and Grade II tumors (22.0%). The majority of cases exhibited high expression (FLI1 score ≥4) at 74.0%, while the remaining cases showed low expression (FLI1 score ≤3) at 26.0%. Grade I and II tumors mostly displayed low FLI1 expression, whereas grade III and IV tumors predominantly exhibited high FLI1 expression. Among the cases with high FLI1 expression, the majority were grade IV tumors (45.9%), followed by grade III tumors (27.0%). Conversely, among the cases 26% with low FLI1 expression, the majority were grade I (46.2%) and grade II tumors (46.2%). The calculated p-value (<0.001) emphasizes the statistical significance of this association.

Conclusion: The results of this study indicate positive association of FLI1 expression with WHO grade in astrocytoma. Low expression of FLI1 was observed in low- grade astrocytomas and high expression of FLI1 was observed in high-grade astrocytoma.

[Journal of Histopathology and Cytopathology, 2026 Jan; 10 (1):27-37]
DOI: https://www.doi.org/10.69950/jhc.2026.10.1.4

Keywords: Astrocytoma, FLI1 expression, WHO Grade.

1. **Dr. Shahana Sultana, MBBS, MD (Pathology), Assistant Professor, Green Life Medical College, Dhaka, shahanashova935@gmail.com,*
2. *Dr. S.M. Rahiduzzaman, MBBS, MD (Cardiology), Resident, Sir Salimullah Medical College, Dhaka. rahid39rpmc@gmail.com*
3. *Dr. Md. Abdullah Yeamin, MBBS, MD (Pathology),Lecturer, Jashore Medical College, Jashore. peyal4794@gmail.com*
4. *Dr. Harun Or Rashid, MBBS, MD (Pathology), Lecturer, Shaheed M Monsur Ali Medical College, Sirajgonj. drharunorrashidjewel@gmail.com*
5. *Dr. Jhuma Akter, MBBS, MD (Pathology), Pathologist, Dhaka Medical College, Dhaka, jhuma.kmc@gmail.com*
6. *Dr. Mainul Islam, MBBS, MD (Pathology), Assistant Professor, Department Of Pathology, Dhaka Medical College, Dhaka. mainul.62.34@gmail.com*
7. *Dr. Ruksana Jeba, MBBS, MD (Pathology), Professor and head, Department of Pathology, Dhaka Medical College, Dhaka, ruksanajeba67@gmail.com*

**For correspondence*

Introduction

Astrocytic tumors represent 64% of central nervous system (CNS) malignancies, usually affecting the brain and sometimes the spinal cord.[1] Astrocytoma can occur at any age. There is a slight predominance of males. Environmental risk factors such as radiation, infectious and chemical agents, genetic risk factors are the reason to increase chance of this tumor.[2] Incidence rate of astrocytoma is about 1/12500.[3] The age- standardized rate of incidence of gliomas has been reported to be 4.7 per 100,000 person- year.[4] In Bangladesh about 14% of CNS tumor samples are astrocytoma.[5]

Astrocytic tumors consist of a variety of neoplasms that differ in their location in the CNS, morphologic features, progressive and invasive behaviors. According to the WHO classification 2016, astrocytoma is categorized into four grades. WHO Grade I (Pilocytic astrocytoma, subependymal giant cell astrocytoma) is a benign, slow growing and less invasive tumor with relatively well-defined borders. WHO Grade II tumors (Diffuse astrocytoma, pleomorphic xanthoastrocytoma) are types of tumors commonly occur in young adult and is a slow growing tumor. WHO Grade III tumors (anaplastic astrocytoma) are also common in young adults but are faster growing and aggressive than the Grade II astrocytoma. WHO Grade IV tumors (glioblastoma) are the most aggressive astrocytic tumors commonly occurring in older individuals.[4,5]

Prognosis and treatment modalities of astrocytic tumors vary according to the grades. Due to the infiltrative nature, grades 2-4 tumors are not cured by surgical resection only and require further chemo and radiotherapy. [4] Glioblastoma is an aggressive cancer without currently effective therapies. Radiation and temozolomide resistance are major contributors to cancer recurrence and failed therapy. Heat shock proteins, through regulation of extracellular matrix remodeling and epithelial mesenchymal transition, provide mechanistic pathways contributing to the development of GBM and radio/TMZ-resistant GBM.[6] Current Standards of Care in glioblastoma therapy consists of safe surgical resection, then radiotherapy with concomitant and maintenance temozolomide. Moreover, biological behavior of astrocytoma and chance of recurrence cannot be ruled out by histopathological evaluation alone. So, new predictive markers for determining tumor progression, and chance of recurrence as well as prognostic markers for better outcome is needed.[7]

The transcription factor Friend leukemia virus integration 1 (FLI1), also known as transcription factor ERGB, is encoded by the FLI1 gene, a protooncogene and features a 98-amino-acid DNA binding domain, also an important member of the E26 transformation specific family (8). E26 transformation specific family (ETS) groups of transcription factors regulate the expression of oncogenes, tumor suppressor genes, and other genes related to vessel formation, invasion, and metastasis and expression of these factors often correlate with poor survival.[1] FLI1 binds to the promoter/enhancer of the target genes and participates in critical roles in normal development, hematopoiesis, and oncogenesis through its dual functions as a transcriptional activator and repressor.[9]

There is a relation between the expression of FLI1 gene with specific biological functions and characteristics of the tissue in which it is located. In tumor research, FLI1 gene is used as a specific marker for the occurrence, metastasis, efficacy, and prognosis of tumors, thus, it's a potential new target for tumor diagnosis and treatment.[10] Therefore, regulating the expression of the FLI1 may become a new target for the clinical treatment of cancer, thus potentially improving

patients' survival.[11] The FLI1 signaling network has been implicated in oncogenesis in GBM, making it an appealing target for advancing novel therapeutics. Expression screening for FLI1 inhibitors identified lumefantrine, an antimalarial drug, as a probable FLI1 inhibitor.[12]

Rationale

Study of FLI1 expression might aid in diagnosis of different grades of astrocytic tumor, guiding therapeutic strategies and improving outcome of the patient. So far, no study has yet been conducted in our country regarding this matter, so it is worth conducting studies to investigate the association of FLI1 protein in astrocytic tumors with WHO grades. This evaluation can provide insights into tumor biology, prognosis, and its potential role in improving patients' survival, thereby offering prognostic value that may aid in further therapeutic advancements in the future.

Objective

To see the FLI1 expression in Astrocytoma and its association with WHO grades.

Methods

This was a cross-sectional observational descriptive study.This study was carried out at the Department of Pathology, Dhaka Medical College from March 2022 to February 2024. Immunohistochemistry was done in a private laboratory. Paraffin blocks of histopathologically diagnosed astrocytoma cases were collected from the department of Pathology of Dhaka Medical College (DMC). Total of 50 samples were collected for this study. Purposive sampling method was followed. Primary CNS tumors of the patient histologically diagnosed as astrocytic brain tumor of various WHO grade irrespective of age and sex were included in this study. Patient who received preoperative chemo/radiotherapy were excluded. The variables are age, sex of the patient, WHO grade of astrocytoma and FLI1 immunoexpression. After getting permission from the ethical review committee, a total of 50 histologically diagnosed cases of astrocytoma were selected for the study. Corresponding slides and paraffin blocks were collected. Representative sections from each paraffin block (paraffin blocks with maximum tumor bulk were chosen) and subsequently IHC stain with FLI1 was done. Tissue was processed employing paraffin embedded method and stained by Hematoxylin and Eosin (H&E) as routine stain.

Immunohistochemical analysis for FLI1

Sections were cut to 3-4µm thick from paraffin block, mounted on poly-L-lysine coated slide. Paraffin-embedded sections will be immunostained using a standard labeled streptavidin-biotin system (Genemed, CA 94080, USA, South San Francisco) with FLI1 polyclonal antibody (Chongqing Biospes Co., Ltd, China) at a dilution of 1:50, at room temperature overnight. Immunodetection will be carried out using detection kits (Dako, Glostrup, Denmark). After dewaxing, Antigen retrieval was done by using 10 mmol/L citrate monohydrate buffer (PH 6.0) and heated for 20 minutes in the microwave. DAB was used as chromogen.

Microscopic Findings

Immunohistochemical Evaluation of FLI1

Sections stained for FLI1 were examined and percentage of immunostained cells was determined. Positiveness was considered as brownish nuclear staining of tumor cells.

The percentage of positive tumor cells was classified as:

0: no positive tumor cells,
1: less than 10% positive tumor cells,
2: 10–50% positive cells,
3: more than 50% positive cells.

The staining intensity was classified as:
0: no staining,
1: weak staining,
2: moderate staining,
3: strong staining.

The FLI1 score was calculated by multiplying the intensity and percentage of positive tumor cells in each sample to yield possible scores of 0, 1, 2, 3, 4, 6, and 9. Immunohistochemical staining result was classified as low-level expression and high-level expression (low and high FLI1 scores). A total score of 4 was set as a cut-off; so-High FLI1 score: ≥4, Low FLI1 score: ≤3 (1). Normal tonsillar tissue was used as external positive control (1).

Data Management and analysis
Data was analyzed by using the IBM SPSS statistical package (version 26). Numerical data was expressed as mean±SD, maximum and minimum. Qualitative data was expressed as frequency and percentage. The chi-square test was used to examine the relation between qualitative variables. A *p*-value equal or less than 0.05 was considered as significant.

Ethical Implication
Ethical clearance, informed written consent and confidentiality were followed properly.

Observation and Results
The age of the patients ranged from 4-92 years with a mean age of 37.8 ± 19.9 years. For statistical analysis, the study population was divided into nine age groups. The majority of the patients belonged to the age group of 31-40 years (24.0%) (Figure 1).

Table I presents the histological diagnosis of tumors. The most common tumor was glioblastoma (36.0%), followed by pilocytic astrocytoma (20.0%) and anaplastic astrocytoma (20.0%).

Table I: Histological diagnosis of tumor of the study cases (n=50)

Histological diagnosis of tumor	Number of cases	Percentage (%)
Glioblastoma	18	36.0
Pilocytic astrocytoma	10	20.0
Anaplastic astrocytoma	10	20.0
Subependymal giant cell astrocytoma	1	2.0
Diffuse fibrillary astrocytoma	6	12.0
Pleomorphic xanthoastrocytoma	2	4.0
Gemistocytic astrocytoma	3	6.0
Total	50	100.0

Table II presents the distribution of study cases based on WHO grade. The majoritywere Grade IV tumors (36.0%), followed by Grade I tumors (22.0%), and Grade II tumors (22.0%). More than 50% tumors were high grade (Grade III and IV).

Table II: Distribution of study cases according to WHO grade (n=50)

WHO grade of tumor	Number of cases	Percentage (%)
Grade I tumor	11	22.0
Grade II tumor	11	22.0
Grade III tumor	10	20.0
Grade IV tumor	18	36.0

Total	50	100.0

Table III illustrates the distribution of study cases based on FLI1 expression. The majority (74.0%) exhibited high expression (FLI1 score ≥4) and remaining 26.0% exhibited low expression (FLI1 score ≤3).

Table III: Distribution of study cases according to FLI1 expression (n=50)

FLI1 expression	Number of patients (n)	Percentage (%)
High (FLI1 score ≥4)	37	74.0
Low (FLI1 score ≤3)	13	26.0
Total	50	100.0

Figure 3 shows that most of the cases of Grade I and 2 tumors showed low FLI1 expression, whereas in Grade III and IV tumors, most of the cases showed high FLI1 expression.

Table IV examines the association between WHO grade and FLI1 expression. Among the cases with high FLI1 expression the majority were Grade IV tumors (45.9%), followed by Grade III tumors (27.0%). On the other hand, among the cases with low FLI1 expression, the majority were Grade I (46.2%) and Grade II tumors (46.2%). The calculated p-value (<0.001) using Chi Square test emphasizes the statistical significance of this association.

Table IV: Association between FLI1 expression and WHO grade (n=50)

WHO Grade	FLI1 expression				P value
	High (n=37)		Low (n=13)		
	n	%	n	%	
Grade I tumor	5	13.5	6	46.2	<0.001[s]
Grade II tumor	5	13.5	6	46.2	
Grade III tumor	10	27.0	0	0.0	
Grade IV tumor	17	45.9	1	7.7	

s= significant

p value reached from Chi Square test

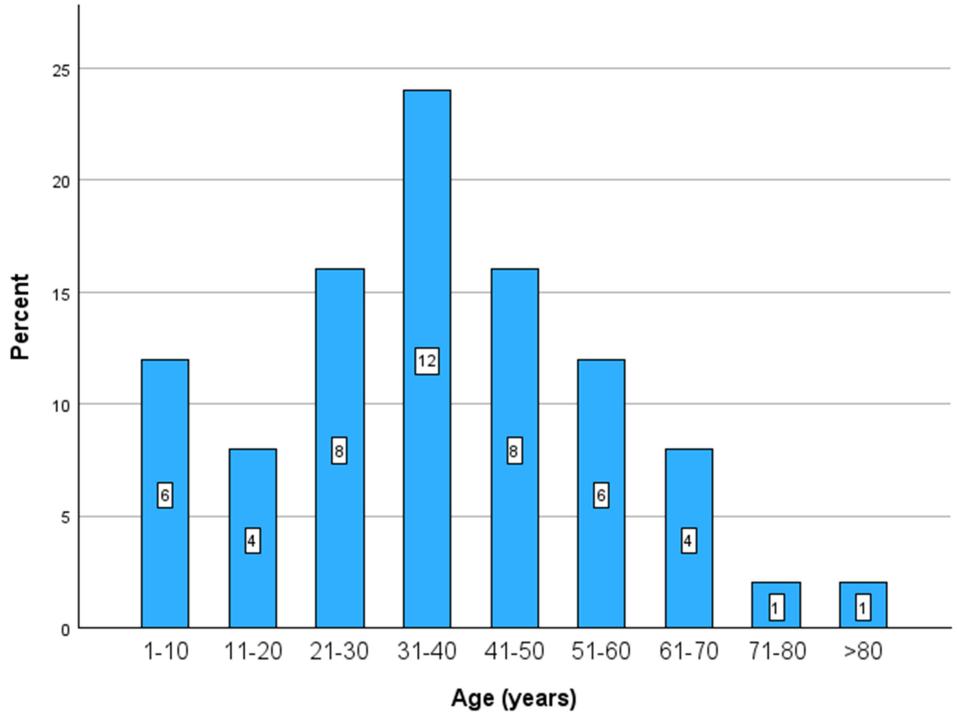

Figure 1. Bar diagram showing age distribution of the study cases (n=50)

In this study out of 50 cases, 30 (60.0%) cases were males, and 20 (40.0%) cases were females with male to female ratio of 1.5:1 (Figure 2).

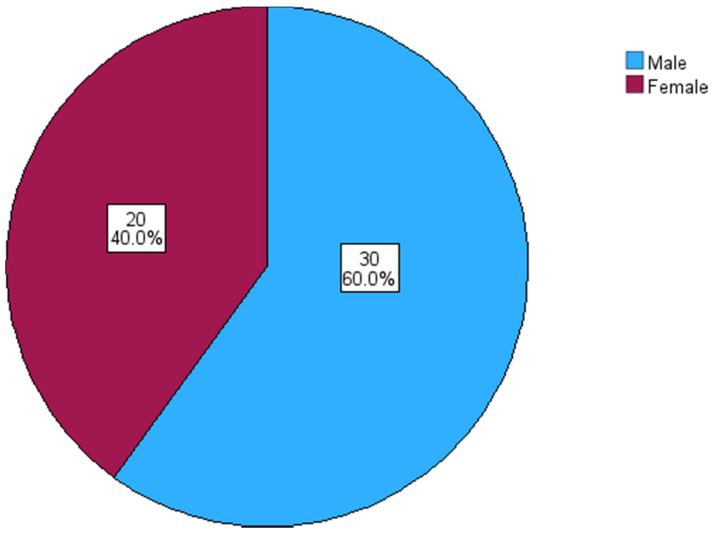

Figure 2. Pie chart showing sex distribution of study cases (n=50)

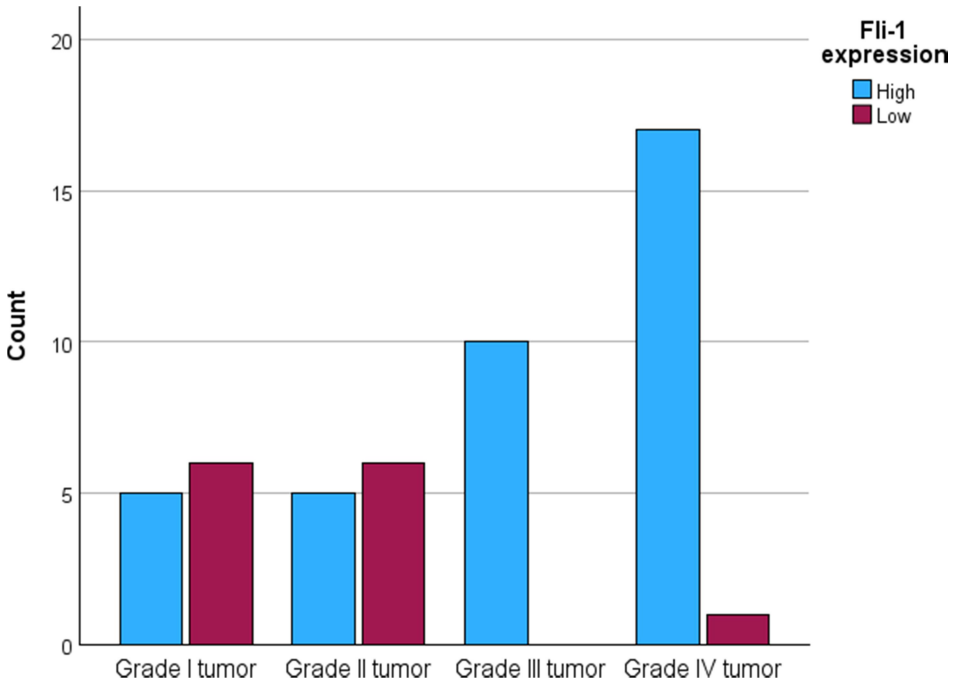

Figure 3. Bar diagram showing pattern of expression of FLI1 in different astrocytomas (n=50)

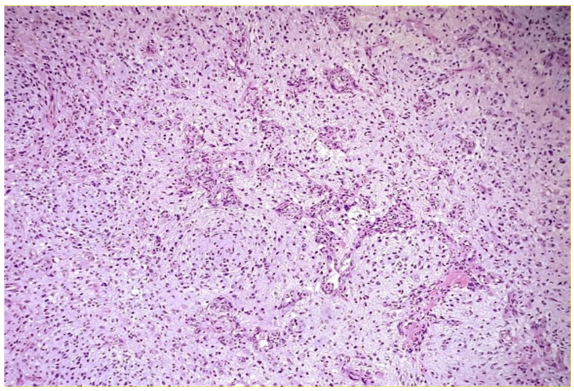

Figure 4. Photomicrograph shows glioblastoma (grade-IV)

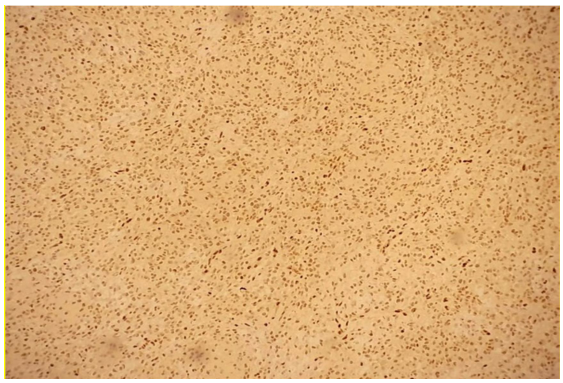

Figure 5. Photomicrograph showing high expression of FLI1 in Glioblastoma

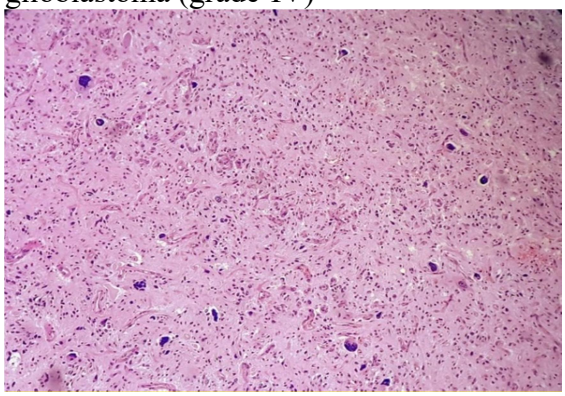

Figure 6. Photomicrograph showing glioblastoma (grade-IV)

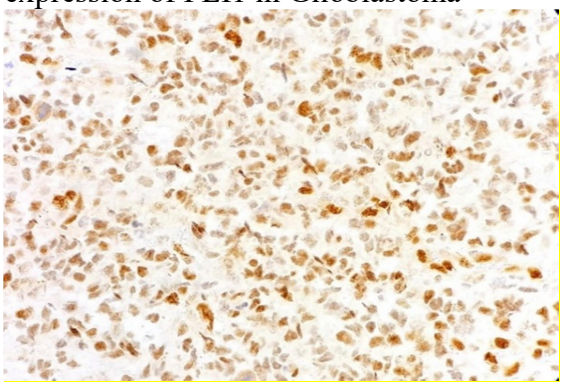

Figure 7. Photomicrograph showing high expression of FLI1 in Glioblastoma

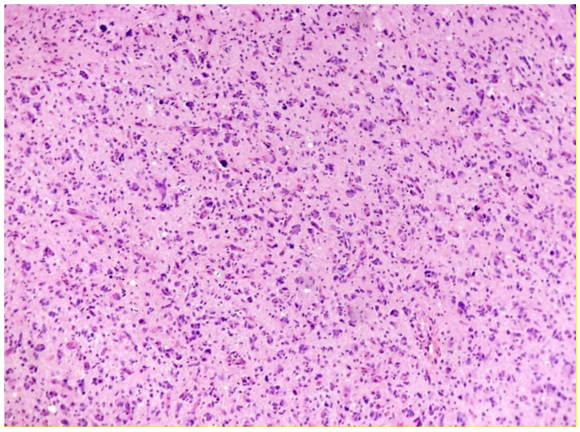

Figure 8. Photomicrograph showing anaplastic astrocytoma (Grade-III)

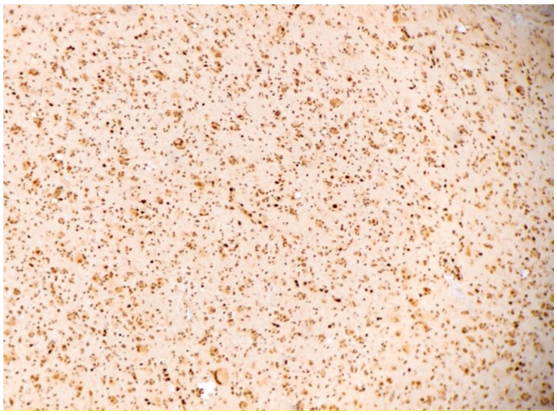

Figure 9. Photomicrograph showing high expression of FLI1 immunostain in anaplastic astrocytoma

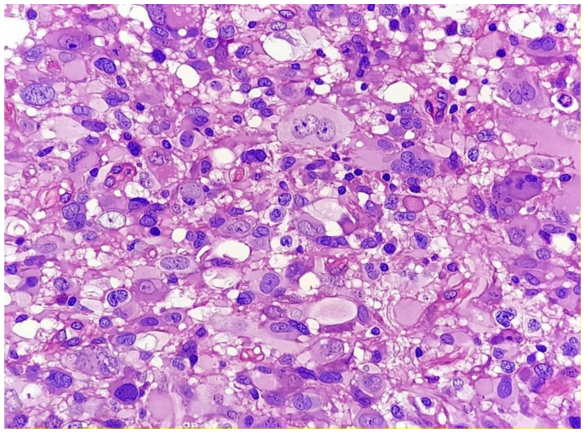

Figure 10: Photomicrograph showing Pleomorphic xanthoastrocytoma (grade-II)

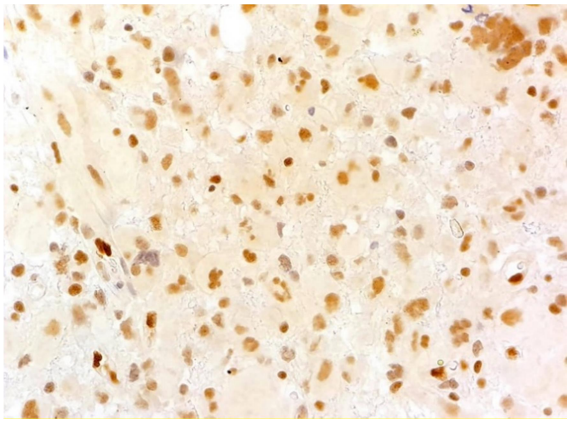

Figure 11: Photomicrograph showing high expression of FLI1 immunostain in Pleomorphic xanthoastrocytoma

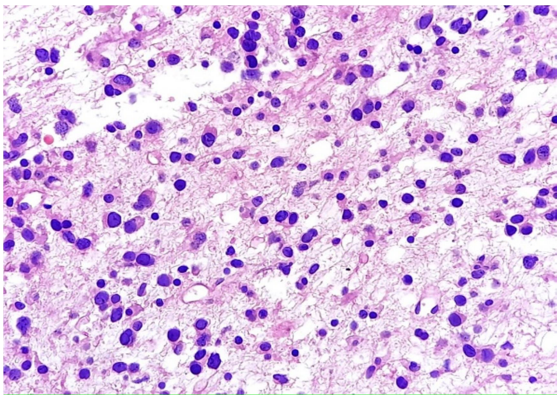

Figure 12. Photomicrograph showing Diffuse fibrillary astrocytoma (grade-II)

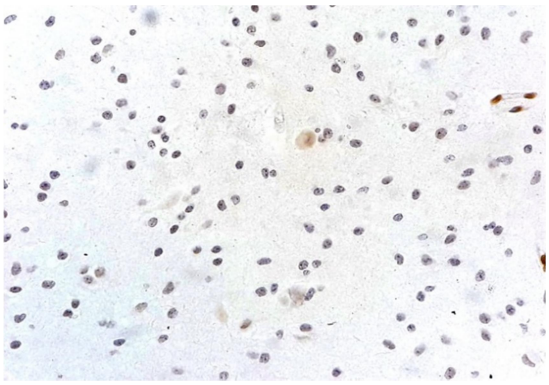

Figure 13. Photomicrograph showing low expression of FLI1 immunostain in Diffuse fibrillary astrocytoma

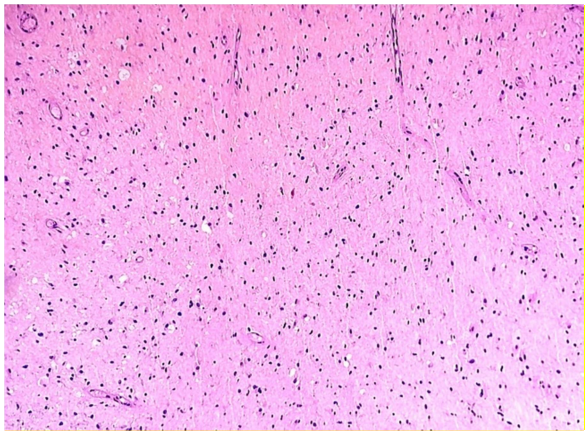

Figure 14. Photomicrograph showing Pilocytic astrocytoma (grade-I)

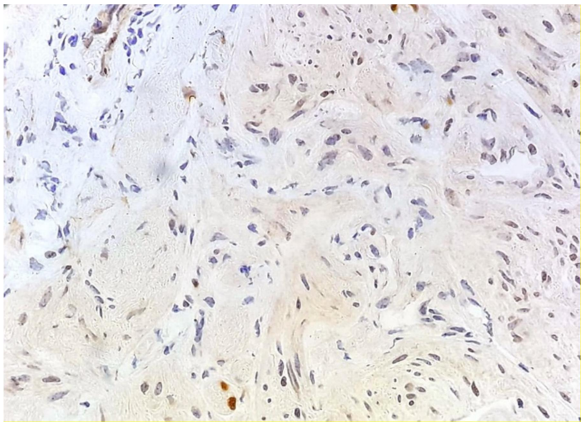

Figure 15. Photomicrograph showing low expression of FLI1 immunostain in Pilocytic astrocytoma

Discussion

In this study, regarding the age distribution of the study population, it was observed that majority of cases with Grade I tumor belonged to age range of 4-40 years and the mean age was 15.8 ± 12.0 years. In Grade II, most of the cases were in the 26 – 60 years age range and the mean was 39.8 ± 12.0 years. In Grade III, the most common age group was 13 -55 years with the mean of 34.6 ± 11.6 years. The highest number of samples with Grade IV tumor were from age range of 5 – 92 years with the mean of 54.4 ± 21.2 years. The mean age of total astrocytic tumors in this study was 37.8 ± 19.9 years when the study population was divided into nine age groups. Most of the patients belonged to the age group of 31-40 years and the lowest number of patients belonged to 71-80 years and >80 years age group. In Bangladesh, Biswas (2019) found highest number of patients age ranged from 21-30 years and lowest number of patients ranged from 61-70 years.[13]In Bangladesh, Islam et al. (2020) found in their study that the common age group of pilocytic astrocytoma was 1-20 years, diffuse astrocytoma was 21-40 years, and glioblastoma was 41-60 years (5). Mallick et al. (2022) reported that maximum number of glioblastoma patients were aged between 41 and 50 years (40%).[14] In another study, they stated that the common age group for pilocytic astrocytoma was first two-decade, diffuse astrocytoma was 30-40 and anaplastic astrocytoma had a mean age of about 40 years .[15]All these studies show a little variation from the present study may be due to different sample size, variation in tumor type and different region.

In this study out of 50 cases, 30 (60.0%) cases were males, and 20 (40.0%) cases were females with male to female ratio of 1.5:1. In Bangladesh, Biswas (2019) found male to female ratio 1.6:1(13). Another study of Bangladesh, Islam et al. (2019) reported that among 567 cases, total male were 346 (61%) and female 221 (39%), male to female ratio was 1.6:1 .[5] In a similar study, Mahzouni and Taheri had total 100 cases of which 59 patients (59%) were male, 41 patients (41%) were female and the male to female ratio was 1.4:1 (7) in their study which correlates well with the current study. Females are less commonly affected by astrocytoma female hormones have preventive effects on tumorigenesis in different age groups. It can be explained that brain tumors develop in female brain cells after a series of genetic alterations and exposure to a growth factor.

According to the histological diagnosis of

tumors in the current study, the most common tumor was glioblastoma (36.0%), followed by pilocytic astrocytoma (20.0%) and anaplastic astrocytoma (20.0%). The majority were Grade IV tumors (36.0%), followed by Grade I tumors (22.0%), and Grade II tumors (22.0%). Another study showed that among 100 samples, 10 samples (10%) were determined as Grade I, 25 samples (25%) as Grade II, 5 samples (5%) as Grade III and 60 samples (60%) as Grade IV (7). This was in line with a previous study where it was found that glioblastoma (Grade IV) was the most common astrocytic tumour.[16] Ahmed et al. found that among 45 cases were 10 cases (22.2 %) of Grade I astrocytoma (8 pilocytic astrocytoma cases and 2 subependymal giant cell astrocytoma cases), 13 cases (26.7%) of Grade II astrocytoma (9 diffuse astrocytoma cases and 4 pleomorphic xanthoastrocytoma cases), 6 cases (13.3 %) of Grade III astrocytoma (anaplastic astrocytoma) and 16 cases (37.8%) of Grade IV astrocytoma (glioblastoma).[1] So this can be opinionated that glioblastoma (Grade IV) was the most common astrocytic tumor.

It was observed in this study that the majority exhibited high expression (FLI1 score ≥4) at 74.0% and remaining exhibited low expression (FLI1 score ≤3) at 26.0%. Another study results in total 27 cases (60%) showed high FLI1 score and 18 cases (40 %) showed low FLI1 score (1). Grade I (46.2%) and 2 (46.2%) tumors showed low FLI1 expression, whereas in Grade III (27.0%) and 4 (45.9%), tumors, most of the cases showed high FLI1 expression. The calculated p-value (<0.001) using Chi-square test emphasizes the statistical significance of this association. In another study Grade III, 4 cases had high FLI1 score, while 21.7% of Grade I, II cases had low FLI1 score. FLI1 expression was related to different clinicopathological findings.[1]

FLI1 can act as either a transcriptional activator or a suppressor to regulate genes involved in cell proliferation, survival, or differentiation. FLI1 may be a prognostic marker in astrocytoma to predict recurrence & patient survival.

Conclusion

The results of this study indicate positive association of FLI1 expression with WHO grade in astrocytoma. Low expression of FLI1 was observed in low- grade astrocytoma and high expression of FLI1 was observed in high-grade astrocytoma.

Limitations

Although the WHO grading of astrocytoma was updated in 2021, in this study WHO grading of 2016 classification was used since the proposal was developed earlier. It would be more appropriate to use the WHO 2021 grading.

Recommendations

The study results recommend using FLI1 expression for clinical practice because association between WHO grade and FLI1 expression reveals higher score with higher grades.

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