ORIGINAL RESEARCH

A Clinicopathological Prospective Observational Study of Paediatric Intracranial and Spinal Astrocytic Tumours with Special Reference to the Role of BRAF-V600E and IDH-1 Mutation

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ABSTRACT

Aim: To study pediatric brain tumor with special reference to the role of BRAFV600E and IDH-1 mutation.

Material and Methods: The present prospective observational study was conducted in the department of Neurosurgery, Bangur Institute Of Neurosciences & SSKM Hospital, IPGME & R, KOLKATA among 40 histologically confirmed astrocytoma across all CNS compartment operated in our hospital during July 2021 to December 2022. Clinical, radiological investigations, surgical details, outcome data was recorded. The patients were regularly followed up at 3, 6, 12 and 18 months after operation.

Results: Male were comparatively more as compared to female. 37.5% of the subjects were suffering from grade IV tumor while 12.5%, 27.5% and 22.5% were having grade III, II and I tumor respectively. Seizure, headache and focal weakness was revealed in 77.5%, 45% and 10% of the subjects respectively. IDH1 mutation was found in 45% (n=34) of the cases, out of which 17 (94.44%) showed IDH1immunopositivity. All these immunopositive cases showed heterozygous IDH1 R132H mutation by sequencing. BRAF V600E mutation was found in 7.14% of the cases. Tumor resection was completed in 67.5% of the subjects while incomplete in 32.5% of the cases. Immediately after surgery, patient condition was bettered in 45% of the subjects while it became worse among 35% of the subjects.

Conclusion: Diffuse astrocytoma and anaplastic astrocytoma are characterized by frequent IDH1 mutation, which can be efficiently detected by IDH1 immunohistochemistry; thus avoiding the need of expensive investigations like FISH.

Keywords: Pediatric, Brain Tumor, BRAFV600E, IDH-1 mutation.

INTRODUCTION

Ganglioglioma (GG) and astrocytoma are the rarest of brain tumor types. GGs are well-differentiated, rare CNS tumors, which are characterized by a slow, circumscribed growth and a relatively favorable prognosis. Gangliogliomas are generally benign WHO grade I tumors, most commonly located in the temporal lobes of children and young adults. However, they are both clinically and histologically heterogeneous, and tumor recurrence or anaplastic progression occurs in some cases.[1] Astrocytomas are the second most common IMSCTs observed in adults, but the most common in children.[2,3] Several recent studies have demonstrated a clear prognostic difference between high-grade (WHO grade 3, 4) and low-grade spinal astrocytomas (mainly pilocytic astrocytoma WHO grade 1 and diffuse astrocytoma WHO grade 2).[4,5] Pilocytic trocytoma (PA) are a distinct histologic and biologic subset of gliomas that account for approximately 5.1% of all these tumors. It is the most common pediatric brain tumor in children. Although preferentially located in the cerebellum, PA can arise

anywhere in the CNS. Almost all are generally considered WHO grade I tumors.[6] The most important and well described molecular alteration in the pathogenesis of grade glioma is isocitrate dehydrogenase (IDH) mutation. It is now believed that IDH mutation is responsible for initiation of glioma genesis.[7,9] IDH1 or IDH2 mutation is seen in >90% of astrocytomas and oligodendrogliomas. Oligodendrogliomas are characterized by 1p/19q co-deletion, whereas ATRX mutation has been recently described in astrocytic tumors. IDH mutation is rarely seen in glioblastomas (GB) and pilocytic astrocytomas (PA).[9] The most commonly described genetic alteration in PA is KIAA-BRAF fusion, while BRAF V600E mutation is observed in <10% cases of PA. According to the molecular signature of these tumors, diffuse astrocytoma is believed to be more closely related to oligodendroglioma, rather than PA. Based upon these information, glial tumors are now classified into three basic categories - pilocytic astrocytoma (WHO grade I), glioblastoma (WHO grade IV), and diffuse gliomas, which include astrocytomas and oligodendrogliomas (WHO grade II and III), and this was adopted in the 2016 update of WHO classification of CNS tumors. These molecular markers not only help to classify the glial tumors into different molecular subgroups, but also provide prognostic information.[10] It has been observed that IDH mutant gliomas carry better prognosis than IDH wild-type tumors. Gliomas with 1p/19q co-deletion behave better than cases that do not show this deletion. Glioblastomas with IDH mutation also show longer survival than IDH wild type GBs. According to the recommendation of ISN-Haarlem 2014 consensus, only histological diagnosis with a WHO grade is no longer sufficient. It is advisable to offer integrated diagnosis, which includes both histological and molecular information. These recommendations have been reflected in the 2016 update of WHO classification of CNS tumors. Most of the information regarding molecular alterations in gliomas is available from the western literature. There is limited data involving these molecules in glial tumors in the Indian population.[11] The frequencies of IDH1 and BRAF, mutations in pediatric brain tumor and their prognostic significance in Indian population has not been published so far. This study was undertaken to determine the frequency of BRAFV600E and IDH-1 mutation alterations in pediatric brain tumor and to correlate with disease outcome.

MATERIAL AND METHODS

The present prospective observational study was conducted in the Department of Neurosurgery, Bangur Institute Of Neurosciences & SSKM Hospital, IPGME & R, Kolkata from July 2021 to December 2022. All histologically confirmed astrocytoma across all CNS compartment operated in the hospital during the study period were recruited. All operated histologically confirmed 40 cases of astrocytoma across all CNS compartments during this time period. Ethical clearance to carry out the study was obtained from the ethical committee of the institute. The study protocol was explained to the patient/guardian and a written informed consent was obtained from each subject to be enrolled in the study.

INCLUSION CRITERIA

All Paediatric cases admitted into Bangur Institute Of Neurosciences & SSKM Hospital, IPGME & R, Kolkata operated for suspected cases of intracranial and spinal astrocytoma of Paediatric age groups i.e. 1-18 year and histologically confirmed thereafter by Pathology Department of SSKM HOSPITAL, IPGMER.

EXCLUSION CRITERIA

1. Patient those who are not willing to participate in the study.
2. Relapsing cases of astrocytoma (Intracranial or spinal)
3. Extraneural metastatic astrocytoma
4. Age more than 18 years

DATA COLLECTION

After written informed consent; age, sex, mode of presentation, clinical symptomatology, clinical findings were recorded in all patients. Radiological investigations, surgical details, outcome data was recorded. The patients were regularly followed up at 3, 6,12,18 months after operation. Details of the patient in terms of clinical history taking, a thorough physical examination with special focus on central nervous system and spine with relevant radiological investigations was recorded. We looked for frozen section or squash cytology positive cases of suspected astrocytoma across all CNS compartments. Size of tumour, intensity in T1W1 and T2W2, enhancement, cyst, calcification, peritumoral edema, intratumoral HMG and MRI of spinal cord plain plus contrast was recorded too. Frozen section or squash cytology and immunohistochemical markers staining BRAF-V600E, IDH-1 was done. Data was collected and subjected to statistical analysis.

RESULTS

Out of 40 subjects, 60% were male and 40% were female. Hence male were comparatively more as compared to female. Mean age among the study subjects was 8.74±4.91 years. Size of tumor viz. <40mm and ≥40mm was revealed in 30% and 70% of the subjects respectively. 37.5% of the subjects were suffering from grade IV tumor while 12.5%, 27.5% and

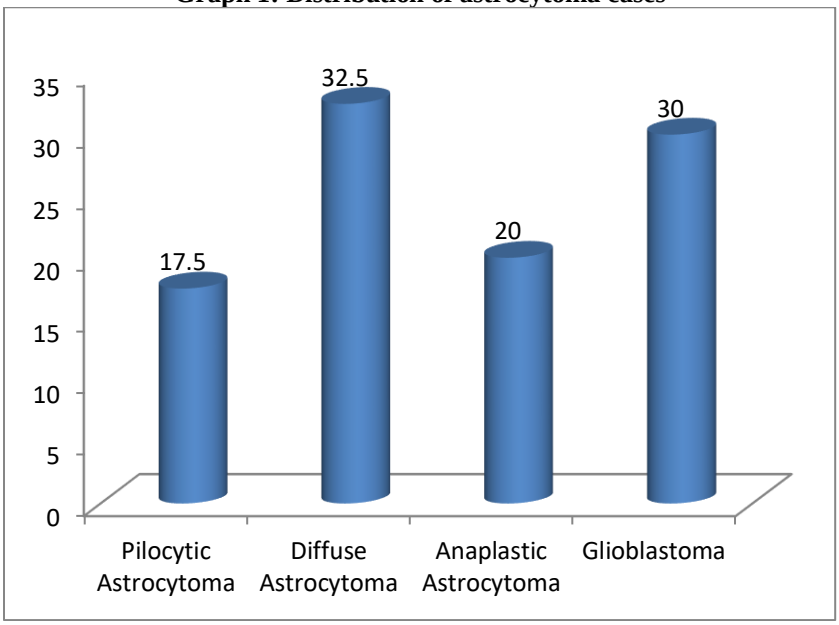
22.5% were having grade III, II and I tumor respectively (table 1).

Table 1: Tumor extension, grade and clinical presentation among the study subjects

Tumor Extension	N=40	%
Localized	35	87.5
Regional	3	7.5
Distant/Invasive	2	5
Tumor Grade		
I	9	22.5
II	11	27.5
III	5	12.5
IV	15	37.5
Presentation		
Seizure	31	77.5
Headache	18	45
Focal Weakness	4	10
Other	7	17.5

Pilocytic astrocytoma, diffuse astrocytoma, anaplastic astrocytoma and glioblastomawas found among 17.5%, 32.5%, 20% and 30% of the subjects respectively (graph 1). Maximum of the astrocytoma cases were located in intracranial region (80%) while 20% of the lesions were of spinal region.

Graph 1: Distribution of astrocytoma cases



IDH1 mutation was found in 45% of the cases. None of thepilocytic astrocytoma cases showed IDH1 mutation while only one Glioblastoma revealed the same. 76.92% and 87.5% of the diffuse and anaplastic astrocytoma cases reported IDH1 mutation. There were total 34 IDH1-mutant cases in this study, out of which 17 (94.44%) showed IDH1immunopositivity. All these immunopositive cases showed heterozygous IDH1 R132H mutation by sequencing. None of the IDH immunonegative cases showed this mutation by sequencing. Thus, IHC showed 100% sensitivity and specificity for detecting IDH1 R132H mutation (table 2).

Table 2: IDH1 mutation and correlation of IHC and sequencing

Type of Astrocytoma	N=40	IDH1 Mutation (Present)	
		N	%
Pilocytic Astrocytoma	7	0	0
Diffuse Astrocytoma	13	10	76.92

Anaplastic Astrocytoma	8	7	87.5
Glioblastoma	12	1	8.33
Total	40	18	45

IDH1-Mutant Cases	**IDH1 Immuno-positivity**
18	17

BRAF V600E mutation was found in 7.14% of the cases. One of the pilocytic astrocytomacases showed BRAF V600E mutation while two Glioblastoma revealed the same (table 3).

Table 3: BRAF V600E mutation

Type of Astrocytoma	**N=40**	**BRAF V600E Mutation (Present)**	
		N	**%**
Pilocytic Astrocytoma	7	1	14.29
Diffuse Astrocytoma	13	0	0
Anaplastic Astrocytoma	8	0	0
Glioblastoma	12	2	16.67
Total	40	3	7.14

Tumor resection was completed in 67.5% of the subjects while incomplete in 32.5% of the cases. Tumor recurrence occurred in 77.5% of the subjects. (table 4).

Table 4: Extent of resection, recurrence

Extent of Resection	**N=40**	**%**
Complete	27	67.5
Incomplete	13	32.5
Recurrence		
Yes	31	77.5
No	9	22.5

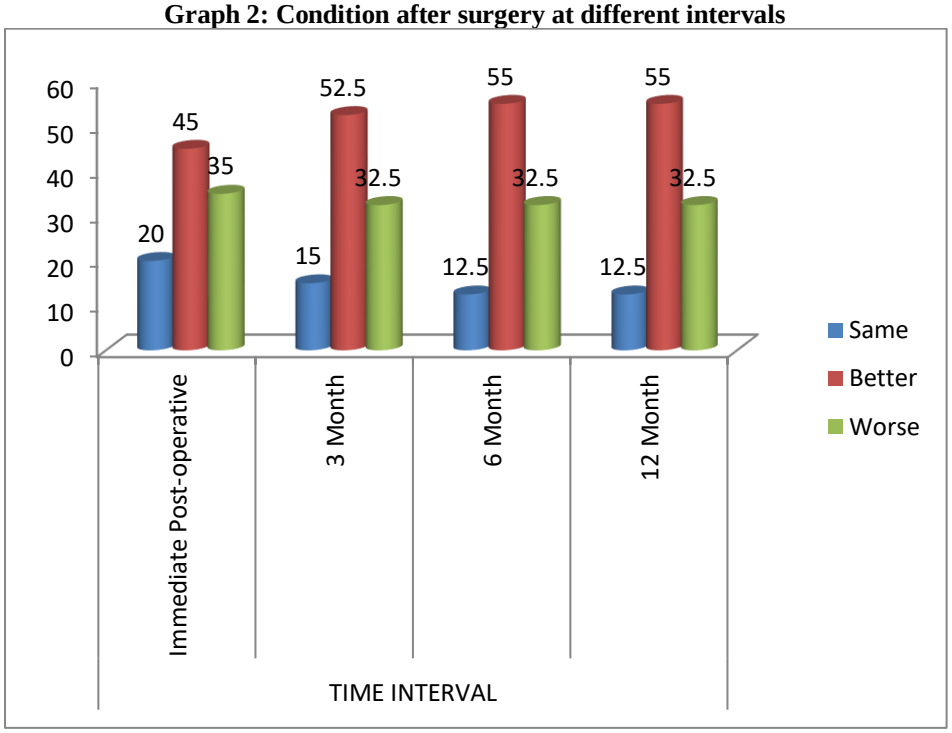
In this study, mortality was reported in 40% of the cases. Maximum mortality was associated maximum with Glioblastoma followed by Anaplastic and Diffuse Astrocytoma cases. No mortality was found in Pilocytic Astrocytoma cases. Most common complication was cranial nerve palsy (27.5%) followed by infection (12.5%). Seizure and weakness was reported in 10% and 7.5% of the subjects respectively (table 5).

Table 5: Mortality and post surgery complications among the study subjects

Type of Astrocytoma	**N=40**	**Mortality (Present)**	
		N	**%**
Pilocytic Astrocytoma	7	0	0
Diffuse Astrocytoma	13	3	23.08
Anaplastic Astrocytoma	8	3	37.5
Glioblastoma	12	10	83.33
Total	40	16	40
Complications			
Infection	5	12.5	
Post ICH	2	5	
Seizure	4	10	
Weakness	3	7.5	
Cranial Nerve Palsy	11	27.5	

Immediately after surgery, patient condition was bettered in 45% of the subjects while it became worse among 35% of the subjects. Approximately similar distribution was found after 3 month. However after 6 and 12 months, condition becomes worse in 32.5% of the subjects (graph 2).

Graph 2: Condition after surgery at different intervals



DISCUSSION

The most important and well described molecular alteration in the pathogenesis of grade glioma is isocitrate dehydrogenase (IDH) mutation. It is now believed that IDH mutation is responsible for initiation of glioma genesis. IDH1 or IDH2 mutation is seen in >90% of astrocytomas and oligodendrogliomas. IDH mutations are rarely seen in glioblastomas (GB) and pilocytic astrocytomas (PA). The most commonly described genetic alteration in PA is KIAA-BRAF fusion, while BRAF V600E mutation is observed in <10% cases of PA. According to the molecular signature of these tumors, diffuse astrocytoma is believed to be more closely related to oligodendroglioma, rather than PA. Most of the information regarding molecular alterations in gliomas is available from the western literature. There is limited data involving these molecules in the Indian population.[12] Hence the present prospective observational study was conducted in the department of Neurosurgery, Bangur Institute Of Neurosciences & SSKM Hospital, IPGME & R, Kolkata among 40 histologically confirmed astrocytoma across all CNS compartment operated in our hospital during July 2021 to December 2022. The aim of the study was to study pediatric brain tumor with special reference to the role of BRAFV600E and IDH-1 mutation. Out of 40 subjects; 60% were male and 40% were female. Hence male were comparatively more as compared to female. Mean age among the study subjects was 8.74±4.91 years in this study. Andrew S. Luksik et al[13] and Debajyoti Chatterjee et al[12] in their study reported similar age and gender distribution. Seizure, headache and focal weakness was revealed in 77.5%, 45% and 10% of the subjects respectively. Similar clinical presentation was found by Sonika Dahiya et al[14] in their study. Pilocytic astrocytoma, diffuse astrocytoma, anaplastic astrocytoma and glioblastoma was found among 17.5%, 32.5%, 20% and 30% of the subjects respectively. Most of the astrocytoma cases were located in supratentorial location (57.5%) while 42.5% of the lesions were of infratentorial region. Similar distribution of astrocytoma cases was found by Debajyoti Chatterjee et al[12] in their study. IDH1 mutation was found in 45% of the cases. None of the pilocytic astrocytoma cases showed IDH1 mutation while only one Glioblastoma revealed the same. 76.92% and 87.5% of the diffuse and anaplastic astrocytoma cases reported IDH1 mutation. There were total 34 IDH1-mutant cases in this study, out of which 17 (94.44%) showed IDH1immunopositivity. All these immunopositive cases showed heterozygous IDH1 R132H mutation by sequencing. None of the IDH immunonegative cases showed this mutation by sequencing. Thus, IHC showed 100% sensitivity and specificity for detecting IDH1 R132H mutation. It is now considered that diffuse astrocytoma and anaplastic astrocytoma share similar genetic profile and they are characterized by IDH and ATRX mutation. Isocitrate dehydrogenase (IDH) is one of the most well recognized and widely described molecular markers in glial tumors, both with astrocytic and oligodendroglial

differentiation. The frequency of IDH mutation in diffuse glioma is variable, ranging from 54% to 90%. IDH mutation is rare in pilocytic astrocytoma[12]. Among the different IDH1 mutations, the commonest is R132H mutation at codon position 132. Other mutations in this position are less frequent[12]. Debajyoti Chatterjee et al[12] in their study reported that there were total 34 IDH1-mutant cases in this series, out of which 32 (94.1%) showed IDH1 immunopositivity. All these immunopositive cases showed heterozygous IDH1 R132H mutation by sequencing. As IDH1 mutation is not seen in reactive conditions, IHC is extremely useful in distinguishing diffuse glioma from reactive gliosis and therapy induced changes, especially in a small biopsy. IDH2 mutation is restricted in codon position 172 (IDH2 R172), and is much less frequent than IDH1 mutation, and is more commonly seen in oligodendroglial tumors compared to astrocytomas. In a large series, Hartmann et al. found IDH2 mutation in 0.9%, 0.9%, 4.7% and 5.2% in grade II astrocytoma, grade III astrocytoma, grade II oligodendroglioma, and grade III oligodendroglioma, respectively. Thus, IDH2 mutation is extremely rare in astrocytic tumors, and is more often associated with an oligodendroglial phenotype. Hence, IDH2 sequencing can be avoided in diffuse gliomas with astrocytic morphology for routine diagnostic purpose. IDH2 mutation has not been reported yet from India. We did not perform IDH2 gene sequencing in this study. Since we included only astrocytic tumors and majority was IDH1-mutant, there was less chance that IDH2 sequencing could have altered the results significantly.[15,16] BRAF V600E mutation was found in 7.14% of the cases. One of the pilocytic astrocytoma cases showed BRAF V600E mutation while two Glioblastoma revealed the same. BRAF V600E mutation was not detected in any astrocytic tumor as mentioned by Debajyoti Chatterjee et al[12] in their study. According to Sonika Dahiya et al[14], positive BRAFV600E staining was observed in 18 of 47 specimens (38.3 %). BRAFV600E mutations have recently been identified in 18 % of ganglioglioma samples by traditional sequencing methods. More sensitive techniques may in fact determine that BRAF mutations are far more common in gangliogliomas. A mutation-specific BRAFV600E monoclonal antibody has recently become available, which accurately identifies the presence of this mutation in pathologic specimens. Immunohistochemical studies using BRAFV600Especific antibodies are 97 % sensitive and 98 % specific for detecting this mutation in other tumor types. More recently, a 94 % concordance has been demonstrated between immunohistochemistry and sequencing in gangliogliomas; this report also showed a frequency of 58 % for the mutant protein using IHC. However, the histologic characteristics and prognostic significance of this mutation have rarely been previously described in pediatric gangliogliomas[17-19].

A recent consensus meeting of neuropathologists has suggested a way to integrate the rapidly emerging molecular information into the diagnostic work up of brain tumors. Treatment strategies directed toward specific subtypes of brain tumors defined by histopathology and molecular diagnostics are being integrated in first-line clinical protocols. With the availability of genetically engineered mouse models and orthotopic xenografts being generated and widely available to research laboratories, there is an added impetus to find newer more effective and less toxic therapies, using high-through point screening for some of the clinically aggressive tumors. The level of optimism in the pediatric neuro-oncology community is unprecedented, in anticipation that these recent discoveries will lead to a paradigm shift in the diagnosis and treatment of pediatric brain tumors.

CONCLUSION

Diffuse astrocytoma and anaplastic astrocytoma are characterized by frequent IDH1 mutation, which can be efficiently detected by IDH1 immunohistochemistry; thus avoiding the need of expensive investigations like FISH. BRAF V600E mutation is extremely rare in astrocytic tumors in our population.

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