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Clinical profile, histopathological, immunohistochemical, and molecular analyses and treatment of pilocytic astrocytoma: an eight year study from a tertiary health care centre in North East India

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Abstract

Background Pilocytic astrocytoma (PA) is a central nervous system (CNS) World Health Organization (WHO) grade 1 glial tumor that is highly prevalent in children and young adults. The main aim of the study was to assess the frequency, clinicopathological features, and treatment of PAs along with their immunohistochemical and molecular analyses in patients from Northeast India. About 144 glial tumors were diagnosed in patients from 3 to 75 yrs of age from January 2015 to March 2022. Nine pediatric PA patients were identified and their clinical data were analyzed. Immunohistochemistry (IHC), fluorescence in-situ hybridization (FISH), and molecular analysis using the real-time polymerase chain reaction (RT-PCR) were performed. Data analysis was performed using the SPSS software.

Results The mean age of the glioma patients was 41.7 yrs ± 18.2 with a male/female ratio of 1.3:1. The most common form of the glial tumor was found to be astrocytoma CNS WHO grade 2 (31.9%). The frequency of PA CNS WHO grade 1 was 6.9%. The pediatric PA cohort had a mean age of 9.2 yrs ± 4.9 with a male/female ratio of 2:1. Glial fibrillary acidic protein (GFAP) positive immunostaining and retention of transcriptional regulator alpha-thalassemia mental retardation X-linked protein (ATRX) expression was seen in all the tested PAs. The KIAA1459-BRAF fusion was detected in four PAs. Surgical intervention with total or radical tumor excision was performed for the PA patients. Eight PA patients exhibited improved condition post-surgery.

Conclusion With the advent of healthcare and newer diagnostic facilities there is an increased incidence of glial tumors in developing countries. A combination of histological, immunohistochemical, and molecular analysis is very important for the diagnosis, accurate treatment, and prognosis of PA patients.

Keywords Glial tumors, Immunohistochemistry, Molecular analysis, Pediatric, Pilocytic astrocytoma, Surgical excision

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Background

Malignant and non-malignant brain and other central nervous system (CNS) tumors are heterogeneous group of neoplasms that affects both children and adults [1]. They represent less than 2% of all tumors [1]. In India CNS tumors ranges from 5 to 10 per 100,000 population and accounts for 2% of all malignant neoplasms [2, 3]. The risk factors that are associated with or contribute to CNS tumor pathogenesis include family history, genetic factors, radiation exposure, stress, and weakened immune system [4]. However, the exact causes of CNS tumor are still unclear [4]. Although the rate of incidence of CNS tumors in children is low, however, they constitute around 20% of all childhood tumors [5, 6]. It is known to be the second most common tumor in children after leukaemia [6]. In general the location of brain tumor in children is the posterior fossa also known as the infratentorial region that involves the cerebellum, fourth ventricle, and brain stem (midbrain, pons, and medulla) [7, 8].

Low-grade gliomas and glioneuronal tumors are the most frequently encountered but relatively rare CNS tumors in children with an accountability of approximately 30% of all pediatric CNS neoplasms patients [9]. According to the 2021 5th edition of the World Health Organization (WHO) *Classification of Tumors of the Central Nervous System*, pediatric low-grade gliomas and glioneuronal tumors falls under three distinct families: (1) Pediatric-type diffuse low-grade gliomas, (2) Circumscribed astrocytic gliomas, and (3) Glioneuronal and neuronal tumors [10]. Pilocytic astrocytomas (PA) are usually well-circumscribed and often cystic CNS WHO grade 1 low-grade gliomas of the CNS that occurs most commonly in children and young adults [11, 12]. Symptoms commonly seen in pediatric PA patients include headache, visual problems, gait imbalance, seizures, nausea, and vomiting. However, symptoms may vary depending on tumor size and location [13]. Most pediatric PA cases are sporadic but they might also occur due to germline mutations in mitogen-activated protein kinase (MAPK) pathway gene neurofibromatosis type 1 (NF1) [14]. PA tumor cells have biphasic appearance that includes compact fibrillar areas comprising of elongated nuclei, bipolar hair-like piloid processes, and Rosenthal fibers and loose microcystic areas of round to oval nuclei, cobweb-like processes, and eosinophilic granular bodies [15].

Classical diagnosis of PA has been primarily based on histopathological features and neuroradiological examinations that include a computed tomography (CT) scan and/or magnetic resonance imaging (MRI) [13]. But patients with similar or identical tumors might experience different clinical outcomes and responses to treatments. This is because the underlying genetic characteristics of the tumors differ with respect to age and location. Thus, the need for broad molecular analysis of CNS tumors has vastly increased. With the advent of newer technologies and novel molecular diagnostic tools such as Next Generation Sequencing (NGS) and DNA methylation-based profiling coupled with immunohistochemistry (IHC) has made diagnosis, grading, and prognosis of CNS tumors including PA a lot more convenient. The main aim of the study was to analyze the histopathological, immunohistochemical, and molecular profile of pediatric PA patients admitted in our hospital setup. Also, data on pediatric PA from the Indian subcontinent specifically from the North East is relatively sparse. The study thus provides an overview of the epidemiology of pediatric PA from the North Eastern part of the country.

Methods

Cases

A single centric retrospective cohort study was performed. The glioma cases which were registered and operated in our hospital from January 2015 to March 2022 were investigated. Cases were identified from the medical records department (MRD) of the hospital. A total of 144 glioma cases were included in the study. Grading of the cases was done according to CNS WHO 2021 classification.

Clinical data collection

Data from January 2015 to March 2022 registered glioma cases were collected retrospectively.

There were nine pediatric PA patients that were considered for the study. Medical records of the pediatric PA group were reviewed to retrieve patient information that included age, sex, and clinical features present at the time of consultation. Information on tumor size, location, and treatment modalities were also reviewed. Neuro-radiological imaging of the patients were done that involved an MRI and/or a CT scan.

Histopathology

The tumor samples were fixed in 4% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The H&E slides were viewed under the microscope for examination of the histological features. Tumors were categorized as per the CNS WHO 2021 classification.

Immunohistochemistry

IHC for six selected PAs was carried out by using the primary antibodies against the antigens glial fibrillary acidic protein (GFAP) (Bio SB Inc., US), isocitrate dehydrogenase 1 (IDH1) (dianova, Germany), MIB E3 ubiquitin

protein ligase 1 (MIB-1) (GenomeMe, Canada), tumor protein p53 (p53) (Santa Cruz Inc., US), and alpha-thalassemia mental retardation X-linked protein (ATRX) (dianova, Germany). Cases that stained for GFAP and showed cytoplasmic staining for IDH1 and nuclear staining for MIB-1, p53, and ATRX were considered as positive.

Fluorescence in-situ hybridization

FISH assay for *BRAF* fusion was carried out for the selected PAs. A series of steps that included deparaffinization, dehydration, washing and air drying of the formalin-fixed-paraffin-embedded (FFPE) tissue sections were performed. FISH was undertaken using the commercially available probes (CytoTest Inc. USA) and counter stained with 4′, 6-diamidino-2-phenylindole (DAPI). For each sample ~ 150 to 200 non-overlapping interphase nuclei were examined [16].

Molecular analysis

Total RNA from FFPE sections was extracted using the RNeasy FFPE kit (Qiagen, Germany) following the manufacturer's instructions. The High-capacity RNA to cDNA™ kit (Thermo Fisher Scientific, USA) was for cDNA synthesis as per the manufacturer's protocol. Detection of *KIAA1549* and *BRAF* fusion was performed by real-time PCR (Bio-Rad, USA) using 2.5 µl of cDNA, primers, and fusion probes. The primer sets (Thermo Fisher Scientific, USA) used for (1) 15–9 fusion were: F_p 5′-CGTCCACAACTCAGCCTACA -3′ and R_p 5′-CCTCCATCACCACGAAATCCTT-3′ (2) 16–11 fusion were: F_p 5′-GCCCAGACGGCCAACA-3′ and R_p 5′-ACTCGAGTCCCGTCTACCAA-3′. Probes (Thermo Fisher Scientific, USA) used for (1) 15–9 fusion was: 5′-TCGGGATGCCCAGACTTG-3′ and (2) 16–11 fusion was: 5′-CCCTGCAGTAAAA-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was taken as an internal control. The final concentrations of the primers and probes were 0.9 µmol/L and 0.25 µmol/L, respectively. The reaction conditions used were 2 min at 50 °C, 10 min at 95 °C, 50 cycles for 15 s at 95 °C, and 1 min at 60 °C [16].

Surgical process

Surgical reports were analyzed to review treatment modalities, extent of resection, and results of pre- and post-operative neuroimaging. Surgical procedures that included midline suboccipital craniotomy at the posterior fossa, left suboccipital craniectomy at the left cerebral hemisphere, right suboccipital craniectomy at the right cerebellum, left temporal craniotomy at the left temporal lobe and left parietal occipital craniotomy at the left parietal occipital lobe were performed on the patients with radical tumor excision. Post-operative antibiotics were suggested and a follow-up.

Statistical analysis

Statistical analysis was performed using the Microsoft excel to determine standard deviations for the data.

Results

Demographics of glioma patients

The demographic characteristics of the glioma patients are shown in Table 1. A total of 144 patients were diagnosed with CNS WHO grades 1 to 4 glioma in our hospital from January 2015 to January 2022. The age of the patients ranged from 3 to 75 yrs with a mean age of 41.7 yrs ± 18.2. The patients comprised of 81 males (56.3%) and 63 females (43.8%) with a male/female ratio of 1.3:1. The most common glial tumor was astrocytoma CNS

Table 1 Demographics of glioma patients admitted in our hospital from 2015 to 2022

	WHO grade	Age range	Mean (yrs ± SD)	Male n (%)	Female n (%)	Ratio (M:F)	Total n (%)
All gliomas	1–4	3 to 75 yrs	41.7 ± 18.2	81 (56.3%)	63 (43.8%)	1.3:1	144
Astrocytoma	1	21 to 32 yrs	26.5 ± 7.8	0 (0%)	2 (100%)	–	2 (1.4%)
Astrocytoma	2	7 to 69 yrs	40.7 ± 16.4	24 (52.2%)	22 (47.8%)	1.1:1	46 (31.9%)
Astrocytoma	3	17 to 75 yrs	46.2 ± 14.6	25 (59.5%)	17 (33.3%)	1.5:1	42 (29.2%)
Astrocytoma	4	3 to 73 yrs	52.2 ± 21.1	11 (55%)	9 (45%)	1.2:1	20 (13.9%)
Diffuse astrocytoma	2	22 to 69 yrs	39.2 ± 12.5	7 (63.6%)	4 (36.4%)	1.8:1	11 (7.4%)
Ganglioglioma	2	18 to 66 yrs	42 ± 33.9	0 (0%)	2 (100%)	–	2 (1.4%)
Oligoastrocytoma	2	39 yrs	39 ± 0.0	1 (66.7%)	0 (0%)	–	1 (0.7%)
	3	52 to 66 yrs	59 ± 9.9	1 (50%)	1 (50%)	1:1	2 (1.4%)
Oligodendroglioma	3	27 to 65 yrs	45.8 ± 14.5	5 (83.3%)	1 (16.7%)	5:1	6 (4.2%)
Pilocytic astrocytoma	1	3 to 25 yrs	10.8 ± 6.8	6 (60%)	4 (40%)	1.5:1	10 (6.9%)
Pleomorphic xanthroastrocytoma	3	20 to 40 yrs	30 ± 14.1	1 (50%)	1 (50%)	1:1	2 (1.4%)

WHO grade 2 tumor and it was prevalent in 46 patients (31.9%) having a mean age of 40.7 yrs ± 16.4. The subsequent highly prevalent glial tumor was found to be astrocytoma CNS WHO grade 3 (n = 42; 29.2%) and those patients had a mean age of 46.2 yrs ± 14.6. Twenty patients (13.9%) with a mean age of 52.2 yrs ± 21.1 were detected with astrocytoma CNS WHO grade 4 tumors. There were two female patients (1.4%) with a mean age of 26.5 yrs ± 7.8 who were diagnosed with astrocytoma CNS WHO grade 1 tumor. Diffuse astrocytoma CNS WHO grade 2 tumor was identified in 11 (7.4%) patients and the mean age was 39.2 yrs ± 12.5. One 18 yr old (0.7%) and a 66 yr old female (0.7%) were diagnosed with the rare ganglioglioma CNS WHO grade 2 tumor. One 39 yr old male (0.7%) was diagnosed with oligoastrocytoma CNS WHO grade 2 while another 52 yr old male (0.7%) and a 66 yr old female (0.7%) were seen with oligoastrocytoma CNS WHO grade 3 tumor. Oligodendroglioma CNS WHO grade 3 tumor was seen in 6 patients (4.2%) having a mean age of 45.8 yrs ± 14.5. There were 10 (6.9%) PA grade 1 patients and their mean age was 10.8 yrs ± 6.8. There was one 40 yr old male (0.7%) and a 20 yr old female (0.7%) who belonged to pleomorphic xanthroastrocytoma CNS WHO grade 3 tumor categories. Of all the glial tumors, pediatric glioma was seen in 19 patients (13.2%) with an age range of 3 to 18 yrs that comprised of 11 males (57.9%) and 8 females (42.1%). PA CNS WHO grade 1 tumor was the most common glial tumor in the pediatric group and it was seen in 9 pediatric patients (6.3%) having a mean age of 9.2 yrs ± 4.9. Whereas only one adult was diagnosed with PA CNS WHO grade 1 tumor. Astrocytoma CNS WHO grade 2 tumors was found in five pediatric patients (3.5%) with a mean age of 12.4 yrs ± 4. One 17 yr old young adult (0.7%) was diagnosed with astrocytoma CNS WHO grade 3 tumor. There were three pediatric patients (2.1%) having a mean age of 8.3 yrs ± 5 who were identified with astrocytoma CNS WHO grade 4 tumors. Ganglioglioma CNS WHO grade 3 tumor was detected in an 18 yr old male (0.7%). An overview of the histological distribution of glial tumors in the adult and pediatric group of our study is depicted in Fig. 1.

Baseline characteristics of the pediatric PA patients

Since PA is a common glioma in pediatric population, we therefore, evaluated all the pediatric PA cases of our

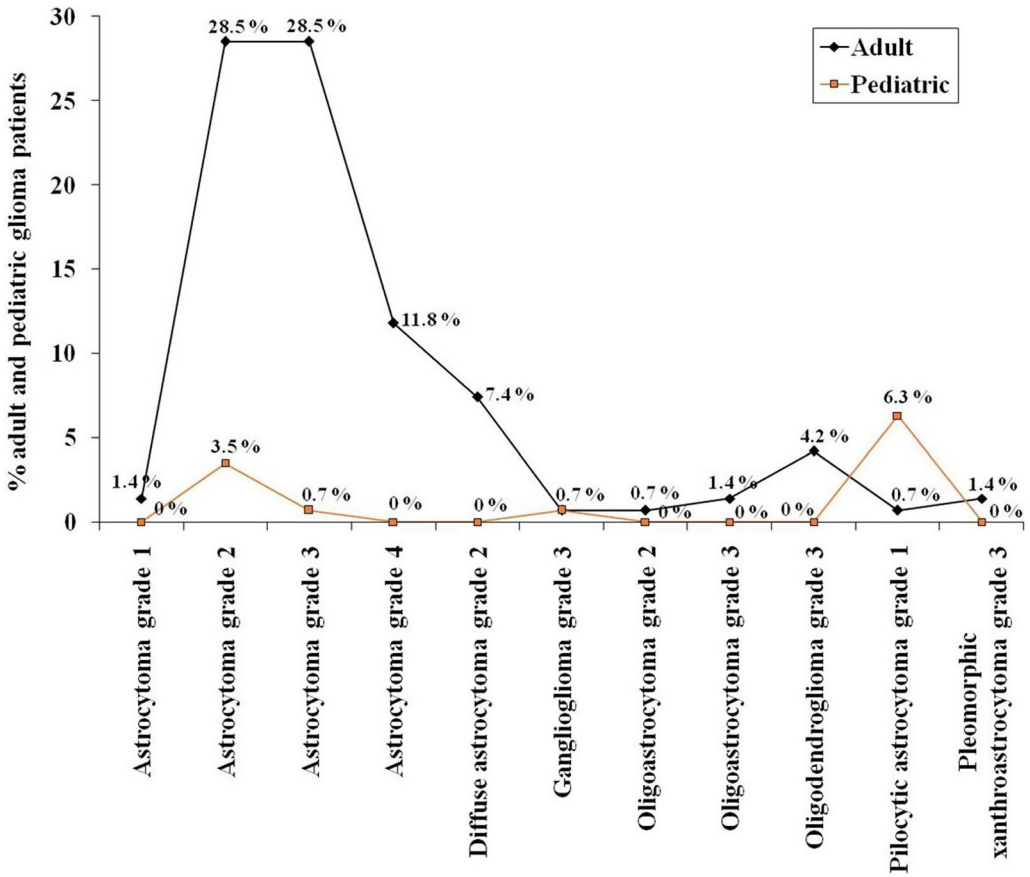

Fig. 1 Histological distribution of glial tumors in adult and pediatric group

study. The baseline characteristics of the pediatric PA patients that were included in our study are illustrated in Table 2. The patients who were diagnosed with PA and admitted in our hospital from 2015 to 2022 were included in the study. As mentioned earlier there were a total of nine pediatric PA patients (6.3%). Patients ranged from 3 to 18 yrs of age with the mean age being 9.2 yrs ± 4.9. The male/female ratio was 2:1. The common clinical presentations in the patients were found to be vomiting (n = 5; 55.6%), visual problems (n = 4; 44.4%), and headache (n = 3; 33.3%). Other symptoms included gait imbalance (n = 1; 11.1%), uncontrolled limb movements (n = 1; 11.1%), weakness of both upper and lower limbs (n = 1; 11.1%), seizures (n = 1; 11.1%); drowsiness (n = 1; 11.1%), and fever (n = 1; 11.1%). There were 2 patients (22.2%) whose symptoms persisted > 1 year until diagnosis, 1 patient (11.1%) had symptoms nearly a year back of diagnosis, and 3 patients (33.3%) showed symptoms < 1 year of diagnosis. Duration of symptoms was uncertain for 3 patients (33.3%). One patient (11.1%) had a history of Left Medium-Pressure Ventriculo Peritoneal (MPVP) shunt surgery to treat hydrocephalus while the remaining 8 patients (88.9%) did not have any past medical or surgical or family or other history. Chest X- ray did not show any anomaly for the patients. Abdominal gastrointestinal (GI) examination showed no signs of abnormalities. Heart rate, breathing, and O_2 saturation levels were found to be in the normal range for all the patients. Low blood pressure of 80/60 mm was found in one patient (11.1%). Heart sound hearing S1S2 was audible in all patients. Neurological assessment was done by the Glasgow Coma Scale (GCS) and all patients at the time of clinical evaluation scored E4V5M6. One patient was lost to follow up. Remaining eight patients were found to be in good condition.

Table 2 Clinical profile of pediatric pilocytic astrocytoma patients included in this study

Clinical characteristics	Cases (n = 9)
Mean age	8.9 ± 5.6 yrs
Gender (M:F)	02:01
Antecedent events	None—0
	Headache—3
	Vomiting- 5
	Seizure—1
	Convulsion—0
	Fever—1
	Visual defects—4
	Gait imbalance—1
	Sensory symptoms—0
	Jerky movement of limbs—1
	Weakness of upper and lower limbs—2
	Drowsiness—1
	Bladder symptoms—0
	Bowel symptoms—0
Duration of symptoms: < 1 year	3
1 year	1
> 1 year	2
Unspecified	3
History of ailment/ surgery/ family/ others	MPVP shunt—1
	None—8
Chest- clear b/l	9
Abdomen- soft, BS + ve	9
Pulse: 60—120/ min range	9
Blood pressure: Normal 120/80 mm	8
Low 80/60 mm	1
O_2 saturation: 95% and higher	9
R/R: 12—20 breaths/min range	9
CVS: S1S2 audible	9
CNS: GCS- E4V5M6	9
Follow up: Alive	8
Dead	0
Lost to follow up	1

Immunohistochemical analysis of pediatric PAs

From the pediatric group, immunohistochemical analyses were performed for six selected PAs. The PAs stained positively for GFAP. IDH1 immunohistochemical expression was not observed in the selected tumor cells. Also, the tumor cells did not stain positively with MIB-1. Similarly, p53 did not show any immunoreactivity in any of the tumor cells. ATRX expression was retained in the selected PAs. The histolopathological features of PAs included presence of cellularity, microcystic changes, Rosenthal fibres and eosinophilic granular bodies. The immunohistochemical results on the PAs are shown in Fig. 2.

Molecular analysis of pediatric PAs

The *KIAA1459-BRAF* fusion is an established diagnostic marker for PAs. *BRAF* fusion was first tested using the FISH assay. From the pediatric group, the analysis was performed for the selected PAs. Using the fusion specific probe set, *KIAA1459-BRAF* fusion was detected in four of the six PAs. Two PAs did not give positive fusion results due to insufficient probe signal. A representative image from the FISH positive fusion results is shown in Fig. 3. *BRAF* fusion RT-PCR assay was also performed for the six PAs (Fig. 4). The analysis revealed that the *BRAF* fusion was positive for four

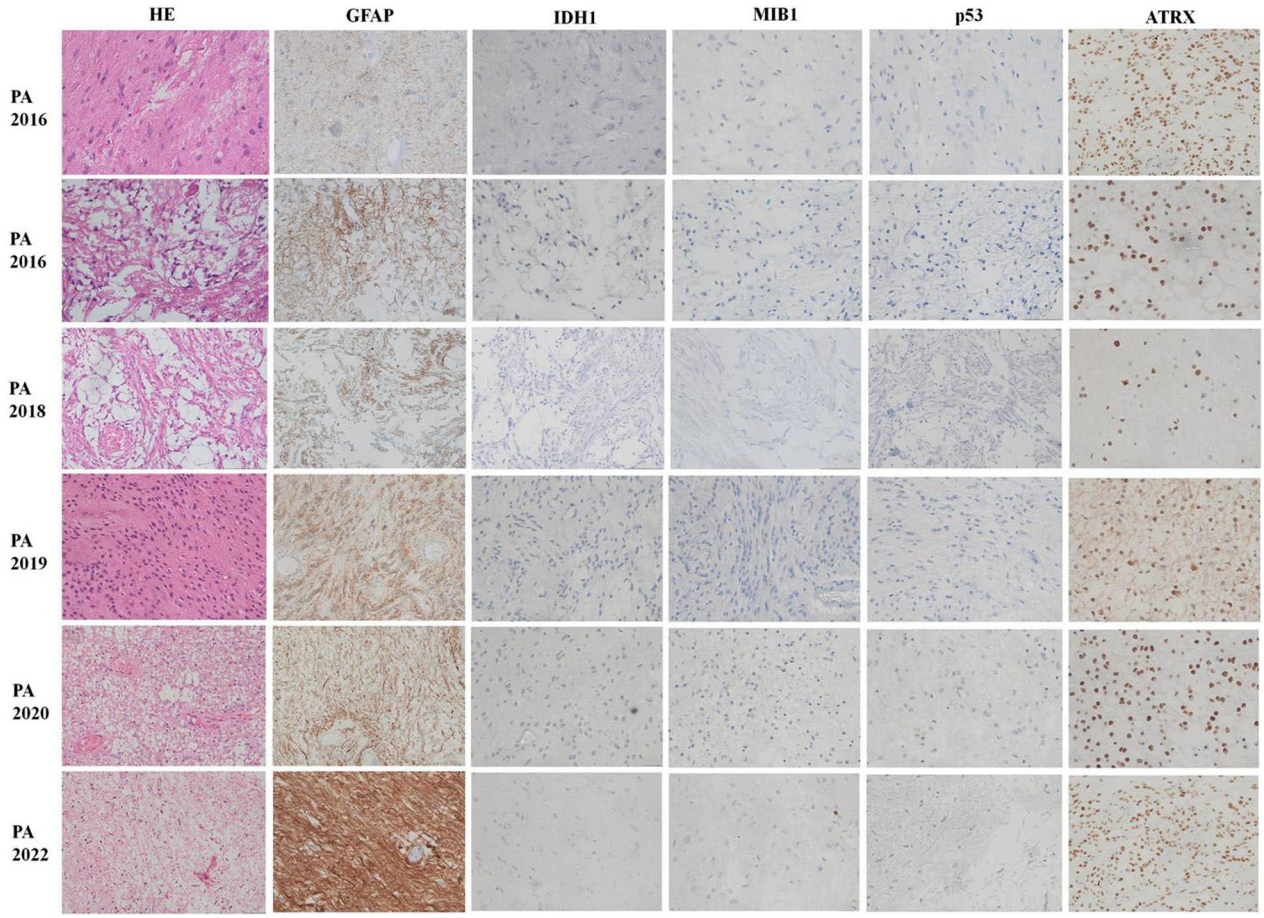

Fig. 2 Photomicrographs showing histological and immunohistochemical analysis of the selected PAs

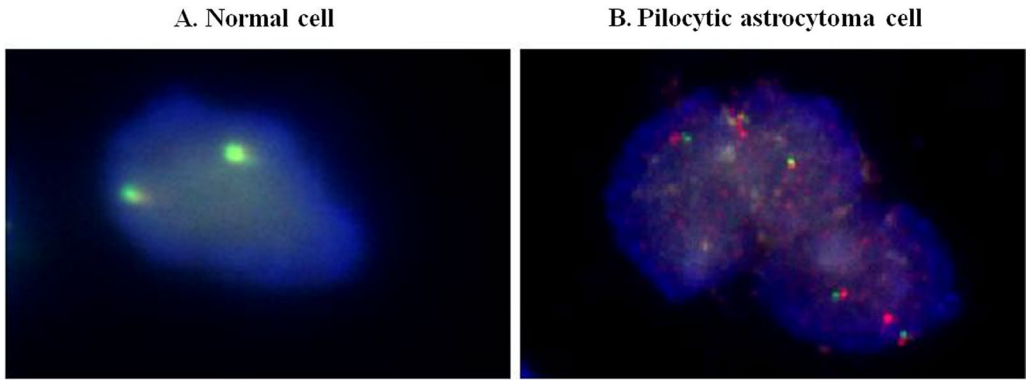

Fig. 3 A representative image of FISH analysis for the BRAFKIAA1549 fusion of the selected case of PA

patients and these patients had only the 15–9 fusion. None of the patients showed the 16–11 fusion. Table 3 describes type of *BRAF* fusion with the tumor location for the PA patients.

Treatment of the pediatric PA patients

Tumor size, location, treatment, and post-treatment condition of the pediatric PA patients are shown in Table 4. After evaluation of the clinical symptoms, brain MRI

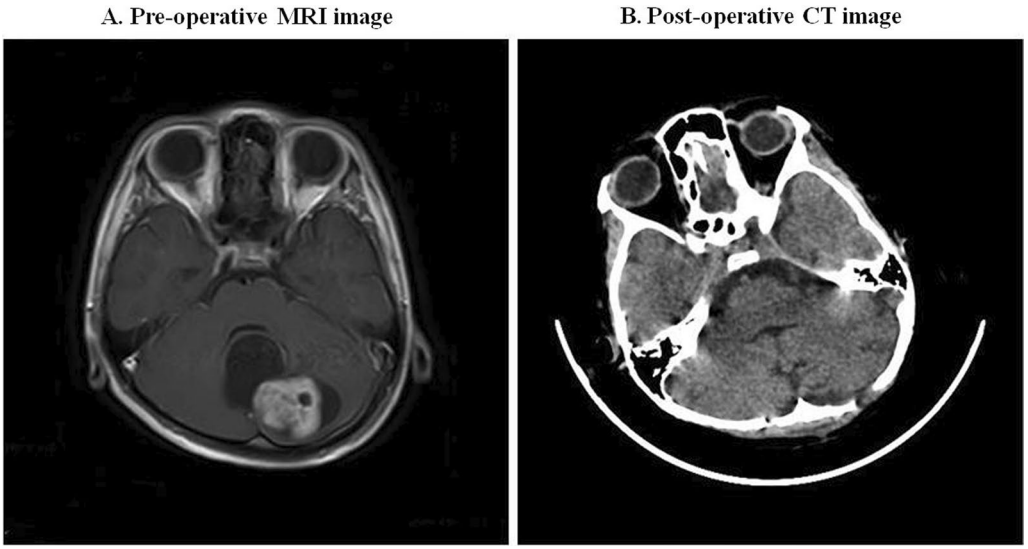

Fig. 4 Pre-operative MRI and post-operative CT images of a selected case of PA. Pre-operative MRI image showed hyper dense mass with cystic component in the left cerebellum. Post-operative CT image showed complete excision of the tumor

Table 3 KIAA1549:BRAF fusion and tumor location

Case	Tumor location	15–9 *KIAA1549:BRAF* fusion		16–11 *KIAA1549:BRAF* fusion
		FISH	RT-PCR	RT-PCR
2016	Posterior fossa in the left cerebral hemisphere	**Positive**	**Positive**	Negative
2016	Vermis	Insufficient probe signal	**Positive**	Negative
2018	Left temporal lobe	Insufficient probe signal	**Positive**	Negative
2019	Midline posterior fossa	**Positive**	Negative	Negative
2020	Midline posterior fossa	**Positive**	Negative	Negative
2022	Left parietal occipital lobe	**Positive**	**Positive**	Negative

and CT scans were performed for the patients. Based on the MRI and CT scans reports, the patients were given supportives that included anticonvulsant, steroids, neuroprotectors, and analgesics and were operated. Of the nine patients in the study, five patients were operated via midline suboccipital craniotomy with radical tumor excision. Left suboccipital craniectomy and radical excision was performed in one patient. Another patient was approached by right suboccipital craniectomy and radical excision of tumor. Left temporal craniotomy and radical excision was done in one patient. Another patient underwent left parietal occipital craniotomy and radical excision. At the time of discharge from the hospital, eight patients showed improvement in their conditions. They did not show any deficit and were found to be afebrile, continent, and ambulatory during their discharge time. However, the patient who was diagnosed in 2019 did not show any improvement in his condition. His GCS score was E3VTM4, pupils were 8 B/L, non-ambulatory, not continent and was on Foley catheter. Post-operative antibiotics were added along with other medications for all the patients. They were advised to follow a normal diet and be rested for ~ 6 weeks. They were also instructed to do a follow up in a neurological or an oncological set up. Pre- and post-operative images of a patient showing radical excision of PA are shown in Fig. 4.

Table 4 Tumor size, location, and treatment of pediatric pilocytic astrocytoma patients included in this study

Case	Year of diagnosis	Age at biopsy (yrs)	Sex	Tumor size (cm)	Tumor location	Surgery	Condition at the time of discharge
1	2015	6	M	6.2×6.5×4.3	Posterior fossa in the left cerebral hemisphere	Left suboccipital craniectomy and radical excision	Improved
							No deficit; afebrile; continent; ambulatory
2	2016	9	F	5.8×6.2×5.4	Vermis	Midline suboccipital craniotomy and radical excision	Improved
							No deficit; afebrile; continent; ambulatory
3	2016	15	M	4.1×5.6×5.2	Posterior fossa	Midline suboccipital craniotomy and radical excision	Improved
							No deficit; afebrile; continent; ambulatory
4	2017	6	F	4.3×3.5×4.2	Right cerebellum	Right suboccipital craniectomy and radical excision	Improved
							No deficit; afebrile; continent; ambulatory
5	2018	3	M	8×6.8×6.5	Left temporal lobe	Left temporal craniotomy and total excision	Improved
							No deficit; afebrile; continent; ambulatory
6	2019	5	M	3×4.5×2.5	Posterior fossa	Midline suboccipital craniotomy and radical excision	Unrestored
							GCS E3VTM4; Pupils 8 B/L; afebrile; non-ambulatory; not continent
7	2020	9	M	6×4×3	Posterior fossa	Midline suboccipital craniotomy and radical excision	Improved
							No deficit; afebrile; continent; ambulatory
8	2022	18	M	4.7×3.6×4	Left parietal occipital lobe	Left parietal occipital craniotomy and radical excision	Improved
							No deficit; afebrile; continent; ambulatory
9	2022	12	F	6.8×5.9×5	Posterior fossa	Midline suboccipital craniotomy and radical excision	Improved
							No deficit; afebrile; continent; ambulatory

Discussion

This is the first study of the Indian population from the North East region of the country regarding spectrum of low grade and high grade gliomas with special emphasis on the pediatric PAs. In our study, glial tumor was detected in patients that comprised of 3 to 75 yrs of age. More males were diagnosed with gliomas than females. According to CNS WHO 2021 classification, the gliomas belonged to grades 1 to 4. We observed that among the adult cohort in our study, astrocytoma CNS WHO grade 2 and grade 3 were the most common glial tumor. Astrocytoma CNS WHO grade 4, diffuse astrocytoma CNS WHO grade 2, and oligodendroglioma CNS WHO grade 3, oligoastrocytoma CNS WHO grade 3, and pleomorphic xanthroastrocytoma CNS WHO grade 3 were also seen among the adult group. We found that gangliogliomа CNS WHO grade 2, oligoastrocytoma CNS WHO grade 2, and PA CNS WHO grade 1 were the least commonly occurring tumor among the adult cohort of our study. The most common form of glial tumor in the pediatric cohort was found to be PA CNS WHO grade 1 which was followed by astrocytoma CNS WHO grade 2. Astrocytoma CNS WHO grade 3 and gangliogliomа CNS WHO grade 3 were the other glial tumors seen in the pediatric population of our study.

Most PAs cases are observed in the first two decades of life. But it is mostly seen in 6 to 13 yrs old age range that accounts for 75% of all PA cases [17, 18]. Within

our study period, nine pediatric and one adult PA cases were diagnosed on histopathological examination in our hospital. Two pediatric PA cases (22.2%) were in the age ranges 0–5 yrs, five cases (55.5%) belonged to 6–13 yrs age range, and the rest two cases (22.2%) fell in the 14–18 yrs age range. Even though majority of the studies have shown no gender predilection [15], however, in our study we observed that males were slightly affected more than females. There are no unique clinical features of this low grade tumor [15]. Signs and symptoms usually persist for several months to years and depend on the tumor size, location, and presence of associated hydrocephalus [15]. In cerebellar tumors, symptoms that are seen include gait imbalance, headache, neck pain, visual defects, and vomiting [18, 19]. In supratentorial tumors, seizures are usually seen [19]. Tumors located in the hypothalamic area are associated with hormonal dysfunctions [18, 20]. In optical pathway tumors, visual loss or visual-field deficits are most commonly observed [19, 20]. In the present study too, the patients exhibited various persistent clinical signs and symptoms at the time of hospital admission. Vomiting (55.6%), visual defects (44.4%), and headache (33.3%) were the common symptoms observed in the patients. One patient (11.1%) had a history of MPVP shunt to treat hydrocephaly and another patient (11.1%) exhibited lowered blood pressure.

Histopathological examination has been the standard technique that is followed in most of the laboratories for the diagnosis of PAs. The well-established and common histological features of PAs include biphasic pattern, eosinophilic granular bodies, microcystic areas, moderate to low cellularity areas, piloid and rounded cells, and Rosenthal fibres [19]. Similar histological features were also observed in the H&E stained PA tissue samples of our study. Diagnosis of PAs is usually challenging due to variation in their histomorphological features and is mostly combined with neuroradiological investigations. Because of heterogeneity in their appearance, PAs are frequently misdiagnosed as high grade tumors at the initial diagnosis [21].

In order to eliminate and/or reduce misdiagnoses, immunohistochemical markers along with molecular analysis has been increasingly used for better diagnosis of complicated cases of PAs [22]. This is in particular important for differential diagnosis of PAs including low and high grade gliomas. GFAP is a useful biomarker for distinguishing PAs from other gliomas [22]. Immunohistochemical staining for GFAP was seen in the selected PAs of our study. *IDH1* or *IDH2* mutations are seldom seen in low grade gliomas including PAs [23]. The selected cases of PAs were also negative for IDH1 immunostaining in our study. MIB-1 and p53 immunostaining are useful for histopathological differentiation of PAs from other high grade gliomas [24]. Our immunohistochemical results also showed negative staining for both MIB-1 and p53. Loss of nuclear ATRX expression on IHC is an indicator of *ATRX* gene mutation. *ATRX* mutations rarely occur in PAs [23]. ATRX immunostaining was positive for the selected PAs of our study. The distinctive molecular feature of PAs is BRAF gene alterations that include either the presence of a KIAA1549-BRAF fusion transcript or mutation in the BRAFV600E [24]. Our molecular findings revealed KIAA1459-BRAF fusion for four selected PA cases.

In the pediatric population, PAs mostly occur in the cerebellum but certain cases have showed the tumor location in the brainstem, cerebrum, diencephalon, and optic pathway whereas in adults, PAs are mostly seen in the supratentorial structures [25]. On neuroimaging, we observed that 44.4% of the pediatric cohort in our study had supratentorial tumor location and the rest 55.6% exhibited infratentorial tumor location. PAs has extremely high survival rates with a 10 yr survival rate of upto 95% [25]. Better prognosis is directly related to the extent of surgical resection and tumor location. Many studies have also reported that a total surgical resection of PAs improves the survival rates by 100% and the recurrence rates of 2–5.4% whereas partial resection of PAs has recurrence rates of 42–45% [26]. Even though prognosis of PA cases is excellent, however, there are certain rare cases where the outcome is not encouraging due to factors such as tumor resection and surgical morbidity. Therefore, the neurosurgeons of the hospital performed extensive excision of the tumor mass for greater survivality. Majority of the patients exhibited no neurological deficit after the surgery. They were advised to undergo radiation therapy and chemotherapy if needed during the follow up. For this study, the primary caregivers of the patients were contacted to know their overall well-being. The patient whose conditions were not restored at the time of discharge could not be contacted. Rest all other patients were found to be in good health.

Our hospital is a dedicated Neurosciences research centre in Northeast India apart from the government medical colleges. Hilly terrain, limited resources, and poor connectivity separates Northeastern region from the rest of the country. As such our hospital is the first superspeciality tertiary health care centre in this region and is dedicated for the people of Northeast India. It has adopted an integrated patient care model system to provide affordable rates for quality care to the patients from Northeast India. Geographical location, affordable and better patient care in an otherwise inaccessible region, presence of health workers employed by our organization in remote areas, and the socioeconomic status of the people of this region are the unique features of our

hospital set up. In recent years our centre has given more emphasis on understanding the molecular alterations in different grades of CNS tumors for better characterization and optimization of treatment. This is important as early diagnosis of pediatric low grade gliomas is crucial for increased survival. There are only a limited number of medical centres in Northeast India including ours that uses molecular markers for reporting of routine gliomas.

The limitation of the study includes small size of the sample. Due to small sample size statistical significance could not be determined for the values. In general the data was collected retrospectively. We were not able to do the follow up on survivality due to limited resources. In summary, the present study was conducted to evaluate the pattern of glial tumors in adult and pediatric Indian population from the Northeast with respect to age, frequency, morphological sub type, and tumor location. PAs was predominantly seen in pediatric male patients that exhibited distinct histological and immunohistochemical features. This type of regional study is important for correct diagnosis, treatment, and management of PAs for the pathologists and neurosurgeons of these areas.

Conclusion

The present study highlights the prevalence of glioma among the Indian population particularly from the north east. Glial tumors were found across all age group and gender. The cohort exhibited high prevalence of astrocytoma CNS WHO grade 2. Among the pediatric group, PA was the most common glial tumor. Precise and accurate diagnosis of glial tumors combining histomorphological features along with clinical and radiological imaging information is important for effective treatment, management, and thereby better prognosis of glioma patients. New and advanced diagnostic facilities such as immunohistochemistry and molecular techniques have made tremendous progress in the identification of pediatric gliomas and thus they play a major role in pediatric neuro-oncologic pathology. Our hospital is one of few institutions in Northeast India that has also adopted state-of-the-art diagnostic tools for early diagnosis and treatment of CNS tumors.

Abbreviations

ATRX	Alpha-thalassemia mental retardation X-linked
CNS	Central nervous system
CT	Computed tomography
DAPI	4´, 6-Diamidino-2-phenylindole
FFPE	Formalin-fixed-paraffin-embedded
FISH	Fluorescence in-situ hybridization
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GCS	Glasgow Coma Scale
GFAP	Glial fibrillary acidic protein
GI	Gastro-intestinal
H&E	Hematoxylin and eosin
IHC	Immunohistochemistry
IDH1	Isocitrate dehydrogenase 1
MAPK	Mitogen-activated protein kinase
MIB-1	MIB E3 ubiquitin protein ligase 1
MPVP	Medium-Pressure Ventriculo Peritoneal
MRD	Medical records department
MRI	Magnetic resonance imaging
NF1	Neurofibromatosis type 1
NGS	Next Generation Sequencing
PA	Pilocytic astrocytoma
p53	Tumor protein p53
RT-PCR	Real-time polymerase chain reaction
WHO	World Health Organization

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Author contributions
Concepts, design, and literature: SA, NB, NJB, and IH. Data acquisition: SA, AB and ID. Data analysis: SA, NB, NJB, IH, AB and ID. Statistical analysis: AB. Manuscript preparation: AB. Manuscript review and editing: SA, NB, NJB, IH, and ID. Guarantor: SA.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are not publicly available due [not applicable] but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the ethics committee of GNRC Hospitals.

Consent for publication
The inform consent was taken from all the patients for publication (as per our department policy we take such consent from all the patients at time of admission).

Competing interests
The authors declare that they have no competing interests.

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