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Audit of histomorphology and immunohistochemistry of the brain tumors: Revisited in context to the WHO 2016 molecular classification

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ABSTRACT

Background: The WHO 2016 molecular classification corroborating with the histology has given more significant diagnostic objectivity to the diagnosis of brain tumors and it is more reliable for instituting therapy as the heterogeneity and observer subjectivity are bypassed with the addition of isocitrate dehydrogenase, ATRX, and 1p19q, and other molecular markers. **Aim:** Our aim is to review the histopathology of diagnosed brain tumors and correlate with immunohistochemical (IHC) findings to note for any disparity to reform the diagnosis in order to benefit the patient and report to the clinician if any treatment change is to be considered. **Materials and Methods:** This article is based on studies of screening and diagnostic test. A total of 150 brain tumors were retrospectively analyzed. Age, gender, and the tumor histological type and grade were systematically recorded. We compared our histopathological diagnosis before the introduction of the WHO 2016 molecular classification of central nervous system tumors and later after the relevant IHC and fluorescence *in situ* hybridization studies. **Statistical Analysis:** The statistical analysis was done by using Statistical Package for Social Sciences version recent for Windows. **Results:** Out of the total 150 brain tumor patients, 65 were males and 45 were females. About 37 were glial and the rest were in other categories. **Conclusions:** The molecular diagnosis that substantiated with the histomorphology is more objective and beneficial in the treatment of the patients.

Key words: 1p19q, ATRX, Ependymoma, Glioblastoma multiforme, Isocitrate dehydrogenase

Introduction

Brain tumors show genetic heterogeneity, but in spite of that the histopathology corroborating with the characteristic radiology and immunohistochemistry (IHC) to substantiate or confirm diagnosis and MIB1/Ki67 for grading of tumors was considered to be the gold standard in the diagnosis of these tumors. However, the 2016 WHO classification of brain tumors[1] uses molecular parameters in addition to histology with restructuring and imparting greater diagnostic objectivity, especially in cases of glial tumors, which had observer variance like the diffuse gliomas which are now classified as diffuse and anaplastic astrocytoma and glioblastoma isocitrate dehydrogenase (IDH) mutant, wild, and not otherwise specified (NOS) types corresponding to Grade II, III, IV of earlier classification, epithelioid glioblastoma, glioblastoma with primitive neuronal component diffuse midline glioma H3K27M mutant which occurs in children and was previously called as diffuse intrinsic pontine glioma.

Oligodendroglioma including anaplastic variety IDH mutant, ATRX mutant and 1p19q intact and another type is IDH mutant, ATRX wild and 1p19q co-deleted and NOS, RELA fusion positive ependymomas, medulloblastomas WNT activated, sonic hedgehog (SHH) activated and TP53 wild type and non WNT and non SHH and these are all genetically defined and other medulloblastoma group is histologically defined.

Addition of embryonal tumor multilayered rosettes C19MC altered, embryonal tumor with multilayered rosettes NOS, diffuse leptomeningeal glioneuronal tumors, anaplastic pleomorphic xanthoastrocytoma, and deleting some tumors such as primitive neuroectodermal tumors, gliomatosis cerebri, protoplasmic and fibrillary astrocytoma, cellular ependymoma. Another feature is the addition of brain invasion as a criterion for atypical meningiomas and introduction of grading system I, II, and III for hemangiopericytomas.

A total of 150 brain tumors were retrospectively analyzed in this study. The histopathology was correlated with IHC findings to note the difference in result and correlate the histology with IHC, IDH and ATRX and 1p19 q by fluorescence *in situ* hybridization (FISH) in astrocytomas and oligodendrogliomas as elucidated earlier as IDH mutant then tested for 1p19q codeletion. Out of total 150 patients, 65 were males and 45 were females. Out of the 150 brain tumors the pre WHO 2016 diagnosis rendered were, 37 glial tumors in which there were 05 Grade 1 astrocytoma out which one was protoplasmic astrocytoma, 01 Grade 2 astrocytoma 05 anaplastic astrocytoma. 02 oligodendroglioma, 30 glioblastoma multiforme and one was gemistocytic glioblastoma, 02 mediastinal seminoma in

young males of average age 25 years, 46 meningiomas of which 30 were transitional type and 20 fibroblastic, 19 pituitary adenomas, 01 mediastinal germ cell tumor, 10 ependymomas of which 01 was myxopapillary type and 01 anaplastic type, 06 hemangioblastomas, 03 medulloblastoma, 01 atypical teratoid rhabdoid tumor (ATRT), 10 craniopharyngiomas, 02 cavernous angiomas, 05 neurocytomas, 01 adenocarcinoma deposits, 01 case of tuberculosis. IHC with relevant antibody markers (IDH, ATRX for glial tumors), FISH 1p19q, WNT, SHH, T53 were performed for confirmation of diagnosis, differentiation of the tumors and MIB1 for grading of tumors.

Materials and Methods

This is a study of diagnostic test, a total of 150 brain tumors were retrospectively analyzed. Age, gender, and the tumor histological type and grade were systematically recorded. The histopathology slides stained with hematoxylin and eosin were reviewed by authors and correlated with IHC findings to note the difference in results. In glial tumors, glial fibrillary acidic protein (GFAP), IDH 1 and 2, ATRX, epidermal growth factor receptor (EGFR), IDH 1 and PHH3 and MIB1 were done to assess the proliferating potential and mitotic index for grading the tumors into I–IV. In germ cell tumors, the IHC done was with CD30, CD117, and OCT3/4; in meningiomas, epithelial membrane antigen (EMA) and Ki67, ependymoma GFAP and EMA. In medulloblastomas, IHC done was synaptophysin and neurofilament protein, and in ATRT, INI1 was done. FISH was done with 1p19q for oligodendroglioma and WNT and SHH for medulloblastoma. The statistical analysis was done by using Statistical Package for Social Sciences version recent for Windows.

Results

Out of the total 150 brain tumor patients, (Table 1) 65 were males and 45 were females. About 37 were glial tumors in which there were 05 Grade 1 astrocytoma, 01 Grade 2 astrocytoma, 05 anaplastic astrocytoma, 02 oligodendroglioma, 24 glioblastoma multiforme and one was gemistocytic glioblastoma, 03 mediastinal seminoma in young males of average age 25 years, 46 meningiomas, of which 30 were transitional type and 20 fibroblastic, 19 pituitary adenomas, 10 ependymomas, of which 01 was myxopapillary type and 01 anaplastic type, 06 hemangioblastomas, 03 medulloblastoma, 01 ATRT, 10 craniopharyngiomas, 02 cavernous angiomas, 05 neurocytomas, 01 adenocarcinoma deposits, 01 tuberculosis. Astrocytic and ependymal tumors were common in male patients, with a ratio of 2:1 within the age range from 30 to 60 years, whereas meningiomas were common in female patients in the ratio of 3:1 within the age group of 20–40 years. The metastatic tumor adenocarcinoma was found in older age group of 62–64 years with total 05 cases, of which 03 were males and 02 were females. The primary site was delineated by IHC, and in males, the tumor was positive for thyroid transcription factor-1 in 02 cases, proving primary to be lungs and in one case gastrointestinal primary with CK20 and carcinoembryonic antigen positive. In 02 females, WT1 was positive, proving primary to be ovarian. The most common clinical presentation was headache and vomiting.

The radiology was suggestive of intracranial space-occupying lesions in all the cases. In one case, there was clinicoradiopathological discordance with the diagnosis of tuberculosis and with the presence of caseating necrosis and epithelioid cell granulomas; however, acid-fast bacilli were not demonstrated. GFAP was used for the confirmation of diagnosis of glial tumors in all 37 cases.

EGFR was used to detect mutations which were confirmed by IHC and IDH, ATRX in all cases of astrocytoma, oligodendroglioma, and glioblastoma multiforme. MIB1 and PHH3 for mitotic index are important in astrocytomas grading. Placental alkaline phosphatase, C-Kit (CD117), CD30, and OCT3/4 in combination were used for germ cell tumor differentiation.

IHC positive in ependymomas with GFAP, EMA and in medulloblastoma synaptophysin and neurofilament proteins were positive. Hemangioblastoma was differentiated from metastatic tumors from other site with the use of CD10, inhibin A, and EMA. Claudin and EMA were used in meningiomas for confirmation. Atypical teratoid/rhabdoid tumors (ATRTs) were confirmed with INI1.

Discussion

Recognition and grading of brain tumor morphology is important.[1,2] Colman *et al*.[3,4] studied PHH3 staining with MIB1 and found that the combination of both with regard to grade and prognosis was better which is concordant with our study, in which 36 glial tumors were positive for GFAP with PHH3 and corresponding Ki67 staining; however, the gemistocytic glioblastoma was negative for GFAP.

In our study, there was 1 case of germ cell tumor which was mediastinal germinoma. Germinomas are the most frequent germ cell tumor arising in the pineal and sellar regions. In germinoma, the morphology was classic of uniform population of large polygonal cells with pale-to-clear cytoplasm and central vesicular nucleus. IHC was positive for OCT4, and C-kit (CD117) was used later to substantiate the diagnosis of a germinoma. Takeshima *et al*.[5] reported that IHC, C-kit, CD30, and OCT4 were immunoreactive in germ cell tumors. Cheng *et al*. studied that OCT4 was positive in ovarian dysgerminomas (germ cell tumors) while negative in non-dysgerminomatous tumors.

In our series of central nervous system (CNS) tumors, there were 6 cases of hemangioblastomas which were diagnosed on the basis of histomorphology and then substantiated with IHC,

Table 1: The audit of 150 brain lesions and change of diagnosis as per the WHO 2016 molecular classification (*N*=150)

Brain SOL *N*=150 subtyping	Number of cases (total 150)	Males 65	Mean age (years)	Females 45	Mean age years	Microscopy	Grade of tumor	IHC and FISH (1p19q)	Change in diagnosis
Pilocytic astrocytoma	05	04	01	-		Bipolar cells with long pilocytic (hair like) processes	I	PHH3, MIB-1/KI-67, and p53	No change
Diffuse fibrillary	01	01	60	-		Astrocytic cells	II	IDH, ATRX GFAP, MIB1	Obsolete changed to astrocytoma NOS
Anaplastic astrocytoma	05	03	60	02	40	Astrocytic with cellular atypia	III	IDH, ATRX, GFAP, MIB1	Anaplastic astrocytoma NOS
Glioblastoma	26	20	65	06	45	Necrosis, mitosis increased, and vascular proliferation	IV	IDH, GFAP, MIB1	Glioblastoma NOS
Oligodendroglioma	02	02	60			Round, central nuclei and oval, water-clear cytoplasm, which has been likened to the fried egg with its yolk	I	IDH, 1p19q (FISH) KI67	Oligodendroglioma
Myxopapillary	01	01	10			Tumor cells around vessels with mucoid degeneration	I	S100, GFAP	No change
Anaplastic	01	-		01	05	Hypercellularity with nuclear hyperchromasia and/or nuclear pleomorphism Numerous mitoses seen throughout the lesion Microvascular proliferation Pseudo-palisading necrosis	III	S100, GFAP	No change
Ependymoma	10	08	08	02	10	Perivascular pseudorosettes (anuclear zones formed by radially arranged tumor cell processes surrounding central blood vessels)	I	S100, GFAP	No change
Central neurocytoma	05	03	40	02	42	Sheets of non-pleomorphic cells with modest cytoplasm and neutrophil	I	Synaptophysin, chromogranin, MIB1	No change
Medulloblastoma	03	02	10	01	08	Poor cellular differentiation, nuclear molding, and minimal indistinct cytoplasm	IV	GFAP, S-100 NSE neurofilament	01 WNT activated and 02 classic
ATRT	01			01	10	Large rhabdoid cells	IV	INI1	No change
Meningiomas	46	14	40	32	32	Syncytial or transitional or fibroblastic	Grade I, II	Phosphohistone-H3, MIB-1/KI-67, and claudin-1	05 atypical meningiomas due to brain invasion, 01 papillary aggressive and 01 lymphoplasmacytic type
Seminoma	03	03	20			Uniform cells, vesicular nuclei, prominent nucleoli		CD30, CD117	

(Contd...)

Table 1: *(Continued)*

Brain SOL *N*=150 subtyping	Number of cases (total 150)	Males 65	Mean age (years)	Females 45	Mean age years	Microscopy	Grade of tumor	IHC and FISH (1p19q)	Change in diagnosis
Craniopharyngioma	10	08	32	02	40	Papillae formed by nonkeratinizing squamous epithelium	Low grade	CK	
Pituitary adenomas	19	10	40	09	35	One cell type with lack of reticulin network		TSH, ACTH, PRL	
Cavernous angioma	02	02	34	-	-	Vascular channels		CD34, CD31	
Hemangioblastoma	06	04	40	02	32	Foamy cells with vasculature benign tumor		Inhibin, CD10	
CNS tuberculous	01	01	32			Epithelioid cell granulomas		Ziehl–Neelson stain	
Papillary adenocarcinoma	05	03	64	02	62	Papillary tumor	Mets	CEA, CK20, WT1	

NSE: Neuron-specific enolase, ATRT: Atypical teratoid rhabdoid tumor, PRL: Prolactin, ACTH: Adrenocorticotropic hormone, TSH: Thyrotropin, GFAP: Glial fibrillary acidic protein, CNS: Central nervous system, FISH: Fluorescence *in situ* hybridization, NOS: Not otherwise specified, IDH: Isocitrate dehydrogenase

CD10, EMA, and inhibin to rule out a metastatic carcinoma. This is important for treatment purposes. We had three cases of embryonal tumor, medulloblastomas, which on histopathology, were classic, densely cellular with closely packed primitive cells with fibrillated intercellular matrix, and on IHC were found to be positive in synaptophysin and neurofilament. This finding is consistent with other studies.[6,7]

In our series of 150 CNS tumors, we had one case of ATRTs in young child in the posterior fossa. Microscopically, the abundant eosinophilic cytoplasm of the tumor cells gives the rhabdoid appearance to this embryonal tumor. IHC with vimentin highlights the inclusions, and lack of immunostaining for BAF47 (INI1) is present. INI1 immunostaining indicated in histologically dubious cases.[8-10]

In our series, we had 10 cases of ependymomas which are ependymal tumors arising from neuroepithelium of ventricles[11] and mostly occur as posterior fossa tumors. Microscopically, ependymomas have round blue cells with individual processes in the perivascular pseudorosettes, IHC was positive for EMA and GFAP. Wolfsberger *et al*.[12] studied 103 cases of ependymoma and studied the prognostic and survival patterns which were not analyzed in our study.

Conclusion

Histomorphology is supposed to be the gold standard for the CNS tumors substantiated with IHC and molecular testing by FISH which becomes mandatory for more objective basis of diagnosis. IHC also helps in distinction between hemangioblastoma and metastatic renal cell carcinoma as the treatment and prognosis are different. INI1 is unique in its negative nuclear staining in ATRTs and can be used in indeterminate histological features or in small biopsies. PHH3 and MIB1 are mitosis-specific markers and can be used for predicting prognosis in meningiomas, astrocytoma, and ependymoma. IHC panel can substantiate histopathology to grade and prognosticate the brain tumors, and the molecular diagnosis substantiated with the histomorphology is more objective and beneficial in the treatment of the patients.

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