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Role of Immunohistochemistry in Diagnosis and Grading of Brain Tumors

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Abstract

Introduction: *Brain tumors are heterogenous group of neoplasms with annual incidence of 5 to 10 per 100000 population in India. Classification of tumors is an art of pathology to distinguish between benign and malignant lesions on histological ground. But histological diagnosis of brain tumors is not always straight forward due to not only overlap in morphological features among different categories of tumors but also due to divergent differentiation within the same tumor. So, application of immunohistochemical markers has become necessary for an exact diagnosis and subtyping.*

Materials and Methods: *The study was carried out in Department of Pathology, SMS Medical College, Jaipur (Rajasthan) from June 2016 to June 2017. Based on histopathological examination of Hematoxylin and Eosin stained sections, total of 226 brain tumors were diagnosed. Out of which, immunohistochemical markers were applied on 50 cases for accurate diagnosis and grading.*

Results: *In the present study, most common histological tumor type was astrocytoma followed by meningioma. Out of 226 cases, 176cases were diagnosed by histopathology alone while 50 cases were sent for IHC. Immunohistochemical staining was done on 42 problematic cases to reach the final diagnosis and on 36 cases for grading.*

Conclusion: *The present study show that histopathological examination is a vital tool for diagnosis and grading in most of the tumors but IHC play an important role in difficult cases where diagnosis and grading is not possible only on histological basis.*

Keywords: *Brain tumors, Diagnosis, Grading, Immunohistochemistry.*

Introduction

The annual incidence of brain tumors ranges from 10 to 17 per 100000 population and majority of these tumors are primary tumors, only one fourth to one half are metastatic.[1]About 10% primary brain tumors occurs in pediatric population with most frequent being astrocytomas, medulloblastomas.[2]

Histological diagnosis of brain tumors is not easy due to overlap in morphological features among different categories, divergent differentiation within the same tumor and non-neoplastic lesion can also mimic tumor.[3]Hence, in spite of clinical data, imaging techniques and preoperative findings, application of immune histochemical markers has become necessary for an exact diagnosis and subtyping. In last two decades

diagnostic neuropathology benefited by incorporation of immunohistochemistry (IHC).[4]

The principle of IHC was known since 1930s, but first IHC study was reported in1942. When Coons et al used FITC- labelled antibodies to identify pneumococcal antigen in infected tissue. The fundamental concept behind IHC is the demonstration of antigen within tissue sections by means of specific antibodies. Once Ag-Ab binding occurs, it is visible by light microscopy or flourochromes by ultraviolet light.[5]

Recently our knowledge regarding the genetics of central nervous system tumors has expanded, hence newer antibodies or molecular markers are continuously being developed, which can be used in IHC.[6] The approach to diagnosis relies principally on histological evaluation of H&E stained sections with incorporation of smear preparations, histochemical stains, electron microscopy, and immunohistochemichal preparation as supplemental aid in diagnosis.[7]So, the present study was planned to determine the proportion of occurrence of types of brain tumors and to access the efficacy and utility of immunohistochemistry in the diagnosis and grading of brain tumors.

Materials and Methods

It was a hospital based cross sectional observational study, carried out in Department of Pathology, SMS Medical College, Jaipur (Rajasthan) from June 2016 to June 2017 after obtaining approval from institutional ethics committee. Specimens of brain tumors were received from Department of Neurosurgery of our institute. Total of 226 specimens were received which were classified according to "WHO classification for Central Nervous System Tumors 2007". Demographic, clinical, imaging and histological data of all cases were noted in a predesigned and semi-structured performa.

The tissue was fixed in 10% formalin for histopathological examination. Then paraffin embedded blocks were made in the usual manner and thin sections of 5 microns were cut by using a microtome. Sections are stained by hematoxylin & eosin stains and histological and immunohistochemical analysis was performed to diagnose and classify various brain tumors according to the World Health Organization (2007). IHC was performed on problematic cases where final diagnosis was not given on H&E sections. Using 3µm thick sections on Poly-l-lysin coated slides, antigen retrieval was done using decloaking chamber in citrate buffer at PH 6. Required markers were used for antigen detection. Autolysed specimens were excluded.

Results

In the present study, total 226 brain tumors were diagnosed. Astrocytic tumors were found in highest frequency (42.48%) followed by meningioma (19.91%), schawannoma (7.52%) and oligodendroglial tumors (6.65%) [Table-1]. About 68.14% brain tumors were located in supratentorial region while only 31.86% were located in infratentorial region. The highest age incidence of all brain tumors were observed in age group 31-40 years (23.45%) followed by 41-50 years (19.47%), 21-30 years (15.93%) and 51-60 years (12.39%). Overall M:F ratio observed was 1.26:1.

Highest astrocytic tumors were observed in age group 21-30 years (21.88%) followed by 31-40 years (17.71%), 41-50 years (15.63%) and 51-60 years (14.58%). Maximum astrocytic tumors were of grade IV with M:F ratio 2:1. Highest oligodendroglioma were observed in age group 4th (33.33%) and 5th (53.33%) decade of age with M:F ratio of 2:1.

Out of 226 brain tuomrs, 3 cases of mixed glioma (all among males), 7 cases of ependymal tumors (M:F ratio 2:1), 2 cases of choroid plexus tumors (M:F ratio 1:1) and 2 cases of neuronal and mixed neuronal-glial tuomrs (M:F ratio 1:1) were found.

70%meduloblastoma were observed in 1st decade of life with male-female ratio of 1.5:1 while about 65% schawannoma were found among 4th and 5th decade of life with M:F ratio of 1.3:1. 45 meningeal tumors were observed making 19.91%

of brain tumors. Meningiomas were highest in 4th to 6th decade of life with M:F ratio of 1:2. The most common variant was transitional meningioma followed by meningothelial meningioma. Peak incidence of craniopharyngioma was observed in 1st decade of life (55.55%) with M:F ratio of 2:1.

Out of 226 cases, 176 cases were diagnosed histopathologically while immunohistochemistry was done for 42 problematic cases to reach the final diagnosis and on 36 cases for grading [Table-2].

Table-1: Frequency of brain tumors in our study

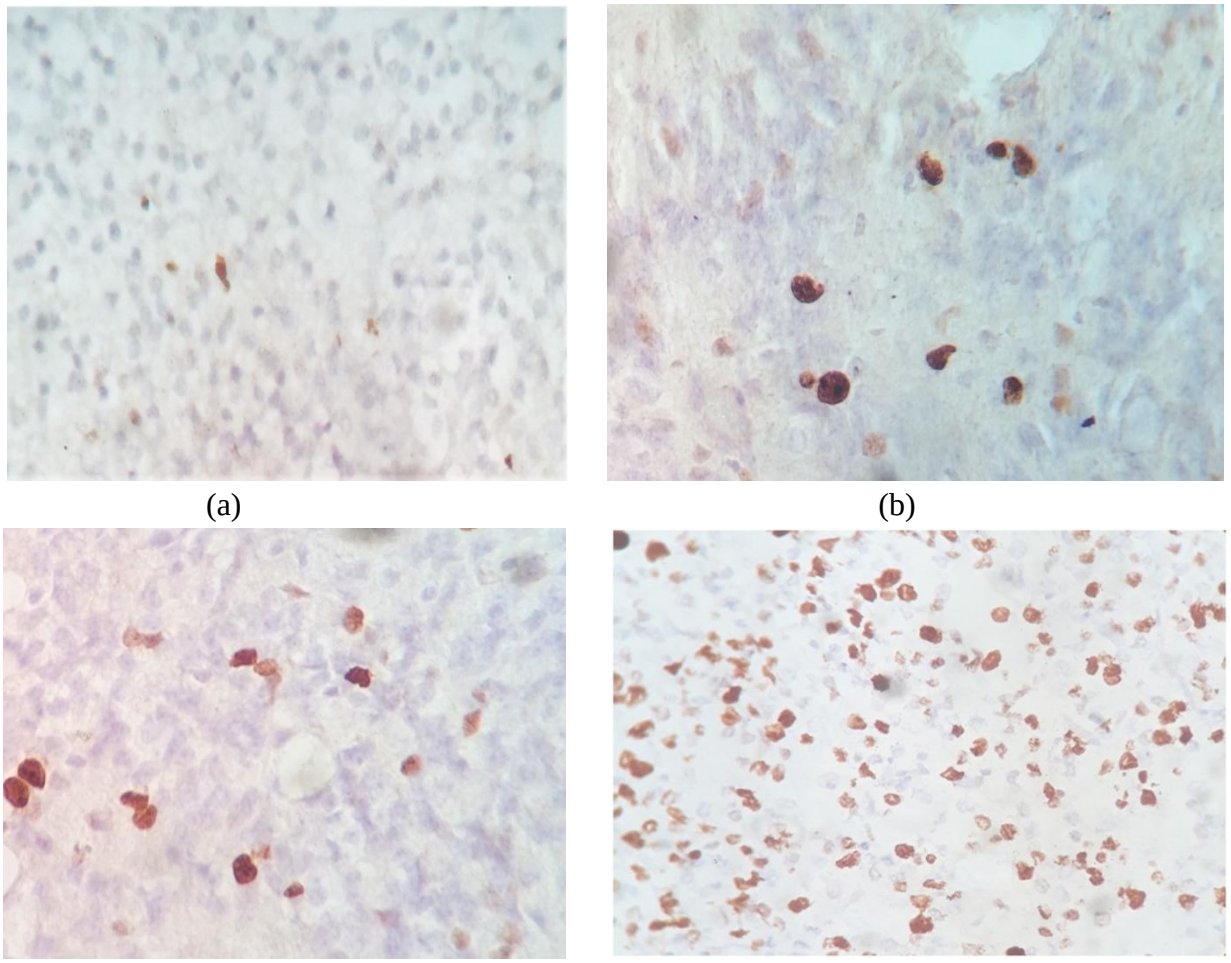
Brain tumors	No of tumors (n)	Percentage (%)
Astrocytic tumors	96	42.48
Oligodendroglial tumors	15	6.65
Oligoastrocytic tumors	3	1.33
Ependymal tumors	7	3.10
Choroid plexus tumors	2	0.88
Neuronal and mixed neuronal-glial tumors	2	0.88
Medulloblastoma	10	4.43
Schawannoma	17	7.52
Meningioma	45	19.91
Hemangioblastoma	2	0.88
Hemangiopericytoma	2	0.88
Lymphomas	6	2.66
Germ cell tumors	1	0.44
Craniopharyngioma	9	3.98
Metastatic tumors	9	3.98
TOTAL	**226**	**100**

Table-2: Immunochemical analysis brain tumors

Clinical data	Histopathological diagnosis	IHC Marker	Final Diagnosis
Male, 50 years, Left temporal mass	High grade astrocytoma	MIB 40%	Glioblastoma WHO GR IV
Male, 67 years, Left frontoparietal mass	High grade glioma	GFAP MIB 60%	Glioblastoma WHO GR IV
Male, 62 years, Left temporal mass	Astrocytoma	GFAP MIB 15%	Glioblastoma WHO GR IV
Male, 57 years, Left frontal mass	High grade astrocytoma	GFAP MIB15%	Glioblastoma WHO GR IV
Female, 65 years, Left frontal mass	Glioblastoma WHO GR IV	MIB 20%	Glioblastoma WHO GR IV
Female, 28 years, Corticomadullary junction mass	Pilocytic astrocytoma WHO GR I	GFAP MIB 3%	Pilocytic astrocytoma WHO GR I
Male, 40 years, Left frontal mass	Diffuse infiltrating astrocytoma WHO GR II	MIB 3%	Diffuse astroctoma WHO GR II
Male, 22 years, Left fronto temporal mass	Low grade astrocytoma	MIB 1%	Diffuse astrocytoma WHO GR II
Female, 12 years, Suprasellar mass	Low grade astrocytoma	GFAP MIB 1%	Diffuse astrocytoma WHO GR II
Male, 42 years, Cerebellar mass	Low grade astrocytoma	MIB 1%	Diifuse astrocytoma WHO GR II
Male, 42 years, Left temporal mass	Astrocytoma WHO GR IV	MIB 18%	GlioblastomaWHO GR IV
Male, 32 years, Left temporal mass	Glioblastoma	MIB 25%	Glioblastoma WHO GR IV
Female, 57 years, Corpus callosal mass	High grade astrocytoma	MIB 18%	Glioblastoma WHO GR IV
Male, 32 years, CP angle mass	High grade astrocytoma	GFAP MIB 8%	Astrocytoma WHO GR III
Female, 8 years, Suprasellar mass	High grade astrocytoma	GFAP MIB10%	Astrocytoma WHO GR III
Male, 16 years, Frontal mass	High grade astrocytoma	GFAP MIB40%	Glioblastoma WHO GR IV
Female, 21 years, Supra tentorial mass	Poorly differentiated malignant neoplasm	GFAP MIB1%	Pleomorphic xantho astrocytoma WHO GR II
Male, 22 years, temporal mass	Pleomorphic xantho astrocytoma	GFAP MIB<1%	Pleomorphic xantho astrocytoma WHO GR II
Male, 30 years, Left frontal mass	High garde astrocytoma	GFAP MIB10%	Astrocytoma WHO GR III
Female, 30 years, Lt frontal mass	Anaplastic oligodendro Glioma	GFAP MIB80%	Oligodendroglioma WHO GR III
Male, 35 years, Right frontal mass	Oligodendroglioma WHO GR II	GFAP MIB 3%	Oligoastro cytoma WHO GR II
Male, 40 years, Frontal mass	Oligodendroglioma WHO GR II	GFAP MIB 4%	Oligoastro cytoma WHO GR II
Male, 47 years, Left temporal mass	Oligoastro Cytoma	GFAP MIB 4%	Oigoastro cytoma WHO GR II

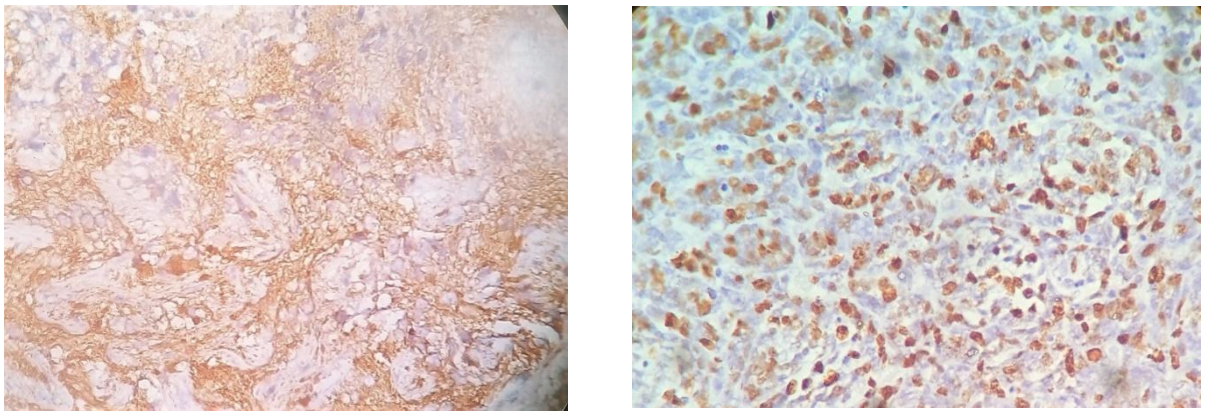
Male, 3 years, Post fossa mass	Ependymoma WHO GR II	EMA GFAP	Ependymoma WHO GR II
Female, 10 years, Left ventricular mass	Ependymoma	GFAP EMA MIB15%	Ependymoma WHO GR II
Male, 5 years, Right cerebellar mass	Neurocytoma	NeuN, Synapto physin, GFAP, MIB 2%	Neurocytoma
Female, 5 years, 4th ventrical mass	Medullo Blastoma	Synapto physin, MIB 60%	Medulloblastoma WHO GR IV
Male, 13 years, Post fossa mass	Medullo blastoma WHO,GR IV	Synapto physin, MIB 60%	Medulloblastoma WHO GR IV
Male, 40 years, Post fossa mass	Medullo blastoma grade WHO GR IV	Synapto physin, MIB 60%	Medulloblastoma WHO GR IV
Male, 10 years, 4th ventrical mass	Medullo Blastoma	Synapto physin, MIB 60%	Medulloblastoma WHO GR IV
Male, 5 years, Right cerebellar mass	Medullo Blastoma	Synapto physin, MIB 80%	Medulloblastoma WHO GR IV
Female, 6 years, Post fossa mass	Medullo blastoma with anaplastic foci IV	Synapto physin, MIB 40%	Medulloblastoma WHO GR IV
Female, 10 years, 4th ventrical mass	Medullo Blastoma	Vimentin, Synapto physin, MIB70%	Medulloblastoma WHO GR IV
Male, 57 years, Left frontal mass	Hemangio blastoma WHO GR I	EMA	Angio matousmenin gioma WHO GR I
Male, 15 years, Dorsal sellar mass	Transitional meningioma WHO GR I	S-00,EMA,	Transitional menin gioma WHO GR I
Female, 40 years, Right frontoparietal mass	Fibrous meningioma WHO GR I	Vimentin, EMA, MIB<1%	Fibroblastic meningioma WHO GR I
Male, 35 years, Right Para sagital mass	Meningo thelial meningioma	MIB 7%	Meningothelial Menin gioma WHO GR I
Male, 19 years, Right cerebellar mass	Hemangio blastoma WHO GR I	S-00,inhibin	Hemangio blastoma WHO GR I
Male, 44 years, Right temporal mass	Hemangio Pericytoma	CD34 Vimentin	Hemangio pericytoma
Female, 30 years, Infra temporal mass	Hemangio pericytoma	CD34 Vimentin	Hemangio pericytoma
Female, 34 years, Frontal mass	Round cell neoplasm	CD20 BCL2 BCL6	DLBCL
Female, 63 years, Right cerebellar mass	Round cell neoplasm	CD20 BCL 2 BCL 6	DLBCL
Male, 53 years, Right parietal mass	Malignant round cell neoplasm	CD20 BCL2 BCL6	DLBCL
Male, 40 years, Frontal mass	Round cell neoplasm	CD20 BCL 2 BCL 6	DLBCL
Male, 20 years, Post fossa mass	Round cell neoplasm	CD20 BCL6 MUM-1 MIB75%	DLBCL

Male, 50 years, Cerebellar mass	Round cell neoplasm	CD20 BCL6 PAX5	DLBCL
Female, 8 years, Suprasellar mass	Glial tissue with Atypical Cells	PLAP CD117	Germinoma
Male, 62 years, Right fronto temporal mass	Metastatic adeno Carcinoma	PSA AMACR	Metastasis from prostate
Male, 55 years, Right parietal mass	Metaststicadeno Carcinoma	TTF1 CD7 EMA	Metastasis from lung/ Thyroid
Female, 35 years, Suprasellar mass	Metaststicadeno Carcinoma	CK,CK7, TTF	Metastasis from lung



(a) (b) (c) (d)

Figure 1 (a): Shows MIB-1 positive nuclei in Astrocytoma Grade-I.Occasional nuclei are stained brown(40x), (b): Shows MIB-1 positive nuclei in Astrocytoma Grade-II. Nuclei are scattered, well defined and compactly stained (40x), (c): Shows MIB-1 positive nuclei in Astrocytoma Grade-III. Nuclei are large and pleomorphic(40x), (d): Shows MIB-I positive nuclei in Astrocytoma Grade-IV (40x)



(a) (b)

Figure 2 (a): Medulloblastoma-tissue section showing immunoreactivity for synaptophysin(40x), (b) Medulloblatoma-Tissue section showing high MIB-1 index(40x)

Discussion

A total 226 cases of brain tumors were studied. Out of 226 cases, 96 cases (42.48%) were astrocytoma followed by meningioma (19.91%) which is similar to the study done by Sajeeb Mondal et al (2016)[8], Khaled R Zalata et al (2011)[9] and M:F ratio of brain tumors was 1.26:1 which is consistent with Sajeeb Mondal et al (2016)[8]. In the present study, 68.14% tumors were supratentorial and 31.86% were infratentorial which is almost same as mentioned in Robin's pathologic basis of disease (J Neurosurg 21;201-206)[10]. Peak incidence of brain tumors were noted in fourth decade of life which is similar to Venugopal Madabhushi et al (2015)[11] whereas Sajeeb Mondal et al (2016)[8] noted peak incidence in 5th decade.

Among 96 cases (42.48%) of astrocytoma, grade IV astrocytoma was most common subtype which is consistent with Sajeeb Mondal et al (2016)[8]. All cases were located supratentorially which is consistent with David N Louis et al (2016)[12]. Oligodendroglioma comprised of 15 cases (6.64%) cases with M:F ratio 2:1. This is close to the result of Jayanti Mehta et al (2017)[13] who reported the incidence of oligodendroglioma as 7.9%, M:F ratio 2:1. Ependymoma comprised of 3.10% which is consistent with the study of Yong-Hyun Chai (2017)[14] (3–9%). Medulloblastoma constituted 4.43%% of all brain tumors which is agreeable to 4.4%incidence reported by Ahmed et al (2001)[15]. M:F ratio 1.5:1 was consistent with Khaled R Zalata et al (2011)[9] (M:F 1.77:1).

In present study schwannoma comprised 17 cases (7.52%) and M:F ratio was 1.12:1, most cases were seen in 4th and 5th decade. All these findings were consistent with David N Louis et al (2016)[12]. 45 cases (19.91%) of meningioma were found in present study and M:F ratio was 1:2, which is very much consistent with Bondy M et al (1996)[16]. Incidence of craniopharyngioma were 3.98% in the present study, which is consistent with Niki Karavitaki et al (2006)[17] (2-5%). About 2.66% cases of lymphomas were found which was consistent with the Uwe Schlegal et al 2009[18] (3%). 3.98% tumors were metastatic brain tumors, which was consistent with Kenneth E Livingston et al (1948)[19].

Out of 226 cases 50 cases were referred to IHC section for grading and diagnosis. For 19 cases of astrocytoma, immunohistochemistry was used. Final diagnosis was made by correlating histopathological and immune histochemical findings. MIB-LI was done on all 19 cases for grading and on 11 cases GFAP was also applied for confirmation of glial differentiation. On reviewing literatures it was found that GFAP marker define neoplastic astroglial differentiation (Fletcher DMCristopher 4thedition)[20]. MIB-LI in grade I (pilocytic astrocytoma) was typically<1% (David N Louis et al 2007)[21] but can vary from 0 to 3.9% (Giannini C et al 1999)[22] which was consistent with our study (MIB-L1 3%). MIB-LI

value varied from <1%-4% for grade II, 8%-10% for grade III, 15%-60% for grade IV astrocytoma which was consistent with the study of David N Louis et al (2016)[12] (grade II MIB-LI is upto 4%, for grade III 5-10% and for grade IV>10%) but MIB-LI has overlapped values so cannot be used alone as diagnostic factor, it should be used with combination of established histological criterias (Johannessen et al 2006)[23]. In one case of oligodendroglioma GFAP was positive and MIB-LI was 80%. Glial protein may be focally present in oligodendroglioma due to presence of mature reactive astrocytes. Minigemistocytes are also positive for GFAP and these are frequently present in anaplastic oligodendrogliomas (David N Louis et al 2007[21], Y Nakagawa et al 1986[24]). MIB-LI is usually >5% for anaplastic oligodendroglioma (David N Louis et al 2016[12]).

3 cases of oligoastrocytoma were positive for GFAP and MIB-LI value varied from 3-4%.GFAP is expressed in astroglial component and variably expressed by oligodendroglial component(David N Louis et al 2007[21] and Rama Goyal et al 2015[25]) and average value of MIB-LI is less than 6% for grade II(David N Louis et al 2016[12]). 2 cases of Ependymoma were positive for GFAP and EMA. In one case with high MIB-LI (15%), close follow up was advised. Ependymoma show immunoreactivity for GFAP and EMA (Kunishio K et al 1991[26]). MIB LI <4% has greater survival and >5% has poor survival (Prayson RA et al 1999)[27].One case of central neurocytoma was positive for synaptophysin, Neu N and GFAP and MIB-LI was 2%. Central neurocytomas express neuronal markers synaptophysin, Neu N and also GFAP (You H et al 2005)[28] MIB-L1 labeling index is usually low(< 2%) (Rades D et al 2004)[29]. 7 cases medulloblastomas showed positivity for synaptophysin, NSE, Neu N and vimentin. MIB labeling index was range from 40-80%. In 5 cases EMA and GFAP were applied to rule out ependymoma, which were negative. Medulloblastoma show immunoreactivity for synaptophysin, NSE, vimentin. MIB-LI is generally >20% (Schiffer D et al 1994[30]).

Out of 45 cases of meningioma only 4 cases were sent to IHC section. EMA, S-100, vimentin were positive in different cases. MIB was applied on 2 cases, values varied from <1% to 7%. Meningioma show EMA, vimentin and S-100 (JM Theaker et al 1986[31]) and MIB-LI >4% have increased recurrence rate (David N Louis et al 2016[12]). One case of hemangioblastoma was positive for inhibin and S-100. According to Shih Ming Jung et al (2005)[33] hemangioblastoma show immunoreactivity for inhibin whereas study of Frank TS et al (1989)[34] show that few cases may be immunoreactivity for S-100 and GFAP (negative in our case). 2 cases of hemangiopericytoma were positive for CD34 and vimentin which was consistent with the study of Middleton LP et al 1998[35]. 6 cases of round cell tumors were positive for CD20, Bcl-2, Bcl-6, MUM-1 and PAX-5 and reported as diffuse large B cell lymphoma. In two cases MIB-LI was 75%. According to Kristopher D M Fletcher 4thedition[20] B cell lineage is positive for CD 20and PAX-5.DLBCL show immunoreactivity for Bcl-6 (60%), Bcl-2 (50%), CD 10 (40%) and MUM-1. Mean MIB LI for DLBCL is 55%. One case of germ cell tumor was positive for PLAP and CD117. According to YupingGao et al (2002)[36] PLAP, CD117 were highly expressed in seminomas/ germinomas. Out of 3 cases of metastatic adenocarcinoma 1 case was positive for PSA and AMACR which was consistent with the study of Hameed et al (2005)[37] and another 2 cases were positive for EMA, TTF-1 and CK 7 which were consistent with Sushila Jaiswal 2016[6].

Conclusion

The present study showed that histopathological examination is vital tool for diagnosis and grading in most of the tumors but immunohistochemistry plays an important role in difficult cases where diagnosis and grading is not possible only on histological basis.

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