Original Research Article

Immunohistochemical expression of idh mutations in gliomas and its clinicopathological correlation

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Abstract

Aim: To study the expression of IDH-1 mutations in gliomas, with histopathological correlation and its clinical significance and also study the prognostic implication of IDH-1 expression by comparing with WHO grading system and with KI-67 expression in glial tumors. **Methodology:** This is a prospective study over a period of 24 months (July 2017 – June 2019) to be conducted at Osmania General Hospital, Hyderabad. Immunohistochemistry (IHC) for IDH1 and KI-67 is performed on three micron sections utilizing standardized protocols IDH1 (clone H09) and KI-67(clone GM001), on all selected cases. **Results:** In the present study, among the 54 cases of gliomas in the present study, IDH-1 mutation were observed in overall 54% of gliomas. History of seizures and frontal location are significantly associated with IDH-1 expression. IDH-1 is expressed mostly in oligoastrocytic tumors, gemistocytic astrocytomas, anaplastic PXA, diffuse astrocytic tumors followed by oligodendroglial tumors, glioblastomas and least expressed in ependymal tumors.These mutations were not seen in pilocytic astrocytomas.Secondary glioblastomas showed more (71%) IDH-1 mutations than primary glioblastomas(16%). IDH-1 expression were most common in WHO grade II and III gliomas and are significantly associated with these mutations. The present study revealed Ki 67 labelling indices were comparable between gliomas of similar malignancy grade, and indices for high-grade gliomas (grade III/IV) were significantly higher than in low-grade (grade I/II) tumors.The p value between gradeII and grade IV was statistically significant. The Ki 67 labelling indices were slightly higher in IDH-1 negative cases i.e IDH-1 wild variants than IDH-1 positive cases. **Conclusion:** IDH-1 mutations in gliomas has potential as an independent prognostic marker and also helps in distinguishing primary from secondary GB & its potential for new therapies, testing for IDH1 mutations can enhance accuracyof glioma diagnosis and treatment.

Keywords: IDH-1, Glioblastomas, Idh-1, Mutations, KI-67, Diagnosis, Prognostic markers

Introduction

Glial tumours represent 42% of all primary central nervous system (CNS) neoplasms of which over 75% are malignant.[1]

Gliomas are primary tumors of the central nervous system that originate from transformed neural stem or progenitor glial cells. They were divided by the World Health Organization (WHO) into low-grade gliomas (LGG ;WHO grades I and II) and high- grade gliomas (HGG; WHO grades III and IV)[2].

LGG are well-differentiated, slow-growing tumors, whereas HGG are less differentiated or anaplastic and diffusive, strongly infiltrating brain parenchyma and making a surgical resection difficult[2].

Histological classification is currently assisted by molecular genetic studies that provide diagnostic, prognostic, and predictive values, and an IDH (isocitrate dehydrogenase) genotype was recently added as one of the key molecular factors to the classification of gliomas.

The new 2016 WHO classification scheme divides diffuse gliomas into LGGs and glioblastomas based on histology. LGGs are further divided into IDH wild type or mutant, which is further classified into either a diffuse astrocytoma that has an intact 1p/19q loci, but is enriched for ATRX and TP53 mutations or an oligodendroglioma (OLIG) that harbors 1p/19q co-deletion [3,4].

Glioblastomas (GB, grade IV) were further divided into an IDH-wild type, which corresponds to primary or de novo GB, and an IDH-mutant type, which refers to secondary or progressive GB.

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IDH, is a citric acid cycle enzyme that catalyses conversion of isocitrate to a- ketoglutarate (a-KG), producing NADP. IDH1 mutations are expressed in 70-80% of grade ll and lll astrocytomas, oligoastrocytomas,oligodendrogliomas and in secondary glioblastomas but less than 10% in primary glioblastomas[5,6], indicating that these IDH mutations play key role in gliomatogenesis [4]. Patients with IDH expression with grade II,III or IV have better overall survival [7,8].

Tumor cells with IDH mutations lose ability to produce a-KG and gain ability to produce 2-hydroxyglutarate (2-HG), which is an a-KG antagonist. These metabolic alterations reduce anti-oxidant function, and cause DNA and histone methylation, which impairs the differenciation of stem cells leading to tumor formation. This discovery of isocitrate dehydrogenase mutations in gliomas have resulted in many novel ideas for therapeutic approaches. The concept of 2-HG being an "oncometabolite"[9] have led many investigators to try to devise strategies to either restore normal IDH function or block production or downstream effects of 2-HG. While there are currently no direct IDH inhibitors but are under clinical trials. [4]

Immunohistochemical determination of proliferative activity (using Ki-67 LI) along with IDH-1 mutations are useful supplement for establishing the histopathological diagnosis of gliomas.

Ki-67 immunostaining is most commonly used and has been shown to correlate positively with tumor grade and prognosis.[10] Despite its widespread use, the procedure still has many uncertain and limiting factors, including problematic overlap of indices between different glioma grades and inherent problems in the immunohistochemical analysis. Thus, publishing data on Ki-67 immunostaining in human gliomas is still worthwhile in order to optimize this method, with the superior goal of achieving a standardized procedure. The aim of this study is to evaluate the expression of IDH-1 mutations and Ki-67 labelling indices (LIs) in a series of gliomas and critically evaluate the findings.

These markers thus serve as effective tools for unequivocal identification of gliomas and their distinction from non-CNS tumors

while helping the pathologist in distinction of different glioma classes.

Aims and objectives

- To study the expression of IDH-1 mutations in gliomas, with histopathologicalcorrelation and its clinical significance.
- To study the prognostic implication of IDH-1 expression by comparing with WHO grading system and with KI-67 expression in glial tumors.

Materials and methods

Study design

This is a prospective study over a period of 24 months (July 2017 – June 2019) to beconducted at Osmania General Hospital, Hyderabad.

Period of study

24MONTHS (July 2017– June 2019)

Place of study

Osmania General Hospital, Hyderabad.

Target population

A total of 54 cases are taken up for study.

Inclusion criteria

- All neuro surgically excised specimens suggestive of gliomas were included.

Exclusion criteria

- All non-glial tumors and metastatic tumors to CNS.

Examination of cases

Due importance was given to record a brief clinical history with age, inpatient registration number, biopsy number, presenting symptoms and signs, CT and MRI findings.

The specimens were received in 10% formalin. Measurements of the specimens were recorded and thorough gross examination was carried out and salient features like haemorrhage, necrosis and calcification were recorded. Depending on the volume of tumour, adequate number of blocks was given. Afterroutine tissue processing H and E staining was done and slides microscopically examined.

Immunohistochemistry

Immunohistochemistry (IHC) for IDH1 and KI-67 is performed on three micron sections utilizing standardized protocols IDH1 (clone H09) and KI-67(clone GM001),on all selected case

Positive control

For Ki-67 – Lymph node with reactive hyperplasia.

Ki 67 Labelling index evaluation

The immune stained sections were scanned using a 40× objective with an eye grid forthe areas with the highest density of labelled tumor cells (hot spots). At least 1000 tumor cells, or alternatively three high power fields (HPF) were examined. Only immunoreactive tumor cell nuclei were counted. Necrotic areas and vascular endothelium were excluded. The Ki-67 LI was defined as the percentage of immunoreactive tumor cell nuclei among the total number of cells.

Statistical Analysis

All information collected in this study was recorded and analysed using SPSS 17 version software. These values were then compared with the Fisher exact test for proportions (p value), set to a 95% confidence interval.

Observations and results

In the present study a total of 54 cases havebeen diagnosed asgliomas (6 pilocytic astrocytomas, 19 diffuse astrocytomas, 2 gemistocytica strocytomas,1 oligoastrocytoma, 3 anaplastic astrocytomas, 1 anaplastic pleomorphic xanthoastrocytoma,5 oligodendrogliomas, 4 ependymomas and 13 Glioblastomas,with a male:female ratio of 1.25:1.

Table 1: Distribution of glial tumors

Pilocytic astrocytoma	6 (11%)
Diffuse astrocytoma	19(35%)
Gemistocytic astrocytoma	02 (4%)
Oligoastrocytoma	01 (2%)
Anaplastic astrocytoma	03 (6%)
Anaplastic PXA	01 (2%)
Glioblastoma	13 (24%)
Oligodendroglioma	05 (9%)
Ependymoma	04 (7%)

Table 2: Sex incidence and association with IDH-1 expression

Sex	No. of Cases	IDH-1 POSITIVE	IDH-1 NEGATIVE	P VALUE
Male	30(56 %)	20	10	0.06
Female	24 (44 %)	09	15	

In this study males were more common than females and showed higher IDH-1 expression, however this difference was statistically insignificant.Male : Female – 1.25:1.

Table 3: Age related association of IDH-1 expression of glial tumors

Age group	Total	IDH-1 POSITIVE	IDH-1 NEGATIVE	P Value
<16 years	13	05	08	0.205
>16 years	41	24	17	

The IDH-1 expression was more commonly seen in adults than in children, however thiswas stastically not significant.

Table 4: Age related incidence of glial tumors

< 15 years	13 (24%)
16-30 years	10 (18%)
31-45 years	17 (31%)*
46-60 years	06 (12%)
>60 years	08 (15%)
Total	54

Table 5: Incidence of presenting symptoms and their associationwith IDH-1 expression

Presenting symptom	Total	IDH-1 POSITIVE	IDH-1 NEGATIVE	P Value
Seizures	26 (48%)	20	06	0.0009*
Headache	15 (29%)	05	10	
Weakness	05 (9%)	02	03	
Ataxia	03 (5%)	01	02	
Hemiparaesis	02 (3%)	01	01	
Altered sensorium	01 (2%)	-	01	
Bowel and Bladder incontinence	01 (2%)	-	01	
Back pain	01 (2%)	-	01	

Out of 54 cases the most common presenting symptom was seizures (48%) followed by headache (29%) and weakness in lower limbs (9%).The other less common symptoms include ataxia, hemiparaesis, altered sensorium.

The history of seizures was associated with IDH-1 expression with a statistically significant p value.

Table 6: Site wise distribution and association with IDH-1 mutations

Site	Total	IDH-1 POSITIVE	IDH-1 NEGATIVE	P Value
Frontal/Frontoparietal	27 (50%)	18	09	0.02*
Temporal/Temporoparietal	10 (19%)	05	05	
Occipital	02 (4%)	01	01	
Intraventricular	04 (7%)	01	03	
Intramedullary	03 (5%)	-	03	
Cerebellar	03 (5%)	02	01	
Others	05 (10%)	02	03	

The most common location for astrocytomas and oligodendroglioma was frontal/fronto parietal (50%) and has association with IDH-1 expression with significant p value.

The ependymal tumours were predominantly seen in the intraventricular region.

Table 7: Showing association of IDH-1 expression in different WHO grades

WHO GRADE	NO.OF CASES	IDH-1 POSITIVE	IDH-1 NEGATIVE	P VALUE
I	06	-	06	
II	31	21	10	
III	04	02	02	P-001*
IV	13	06	07	
TOTAL	54	29	25	

In the present study IDH-1 mutations were most commonly seen in WHO grade II-IIIwith significant p value.

Table 8: Showing association IDH-1 expression in different histological types

HISTO LOGICALTYPE	NO.OFCASES	IDH-1 POSITIVE	%	IDH-1 NEGATIVE	%
Pilocytic Astrocytoma	06	-	-	06	100
Diffuse Astrocytoma	19	14	73	05	27
Gemistocytic Astrocytoma	02	02	100	-	-
Oligoastocytoma	01	01	100	-	-
Anaplastic-astrocytoma	03	01	33	02	67
Anaplastic Pilocyticxantho-astrocytoma	01	01	100	-	-
Oligodendro-glioma	05	03	60	02	40
Ependymoma	04	01	25	03	75
Glioblastoma	13	06	46	07	54

IDH-1 mutations in this study were most commonly observed in oligoastrocytic tumors, gemistocytic astrocytomas, anaplastic PXA,diffuse astrocytomIc tumours ,least in ependymoma and all cases of pilocytic astrocytomas were IDH-1 negative.

Table 9: Relationship between Ki-67 Labelling index with WHO Grade of Gliomas and IDH-1 expression

Grade	HistomorphologicalDiagnosis	No. of Cases	IDH+	Median KI-67 LI	IDH-	MedianKI-67 LI
Grade 1	Pilocytic Astrocytoma	6	-	-	6	1.5
Grade 2	Diffuse Astrocytoma	19	14	3.5	05	4.0
	Oligodendroglioma	5	03	5.5	02	5.5
	Oligoastrocytoma	1	01	3	-	-
	GemistocyticAstrocytoma	2	2	4	-	-
	Ependymoma	4	1	12	03	12
Grade 3	Anaplastic astrocytoma	3	01	23	02	25
	Anaplastic PXA	1	01	12	-	-
Grade 4	Glioblastoma	13	06	26	07	28

The table shows Ki 67 LI range in each grade of glioma and their respective median Ki67 LI. The median Ki 67 LI increases in correlation with the grade of the tumour and slightly higher in cases with IDH negative expression.

P value has been calculated between Ki 67 Labelling index and grade of glioma.P value of < 0.05 is considered significant.

Table 10: p value between Ki 67 LI versus grade 1 and grade 2 gliomas

	Grade 1	Grade 2	p value
Ki-67 <= 6%	**02**	**21**	**0.25**
Ki-67 >6%	**04**	**10**	

The relationship between grade 1 and grade 2 gliomas is not significant.

Table 11: p value between Ki 67 LI versus grade 2 and grade 3 gliomas

	Grade 2	Grade 3	p value
Ki-67 <= 6%	21	01	0.09
Ki-67 >6%	10	03	

The relationship between grade 2 and grade 3 gliomas is not significant.

Table 12: p value between Ki 67 LI versus grade 3 and grade 4 gliomas

	Grade 3	Grade 4	p value
Ki-67 <= 6%	01	02	0.194
Ki-67 >6%	03	11	

The relationship between grade 3 and grade 4 gliomas is not significant.

Table 13: p value between Ki 67 LI versus grade 2 and grade 4 gliomas

	Grade 2	Grade 4	p value
Ki-67 <= 6%	21	02	0.0015
Ki-67 >6%	10	11	

The relationship between grade 2 and grade 4 gliomas is significant.

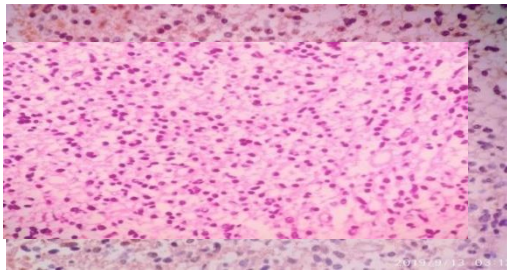
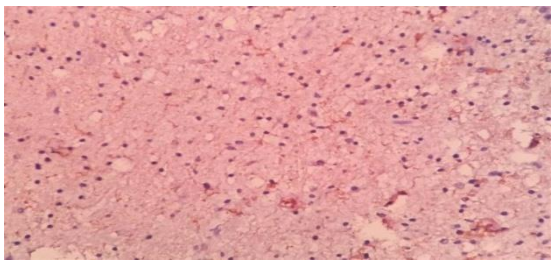


Fig 1: Pilocytic astrocytoma with IDH1 negative expression with Low Ki 67 LI(40X)

IDH-1 NEGATIVE



Low Ki-67 LI

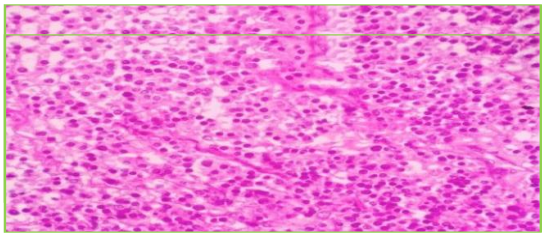
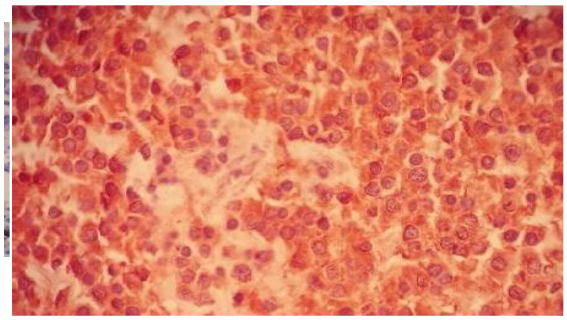


Fig 2 : Oligodendroglioma with IDH-1expression and Ki 67 LI

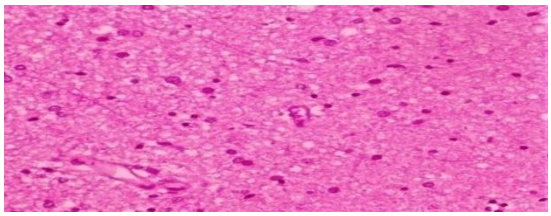
(40x) Fried egg appearance

IDH-1 POSITIVE



Low Ki-67 LI

Fig 3: Diffuse Astrocytoma with IDH-1 Positive and low Ki-67 LI



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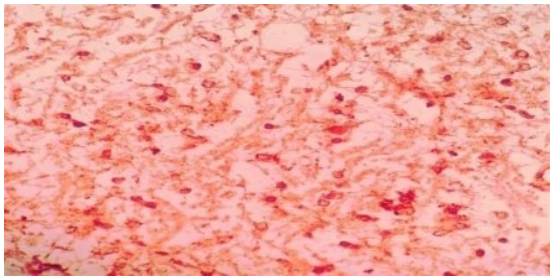
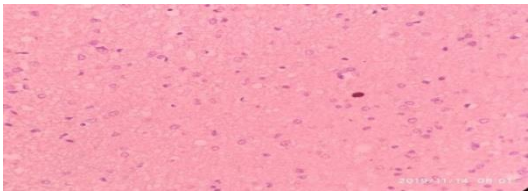
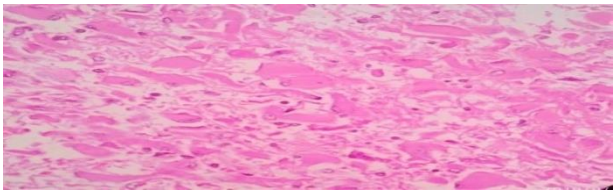


IDH-1 POSITIVE



Low Ki-67 LI

Fig 4: Gemistocytic astrocytoma with IDH-1 Positive expression and Ki 67 LI



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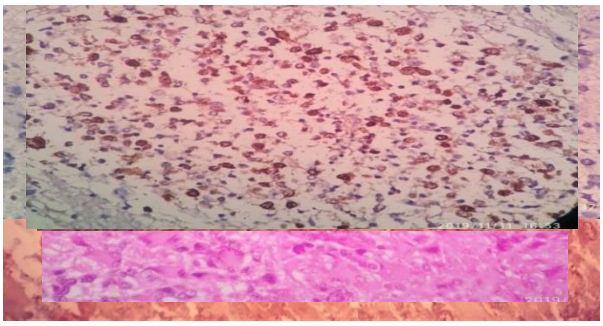
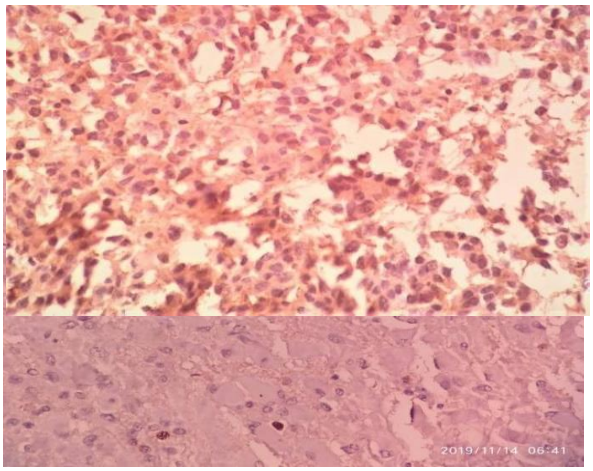


IDH-1 POSITIVE

Low Ki-67 LI

Fig 5: Ependymoma with IDH-1 Positive and low ki-67 LI

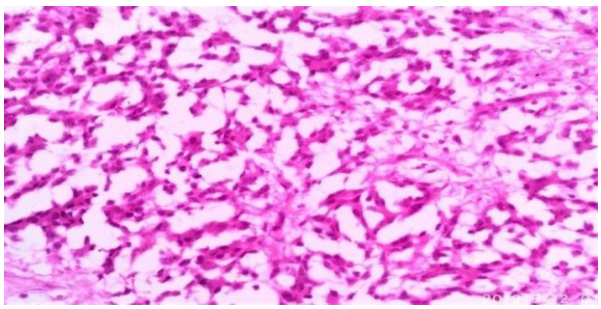
(40x) Ependymal rosettes



IDH-1 POSITIVE

Low Ki-67 LI

Fig 6: Anaplastic Astrocytoma with IDH-1 positive expression and Ki 67 LI

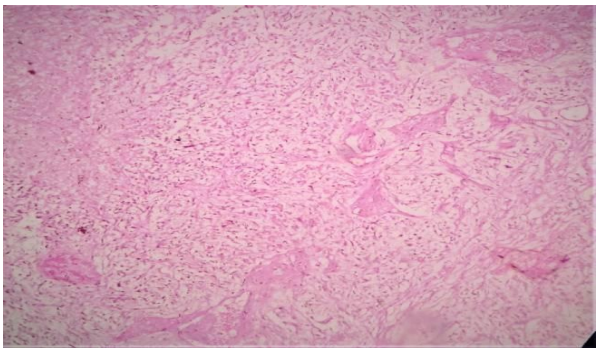


(40x)

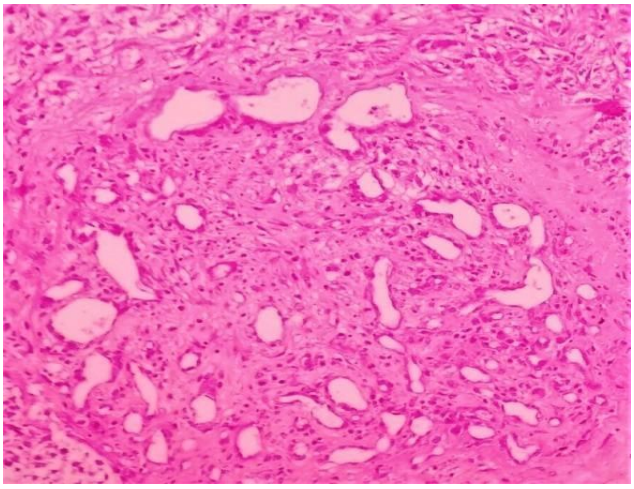
IDH-1 POSITIVE

High Ki-67 LI

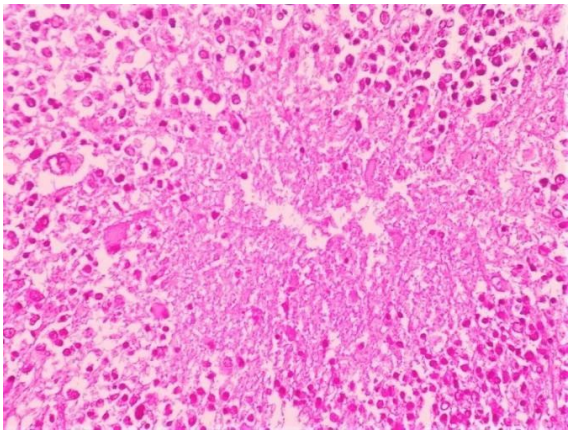
Figure 7: Glioblastoma with characteristic histological features



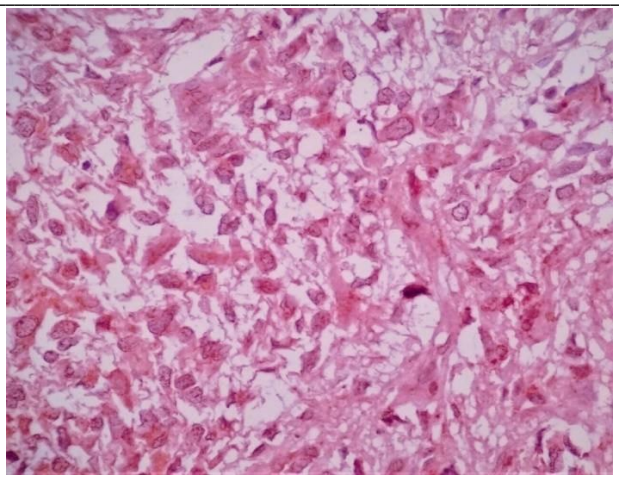
(10x)Glomeruloid type of blood vessels



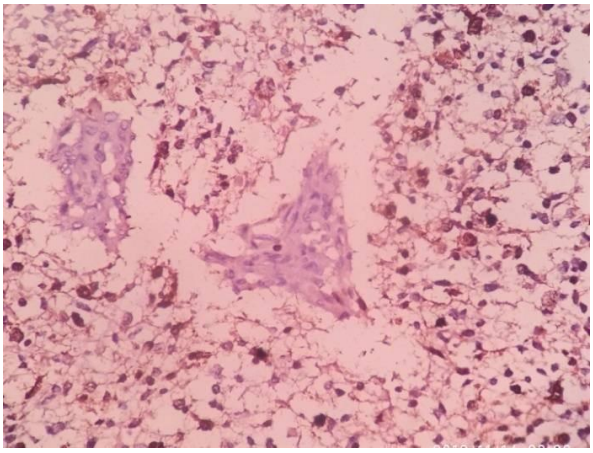
(40x) with cystic change



Pseudo palisading necrosis



IDH-1 POSITIVE



High Ki-67 LI

Discussion

Conventional H and E staining is crucial for diagnostic neuropathology. The art of making a diagnosis in the practice of oncology has moved ahead from microscopic evaluation of the H and E stained slides, to the present era of the application of ancillary techniques in the form of immunohistochemistry and techniques of molecular biology.The use of these adjuvant modalities have not only helped in better understanding of the biological behaviour of malignancies, but have also opened doors to the development of specific targeted therapies.Histological grading of gliomas gives a powerful estimation of the biological behaviour of the tumour and forms the basis for planning adjuvant therapies after surgical resection.

WHO grade I is applied to tumours,which are clearly circum-scribed, benign in their behaviour and can be cured following total surgical resection.

WHO grade II tumours are slowly progressing, low grade malignant tumours that exhibit cellular atypia without extensive proliferation or anaplasia. Typical survival for grade II neoplasms is over 5 years.

WHO grade III tumours show increased cellularity, anaplasia and mitotic figures. Survival for grade III tumours is 2-3 years.

In grade IV tumours the histological hallmarks are angiogenesis and necrosis in addition to grade III features.

Angiogenesis

Glioblastomas are highly vascular tumours and represent the most angiogenetic entity of all solid tumours. From a diagnostic point of view, microvascular proliferation is the essential feature of glioblastomas.[3]

The glioma angiogenesis is driven by a number of molecular pathways, the angiogenic process is complex and dynamic, and different mechanisms can act simultaneously. In addition to the classical concept of neoangiogenesis, involving sprouting and growth of new capillary vessels from pre-existing vessels stimulated by hypoxia-induced growth factors such as VEGF, glioblastoma cells adopt pre-existing vessels and migrate to them.

Necrosis

Another essential feature of glioblastoma is the presence of necrosis, which is commonly used as a differential diagnostic criterion when tumours are evaluated eitherby neuro imagining or microscopically.

Idh mutations in gliomas

Isocitrate dehydrogenases (IDHs) enzymes are main component of the tricarboxylic acid (TCA) cycle that convert isocitrate to α-ketoglutarate (α-KG) with production of NADH and/or NADPH. However, IDH gene was considered as “housekeeping” gene by cancer biologists with previously no defined role in cancer . The exact mechanism of how these IDH mutations contribute to oncogenesis is still unclear. One hypothesis postulates IDH-1 mutations leds to conversion of α-ketoglutarate to 2-hydroxyglutarate, which invariably blocks many enzymes, thereby contributing to tumor development [4].

Various methods are applied for the detection of these IDH-1 and/ or IDH-2 mutations in gliomas like single-strand conformation polymorphism analysis, direct sequencing, , DNA pyrosequencing, PCR-restriction fragment length polymorphism-based assays, and real-time PCR with post-PCR fluorescence melting curve analysis

assays. Especially, the later assays are rapid, inexpensive, and sensitive analysis of IDH-1 and IDH-2 mutations in routinely processed formalin-fixed, paraffin-embedded tumor tissues, even in samples which have low tumor cell content.

Age and Sex distribution

The result of the data analysed in the present study, showed male predominance with a male : female ratio of 1.25:1 and is in agreement with a study by Ganju et al.[11]

In the present study a higher degree of IDH-1 expression in adults (60%) than in children (35%), however, this difference was statistically not significant. Similar in studies conducted by Pollack [12] and Bleeker [13]. However, Antonelli [14] and Buccaliero [15] described absent IDH-1 expression in pediatric age group in their studies. This differential expression of IDH-1 among different age group suggests existence of various age related pathways of tumorigenesis.

Among 54 patients in the present study, age ranges show significant variations; similar to studies conducted by Whitton and Bloom and Jaskơlsky et al.[16]

Inskip et al.[17]described that IDH-1 mutation is more common in gliomas located in frontal lobe [18,19]. It may be because of the fact that frontal lobe being the largest lobe, is the commonest site of gliomas. This finding was noted in the present study also which showed statistically significant association between frontal lobe location and IDH-1 expression (P=0.0236).

In the present study, pilocytic astrocytoma (Grade I astrocytoma) occurred typically in the cerebellum and brainstem in children which was in concordance with studies done by Burkhard et al.[20]

Similar to present study, Simpson et al. observed majority of glioblastomas in the frontal lobe, similar to present study[21].

Presenting Symptoms

In present study, noted that history of seizure (48%) is significantly associated with IDH-1 expression. Similar result was also observed by Stockhammer who postulated that it may be due to altered metabolic profile of IDH-1 mutated cells which have become epileptogenic [22].

The other symptoms include headache, weakness in lower limbs, ataxia, hemiparaesis and altered sensorium.IDH-1 expression was significantly higher in WHO grade II and III tumors in our study (P=0.001). Similar observations were also described by other authors [23]. Our results showed that all of our oligoastrocytomas , Gemistocytic Astrocytoma and Anaplastic Pilocytic xantho-astrocytoma(100%) showed IDH-1 expression followed by diffuse astrocytic tumor (73%) ,oligodendroglial tumors (60%), and ependymal tumor (25%) and all cases of pilocytic astrocytomas did not show IDH-1 expression. This finding was in concordance with the various other studies[24,25].However, few other studies have observed almost equal frequency of IDH-1 expression in astrocytic and oligodendroglial tumors[26,27]. This discrepancy in results may be due to difference in study cohorts in terms of their histological types and grades of tumor.Presence of higher degree of IDH-1 mutation in oligodendroglial, oligoastrocytic and astrocytic tumors, as shown in our study, suggest that probably these tumors have a common glial origin.

It has been studied that IDH-1 mutation does not exist in reactive conditions related to cerebral ischemia or infarctions, viral infections, or radiation change [28]. These findings are of particular diagnostic value because they enable the distinction of reactive gliosis from low-grade diffuse astrocytoma, a diagnostically challenging task, especially in the context of small biopsy samples.

Based on pooled results from multiple studies on IDH mutated gliomas, it has been noted that IDH-1 is affected 96% of the times and IDH-2 is affected in only 4% of cases [29]. Many studies have shown that IDH-1 and IDH-2 mutations are associated with a favorable prognosis and the survival of patients with the mutant form of IDH-1 in astrocytomas or oligodendrogliomass (WHO grade II-III) and glioblastomas is longer than that of their IDH-1 wild-type counterparts [30,31]. However, due to poor follow up data of patients in the present study survival details could not be analalysed.

Ki- 67

Astrocytic tumors have an inherited tendency to progress and recur. The accurate grading of astrocytic tumors is of prime importance because it is critical to the patient management, survival and outcome. As the expression of the Ki-67 antigen changes during the cell cycle [32] ,the intensity of nuclear staining will vary; principally, all types of staining should be regarded as positive.[33]

Counting can be done manually or by digitalized image analysis systems, but manual counting has turned out to be applicable for most diagnostic purposes.[34] Defining a cut- off value is also a topic of interest due to its impact on the determination of patients classified as "high Ki-67", which is indicative of a poorer outcome.

In our material we found that the Ki-67 LIs correlated significantly with increasing tumor grade in all types of gliomas but an overlap occurred between the malignancy groups.

The positive correlations between Ki-67 LI and tumor grade in our series of gliomas are in agreement with the literature.[35]

In the present study, Ki 67 LI showed a range of 1 – 13% in pilocytic astrocytoma with a median of 1.5%.

In case of diffuse astrocytoma Ki 67 LI ranged from 1-20% with a median of 3.5%.

In anaplastic astrocytoma, median Ki 67 LI is 23% whereas glioblastoma had a labelling index of 26%.

Oligodengrogliomas showed a median labelling index of 5.5% and ependymomas showed a median of 12%.The present study had 1 case of anaplastic pleomorphic xanthoastrocytoma which showed Ki 67 Li of 12% .

We found that indices were comparable between gliomas of similar malignancy grade, and indices for high-grade gliomas (grade III/IV) were significantly higher than in low- grade (grade I/II) tumors.

In the present study, the p value between grade 1 and grade 2 tumours was 0.7766 which is not statistically significant.

The p value between grade 2 and grade 3 tumours was 0.0025 and between grade 2 and grade 4 tumours was 0.09 which is not statistically significance.

The present study, could not show any statistical significance between grade 3 and grade 4 tumours with a p value of 0.1946.

The p value between between grade 2 and grade 4 tumours was 0.0015 which is was statistically significant.

Table 14:Comparison of various variables of present study with that of literature

VARIABLE	PRESENT STUDY IDH 1 Expression	OTHER STUDIES
Age	Adults > children;p value :0.542	Pollack[12] &Bleeker[13]
Location	Frontoparietal, p=0.0238*	van den Bent MJ etal[18],Carrillo JA[19]
Clinical symptoms	Seizures,p=0.002*	Stockhammer[22]
WHO Grade	II-III, p=0.002*	Ichimura K et al23], Hartmann C et al[36]

Table 15: Comparison of IDH-1 mutations in various histological types ofpresent study with other studies .

STUDY	IDH-1 EXPRESSION IN GLIOMAS
Balss et al[8]	68% in DA, 69% in OLIG and 88% in sec- GB.
Ichimura et al.[23]	54% of DA and 65% of OLIG but in only 6% of GB (3% of prim-GB and 50% of sec-GB).
Nobusawa et al.[31]	36/407 (8.8%) of GB and that GB patients with IDH1 mutations were younger and were associated with a longersurvival.

Capper et al.[37]	44/53 (83%) DA, 9/10 (90%) OLIG, 0/21 PA, 5/7 (71%) sec-GB and 2/56 (3.6%) prim-GB.
Present study	14/19(73%) DA, 0/6 PA, 3/5(60%) OLIG, 1/4(25%) EPEN,6/13(46.1%) GB of which 1/6 (16.6%) prim-GB & 5/7(71.4.%) sec-GB.

Table 16: Comparison of Median Ki-67 LI of present study with other studies.

	kjulsvik et al[38]	hotakura M et al[39]	bise et al [40]	Present study in cases with IDH-1 expression
Median Ki 67 LI	Grade 1 – 1.9 Grade 2 – 4.5 Grade 3 – 14.5 Grade 4 – 19	Grade 1 – 0.2 Grade 2 – 06 Grade 3 – 32 Grade 4 – 40	Grade 1 – 2.4 Grade 2 – 2.3 Grade 3 – 5.3 Grade 4 – 9.8	Grade 1 – 1.5 Grade 2 – 3.5 Grade 3 – 23 Grade 4 – 26

Thus, Ki-67 is useful for differentiating between high and low-grade gliomas, but differentiating between grade I and grade II or grade III and grade IV is more problematic due to the overlap of values between the different tumor grades.

Pilocytic astrocytomas (Grade I) have distinct clinical, pathological, and prognostic characteristics when compared to diffuse astrocytomas (Grade II).[35]A very low LI for pilocytic astrocytomas was noted and all cases had negative IDH1 expression. No significant prognostic role has been observed for Ki-67 LI in pilocytic astrocytomas, and there is a limited role for Ki-67 LI in determining the diagnosis in pilocytic astrocytoma.[35]In the present study there were significant differences in Ki 67 LI between low grade (Grade II) and high grade (III-IV) gliomas and slightly higher median ki-67 index was observed in IDH1 negative cases which is in agreement with most of the other studies.

Table 17: Comparison of median Ki 67 LI between different grades of gliomas ofpresent study with other studies

Authors	Number of Cases	Median Ki 67 LI
Ralte et al.[40]	Grade II – 30 Grade III - 11Grade IV – 15	Grade II – 3.73 Grade III – 9.65 Grade IV – 10.33
Torp et al.[41]	Grade II – 22Grade III - 10 Grade IV – 09	Grade II – 2.7 Grade III – 13.9 Grade IV – 12.1
Wakimoto et al.[42]	Grade II – 19 Grade III - 25Grade IV – 28	Grade II – 3.8 Grade III – 18.4Grade IV – 31.6
Present Study	Grade II – 31Grade III - 4 Grade IV – 13	Grade II – 4.2Grade III – 23 Grade IV – 26

Although few studies have found a significant difference in Ki-67 LI between Grade III and Grade IV astrocytomas [43] ,majority of them including the present study could not find a significant difference between them.

The variations in the Ki-67 LI in various studies can be attributed to many factors such as fixative used, immunohistochemical procedures, especially antigen retrieval and interpretation of immunostaining [44].

A low Ki-67 LI in high-grade astrocytoma could result from faulty sampling techniques and heterogeneity of the tumor. Retrieval of antigen can be better with hydrated autoclave treatment than with microwave treatment, which can result in higher Ki-67 LI possibly resulting from successful denaturation of formalin-fixed antigens [44].

Ki-67 immunostaining to distinguish gliosis and low-grade gliomas should be interpreted with caution[34]. Normally, reactive astrocytes do not exhibit proliferative activity, but in some non-neoplastic conditions reactive astrocytes may have aroliferation rate of 1-5%.[45].

In such cases, immunohistochemical analyses for mutated p53 and isocitrate dehydrogenase (IDH) proteins can be useful, though p53 immunoreactivity may occur in both settings, and there are gliomas without IDH mutation [46].

The procedure for Ki-67 immunostaining is not standardized and has various analytical and clinical elements of uncertainty. [47]

Nevertheless, the method is regarded as being robust ,[33] which is also in accordance with our experience during several years with both clinical and experimental use.

The recommended fixative is buffered formalin, and storage time, delay in fixation and fixation time does not seem to substantially affect the staining results. [32]

Loss of immunoreactivity has been described if cut sections are exposed to room air for some months.[10] A prerequisite for satisfactory immunostaining is adequate antigen retrieval.[32] Though Ki 67 LI has a few limitations, it serves as a useful supplement to the histopathological diagnosis of human gliomas.

Conclusion

Monoclonal antibody to IDH1 (R132) combined with Ki-67 LI are useful and less-labor-intensive method to detect mutations in gliomas, to differentiate reactive gliosis from low-grade astrocytoma, and categorise gliomas into clinically meaningful and prognostically distinct subgroups.

IDH-1 mutations were expressed mostly in diffuse astrocytomas followed by oligodendroglial tumors, glioblastomas and least expressed in ependymal tumorsand absent in pilocytic

astrocytomas.In this present study IDH-1 expression were most common in WHO grade II and III gliomas and rare in grade I gliomas. Median Ki-67 LI in IDH-1 negative cases showed slightly higher values when compared with IDH-1 mutant variants of similar grade indicating poorer prognosis.

IDH-1 mutations in gliomas has potential as an independent prognostic marker and also helps in distinguishing primary from secondary GB & its potential for new therapies, testing for IDH1 mutations can enhance accuracyof glioma diagnosis and treatment.

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