Expression of FLI1 in Astrocytoma and its Association with Histological Parameters

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Abstract

Background: Astrocytomas represent the most prevalent type of glial tumor. This category encompasses a diverse array of neoplasms that vary in their central nervous system (CNS) locations, morphological characteristics, and patterns of progression and invasiveness. FLI1, a nuclear transcription factor, plays a significant role in enhancing cellular proliferation and tumorigenesis, thereby serving as a prognostic indicator for numerous human tumors.

Objective: To see FLI1 expression in astrocytoma and its association with histological parameters (cellularity, nuclear atypia, mitosis, microvascular proliferation and necrosis) of astrocytic tumors.

Methods: 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 histomorphologically diagnosed astrocytoma cases within the period from March 2022 to February 2024. The collected 50 paraffin blocks were sectioned, stained with hematoxylin and eosin (H&E). Immunostaining with FLI1 was done in all cases. Relevant information were 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. The most common tumor location was in the frontal lobe (28.0%), approximately half showing moderate cellularity (48.0%), moderate atypia (36.0%), mitosis (56.0%), and necrosis (32.0%). 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%. FLI1 expression was associated with atypia ($p < 0.001$), mitosis ($p < 0.001$), and necrosis ($p= 0.029$). The calculated p-value (<0.001) emphasizes the statistical significance of this association.

Conclusion: The findings of this study suggest that elevated expression of FLI1 is positively correlated with atypical features, mitotic activity, and necrosis in astrocytoma. Assessing FLI1 may serve as a predictive indicator for tumor advancement and a prognostic marker for individuals diagnosed with astrocytoma.

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Introduction

Intracranial neoplasms represent a major cause of tumor-related morbidity and mortality among children and adolescents.[1] Gliomas are the most prevalent type of intracranial neoplasm, with astrocytoma being the most frequently encountered variant. These tumors originate from astrocyte precursors, which are the supportive cells of the brain, characterized by their star-like appearance. Astrocytic tumors account for 64% of malignancies within the human central nervous system (CNS), predominantly affecting the brain and, in some cases, the spinal cord.[2]

The incidence rate of astrocytoma is approximately 1 in 12,500 individuals. The age-standardized incidence rate of gliomas has been documented at 4.7 per 100,000 person-years.[3] In Bangladesh, around 14% of CNS tumor samples are identified as astrocytoma.[4] The prevalence of CNS neoplasms exhibits significant variability, with regional incidence rates generally being higher in developed nations compared to developing ones.[5] Astrocytoma can manifest at any age, with a slight male predominance. Various environmental risk factors, including radiation exposure, infectious agents, and chemical substances, along with genetic predispositions, contribute to an increased likelihood of developing this type of tumor.[6]Astrocytic tumors encompass a diverse array of neoplasms that vary in their anatomical locations within the CNS, morphological characteristics, and patterns of progression and invasiveness.

According to the WHO classification, Grade I tumors represent the most benign, slow-growing and cure by surgical resection. Grade II astrocytoma comprises relatively slow-growing diffuse tumors. Grade III astrocytoma is mitotically active in histopathology. Grade IV astrocytoma or glioblastoma is the most aggressive human malignant primary brain tumor and is characterized by histopathologic features, such as necrosis and microvascular proliferation. Due to the infiltrative nature, grades 2-4 tumors are not cured by surgical resection only and require further chemo and radiotherapy.[3,7] Glioblastoma is an almost invariably fatal disease, with most patients dying within 15-18 months after diagnosis, and <5% of patients alive after 5 years.[7] Moreover, biological behavior of astrocytoma and chance of recurrence cannot be ruled out by histopathological evaluation alone. So, new predictive marker for determining tumor progression, chance of recurrence as well as prognostic marker for better outcome is needed.[8]

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.[9,10] The FLI1 is expressed in normal cells such as hematopoietic stem cells and vascular endothelial cells, and also abnormally expressed in different malignant tumors including Ewing sarcoma, squamous cell carcinoma, adenocarcinoma, bladder cancer, leukemia and lymphoma.[11] FLI1 binds to the promoter/enhancer of the target genes and participates in a variety of pathophysiological processes of tumor cells, including cell growth, proliferation, differentiation, and apoptosis.[12] This affects cellular proliferation and tumorigenesis in Ewing sarcoma and primitive neuroectodermal tumors and additionally plays critical roles in normal development, hematopoiesis, and oncogenesis through its dual functions as a transcriptional activator and repressor.[13] 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.[14]

Glioblastoma is a highly aggressive form of cancer for which effective treatments are currently lacking. Resistance to radiation and temozolomide significantly contributes to the recurrence of the disease and the failure of therapeutic interventions. The FLI1 signaling network has been associated with the development of glioblastoma, positioning it as a promising target for the development of new therapeutic strategies.

Rationale

Elevated levels of FLI1 are correlated with high-grade astrocytic tumors and a poor prognosis. Consequently, investigating FLI1 expression may facilitate the diagnosis of various grades of astrocytic tumors, thereby informing therapeutic approaches and enhancing patient outcomes. Prior research has demonstrated that a reduction in FLI1 levels results in significant growth inhibition and apoptosis in erythroleukemic cells, suggesting the potential of FLI1 as a therapeutic target for tumor suppression. Additionally, other studies have recognized FLI1 overexpression as a biomarker for specific cancers, including melanoma, endometrial cancer, ovarian cancer, breast cancer, and nasopharyngeal carcinoma. There is a deficiency of data concerning the relationship between FLI1 protein expression and the clinical parameters linked to astrocytoma. The current study aims to assess the expression of FLI1 in astrocytic brain tumors and its correlation with the clinical parameters related to astrocytoma. This research may provide valuable insights that could inform therapeutic strategies and enhance patient outcomes.

Objective

To find out the FLI1 expression in astrocytomas and its association with histological parameters (cellularity, nuclear atypia, mitosis, microvascular proliferation and necrosis).

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). 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 and sex of the patients, cellularity ,mitosis, microvascular proliferation, necrosis 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 were 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: (Point)

Sections stained for FLI1 were examined and percentage of immunostained cells was determined. Positiveness was considered as brownish nuclear staining of tumor cells. To score density in a semiquantitative and reproducible way, the results of immunohistochemical staining were scored on a scale of 0 to 9.

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 (2).

Control

Normal tonsillar tissue was used as external positive control.

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.

Results

Age Distribution of the Study Cases

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).

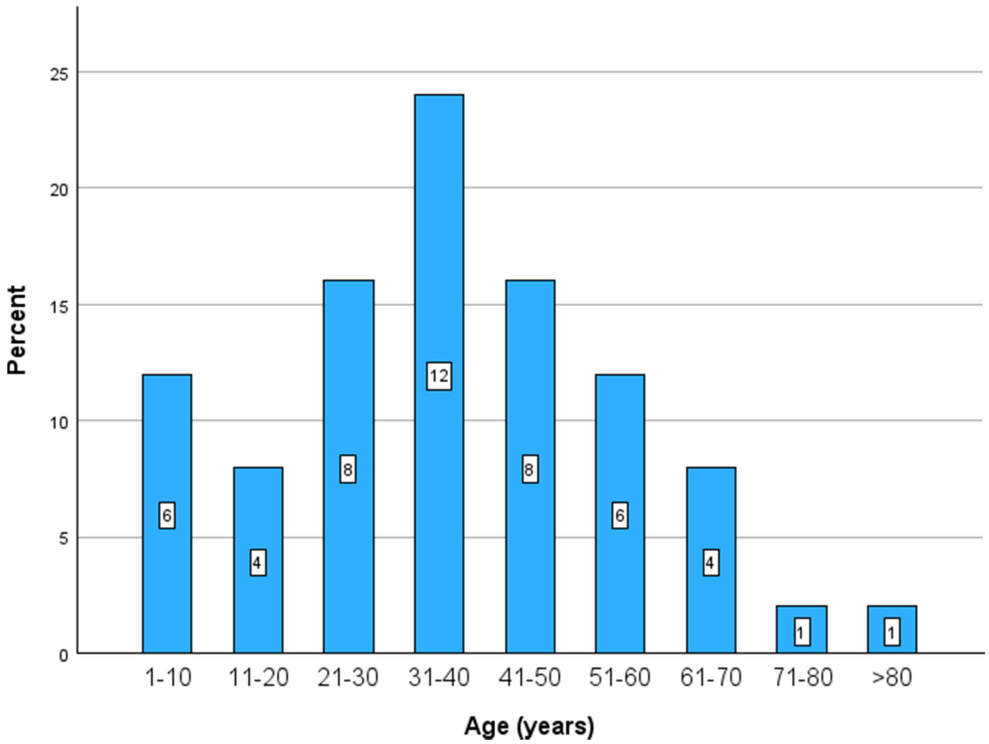

Figure 1. Bar diagram showing age distribution of the study cases (n=50)

Sex distribution of the study cases

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 4.2).

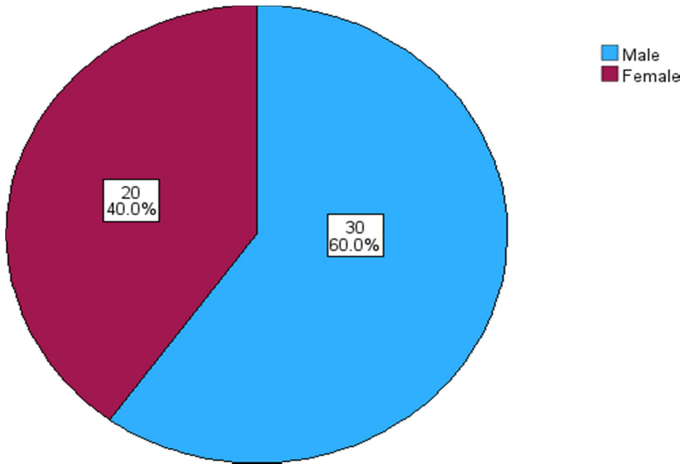

Figure 2. Pie chart showing sex distribution of study cases (n=50) Histological diagnosis of tumor

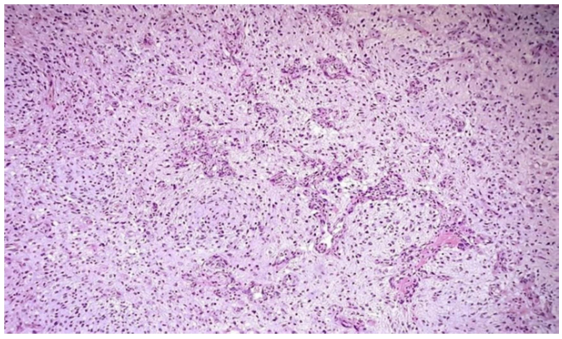

Figure 3. Photomicrograph shows glioblastoma (grade-IV)

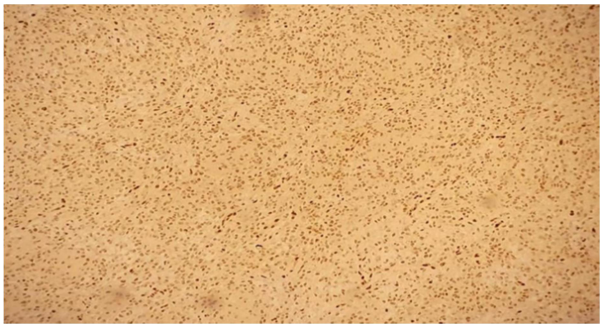

Figure 4. Photomicrograph showing high expression of FLI1 immunostain in glioblastoma

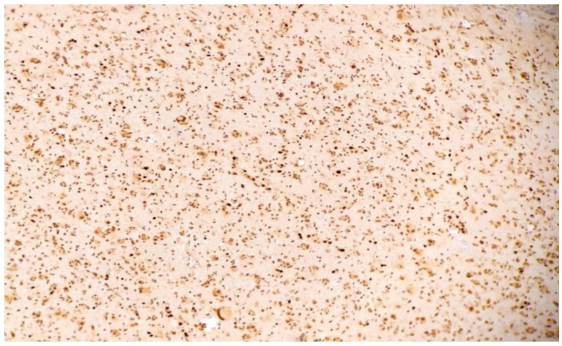

Figure 5. Photomicrograph showing high expression of FLI1 immunostain in anaplastic astrocytoma

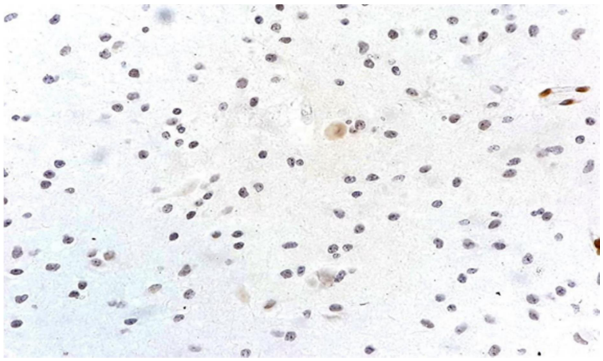

Figure 6 Photomicrograph showing low expression of FLI1 immunostain in diffuse fibrillary astrocytoma

Table III 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 III: 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

Histomorphologic Criteria

Table IVreveals that approximately half (48.0%) of the cases exhibited moderate cellular characteristics, while 40.0% displayed marked cellular features. Furthermore, 34.0% of cases demonstrated mild atypia, 36.0% exhibited moderate atypia, and 30.0% presented

marked atypia. Mitoses were observed in 56.0% of cases. Microvascular proliferation was identified in 40% of cases, with 14.0%, 14.0%, and 12.0% representing mild, moderate, and marked levels, respectively. Additionally, necrosis was evident in 32.0% of cases.

Table IV: Distribution of study cases according to histomorphology (n=50)

Histomorphology	Number of patients	Percentage (%)
Cellularity		
Mild	6	12.0
Moderate	24	48.0
Marked	20	40.0
Atypia		
Mild	17	34.0
Moderate	18	36.0
Marked	15	30.0
Mitosis		
Present	28	56.0
Absent	22	44.0
Microvascular proliferation		
Mild	7	14.0
Moderate	7	14.0
Marked	6	12.0
Absent	30	60.0
Necrosis		
Present	16	32.0
Absent	34	68.0
Total	50	100.0

Expression of FLI1

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

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

Table VI shows that FLI1 expression was significantly associated with nuclear atypia, mitosis, and necrosis but not associated with cellularity and microvascular proliferation. Results were yielded through Chi Square test.

Table VI: Association of cellularity, nuclear atypia, mitosis, microvascular proliferation and necrosis with FLI1 expression (n=50)

Cellularity	FLI1 expression				P value
	High (n=37)		Low (n=13)		
	n	%	n	%	
Cellularity					
Mild	3	8.1	3	23.1	
Moderate	16	43.2	8	61.5	<0.077[ns]
Marked	18	46.6	2	15.4	
Nuclear atypia					
Mild	7	18.9	10	76.9	
Moderate	17	45.9	1	7.7	<0.001[s]
Marked	13	35.1	2	15.4	
Mitosis					
Present	27	73.0	1	7.7	<0.001[s]
Absent	10	27.0	12	92.3	
Microvascular proliferation					
Absent	19	51.4	11	84.6	0.171[ns]
Mild	7	18.9	0	0.0	
Moderate	6	16.6	1	7.7	
Marked	5	13.5	1	7.7	
Necrosis					
Present	15	40.5	1	7.7	0.029[s]
Abscent	22	79.5	12	92.3	

ns= not significant s= significant
p value reached from Chi Square test

Discussion

This cross-sectional study was conducted at the Department of Pathology of DMC to evaluate the expression of FLI1 in astrocytoma and its association with WHO grade. A total of 50 samples histologically confirmed as cases of astrocytoma were included in the study.

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.[16] 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.[4] Mallick et al. (2022) reported that maximum number of

glioblastoma patients were aged between 41 and 50 years (40%).[17] 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 (18). According to the Surveillance, Epidemiology, and End Results (SEER) program database (1999-2010) the mean age of occurrence of astrocytoma was 37.77 years.[19] 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.[16] 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.[4] 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.[8] Females are less commonly affected by astrocytoma female hormones have preventive effects on tumorigenesis in different age groups. It can be explained that brain tumor develops in female brain cells after a series of genetic alterations and exposure to a growth factor.

The current study showed most of the cases with low FLI1 expression (61.5%) had moderate cellularity, while the most cases with high FLI1 expression (46.6%) had marked cellularity. The difference was not statistically significant ($p<0.077$). Among the cases with high FLI1 expression, the majority had moderate atypia (45.9%), with among the cases with low FLI1 expression, the majority had mild atypia (76.9%). The difference was statistically significant ($p<0.001$). That majority of the mitotically inactive cases (92.3%) showed low FLI1 expression, whereas majority of mitotically active cases (73.0%) showed high FLI1 expression. The difference was statistically significant ($p<0.001$). Among the cases with high FLI1 expression, approximately half (51.4%) had absent microvascular proliferation. In contrast, among the cases with low FLI1 expression, about 84.6% showed absent microvascular proliferation. However, this difference was not statistically significant. Among the cases with high FLI1 expression, necrosis was present in 40.5%, whereas among the cases with low FLI1 expression, necrosis was present in 7.7% of cases. The observed difference was statistically significant ($p=0.029$).

Conclusion

The results of this study indicate high FLI1 expression was positively associated with atypia, mitosis and necrosis of astrocytoma. Evaluation of FLI1 can act as a predictive marker for tumor progression and prognostic marker for patients diagnosed as 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 FLI1 expression with different histological parameters reveal high expression in higher grade with poor Prognosis

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