Original Article

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Glioblastoma Multiforme: A clinico-pathological analysis

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ABSTRACT

Background: Aim of the study was to classify Glioblastoma Multiforme (GBM) and its variants on histology and analyze their clinico-pathological features. Better understanding of the heterogeneous nature of GBM and its variants may provide improved treatment paradigms, prognostic classification and approaches towards molecular targeted treatments.

Methods: 40 cases of GBM were analyzed retrospectively and prospectively, covering a period of 8 years (January 2008- July2015). Cases were analyzed on the basis of clinical presentation, histopathological features and radiological reports.

Results: Of the 40 cases of GBM, the Conventional GBM, GBM with oligodendroglial component, Giant Cell GBM accounted for 26, 9 and 3 cases respectively. While there was 1 case each of Gliosarcoma and P-NET variants. Conventional GBM most commonly presented with Headache (69.2%) and paresis (57.7%) whereas GBMO patients presented with convulsions. On radiology, the variants of GBM cannot be distinguished as the findings were similar in all of them. Non-palisading necrosis was predominantly present in Conventional GBM (61.5%) and palisading necrosis in GBMO (66.7%).

Conclusion: Histopathology remains the main tool for differentiating the variants as radiological differentiation is not possible due to similar appearing features.

Keywords: *Glioblastoma Multiforme, Glioblastoma Multiforme Variants.*

Introduction

GLIOBLASTOMA synonym (Glioblastoma Multiforme - GBM) is the most malignant neoplasm of the central nervous system with predominant astrocytic differentiation, affecting adults and preferentially located in the cerebral hemispheres [1] . Glioblastoma can be primary (De Novo), or secondary on the background of pre-existing astrocytic neoplasm. This is a morphologically diverse neoplasm with a dismal prognosis. Though three morphologically different variants have been recognized, additional variants which have significant morphology overlap with tumors having more favourable prognosis and treatment response rates, have been described. Even though Glioblastoma Multiforme is a quite rare tumor with a global incidence rate of only 3.17 per 100,000 [2] it significantly impacts the life of the affected patients due to its poor prognosis with a median survival time of only 12-15 months from the time of diagnosis. [3]

Materials and Methods:

After approval from the institutional ethics committee for this retrospective and prospective study, 40 cases from January 2008 to July 2015 were analyzed. Retrospective study was done using the blocks and slides available. For prospective study biopsy tissue was fixed overnight in 10% buffered formalin and submitted entirely for processing. Intraoperative tissues sent were processed as squash preparation and frozen section on cryostat while still in an unfixed state and H&E staining was performed. After frozen section diagnosis, remaining tissue was transferred to a fixative and processed routinely. Paraffin sections were cut 4 to 6 microns in thickness and routine H&E staining was performed, special stains were performed wherever necessary.

The clinical and radiological data was obtained from patient's proforma available in the Pathology Department. Anatomical location of the tumor was based on radiological imaging and or operative findings. GBM was diagnosed and classified as per the 2007 WHO.

Results

In the 8 years study period (January 2008 to July 2015), there were 433 cases of central nervous system space occupying lesions, out of which 40 were diagnosed as GBM. The overall incidence of GBM was 9.2%.The average number of GBM cases received was 5 per year.

The maximum number of cases were diagnosed in the 4th and 5th decade, 9 cases each (22.5 % each). In our study, a male predominance was found (Male: female=2.07:1). The youngest patient was 5 years old while the oldest one was 73 years old. GBM was found to be more common in males

in the 41-50 years age group and in females in the 51-60 years age group.

Headache and paresis were the commonest symptoms encountered, 24 cases (60%) and 21 cases (52.5%) respectively. Majority of the patients (22.5%) had duration of symptoms from 15-30 days. 29 cases (72.5 %) presented in less than 3 months. 95% of cases had supratentorial GBM and 5 % had infratentorial. The most common locations of the tumour were frontal lobe and temporo-parietal region. (17.5% each). 95 % (38/40) of the tumors were present in cerebral hemisphere whereas 2.5 % tumor involved cerebello-pontine angle and brain stem. Out of 38 Glioblastomas in cerebral hemisphere 18 involved only single lobe whereas 17 involved 2 lobes and 3 involved thalamocapsular ganglion.

CT findings were available in 25 cases and MRI findings in 15 cases. On CT scan, tumors were iso to hypodense (84%), heterogeneous enhancing (95%) and showed hemorrhage and necrosis (100%), peritumoral oedema (97.5%).

On histopathology majority of the cases showed marked cellularity (72.5%), moderate pleomorphism (90%), microvascular proliferation (95%), non-palisading necrosis (55%) and mitosis (100%). These are the defining histological features of GBM. [Table-1, Figure-1] Predominant types of cells were small cells (72.5%). [Table-2] 5 pediatric cases were seen. Histologic features of pediatric GBM are similar to adult GBM. [Table-3]

Of the 40 cases, 34 cases were analyzed on the frozen section .All these were given the diagnosis of high grade glioma on the basis of micro vascular proliferation and necrosis. On further histopathological examination, these were given the final diagnosis as Conventional GBM (26 cases) or GBMO (9 cases). [Figure-2] In the present study, 5 variants of GBM were obtained. [Table-4, Figure-3]

Conventional GBM (69.3%) and Glioblastoma with oligodendroglial component (GBM-O) (44.4%) were present in the elderly age group of 40-70 years. Only single case of Gliosarcoma presented in 50-60 year of age group. In contrast to these, the only case of Glioblastoma with Primitive Neuroectodermal Tumor(GBM-PNET) and 66.6% of Giant Cell GBM (gcGBM) presented in a younger age group (10-30 years). Both Conventional and GBMO had male predominance which was more marked in Conventional (76.9%) as compared to GBMO (66.6%).

Headache (69.2%) and paresis (57.7%) were the main presenting symptom in most cases of conventional GBM. GBMO (33%) more commonly presented with convulsions than Conventional GBM (7.7%). Both Conventional GBM (76.9%) and GBMO (66.7%) presented acutely i.e. within 3 months of onset of symptoms. Conventional GBM was present predominantly in frontal region (19.2%) and GBMO was equally present in fronto-temporal, temporo-parietal and parieto-occipital region (22.2%).

CT findings were available in 25 cases (16 of Conventional GBM, 6 of GBMO and 3 of Giant-cell GBM). MRI findings were available for 15 cases (10 of Conventional GBM, 3 of GBMO and 1 each of GBM-PNET and Gliosarcoma). Conventional GBM and GBMO showed iso to hypodensity on CT (87.5% and 66.5% respectively). Hemorrhagic necrosis was present in all the cases. Non-palisading necrosis was predominantly present in Conventional GBM (61.5%) and palisading necrosis in GBMO (66.7%). [Table-5]

Table 1: Defining histological features in GBM.

Histology		Total n=40	Percentage%
Cellularity n=40	Marked	29	72.5
	Moderate	11	27.5
Pleomorphism n=40	Marked	4	10
	Moderate	36	90
Microvascular proliferation n=40	Present	40	100
	Absent	0	0
Necrosis n=40	Palisading	14	35
	Non palisading	22	55
	P +np	4	10
Mitosis n=40	Present	40	100
	Absent	0	0

Table 2: Additional histological features in GBM.

	Microscopy	Total	Percentage%
Type of cells n=40	large cell type	6	15
	small cell	29	72.5
	giant cell	5	12.5
Oligocomponent n=40	Present	9	22.5
	Absent	31	77.5
Secondary structure n=40	Present	3	7.5
	Absent	37	92.5
Gemistocytes n=40	Present	11	27.5
	Absent	29	72.5
Perivascular lymphocytes n=40	Present	3	7.5
	Absent	37	92.5
Thick walled blood vessels n=40	Present	3	7.5
	Absent	37	92.5
Thrombosed vessel n=40	Present	5	12.5
	Absent	35	87.5

Table 3: Histology of Pediatric vs. Adult GBM cases.

Histology		Pediatric	Adult
		n =5	n=35
Pleomorphism	marked	0	4 (11.45%)
	moderate	5 (100%)	31(88.6%)
Microvascular proliferation	present	5(100%)	33(94.2%)
	absent	0	0
Necrosis	present	5(100%)	35(100%)
	absent	0	0
Mitosis	present	5(100%)	35(100%)
	absent	0	0

Table 4: Variants of GBM found in the study (n=40):

Variant	Number	Percentage
Conventional	26	65
GBMO	9	22.5
Giant Cell GBM	3	7.5
Gliosarcoma	1	2.5
GBM PNET	1	2.5
Total	**40**	**100**

Table 5: Immunohistochemical findings – GBM-PNET:

IHC stains	Small Cell GBM		GBM- PNET		sPNET
	Astrocytic component	Small cell component	Astrocytic component	Small-cell component	Small-cell component
GFAP	+	+	+	-	-
Synaptophysin	-	-	-	+	+
CD 56	+	+	+	+	+
Vimentin	+	+	+	-	-

**sPNET are Supra-tentorial PNET which lack glial component.*

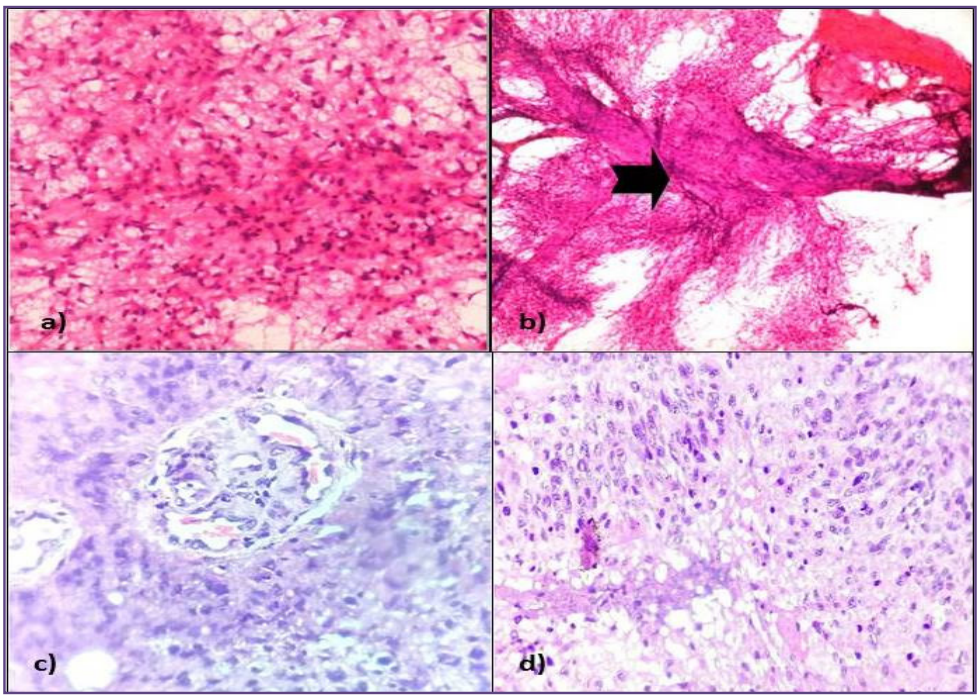

Fig. 1: Frozen section: (a) Squash smear of glioblastoma showing glial cells with hyperchromatic and pleomorphic nuclei. (HE X 400) (b) Squash smear of Glioblastoma showing microvascular proliferation. (HE 400X) (c) Paraffin section of Glioblastoma showing microvascular proliferation (HE 400X) (d) Paraffin section of Glioblastoma showing pseudo-palisading necrosis. (HE 400X).

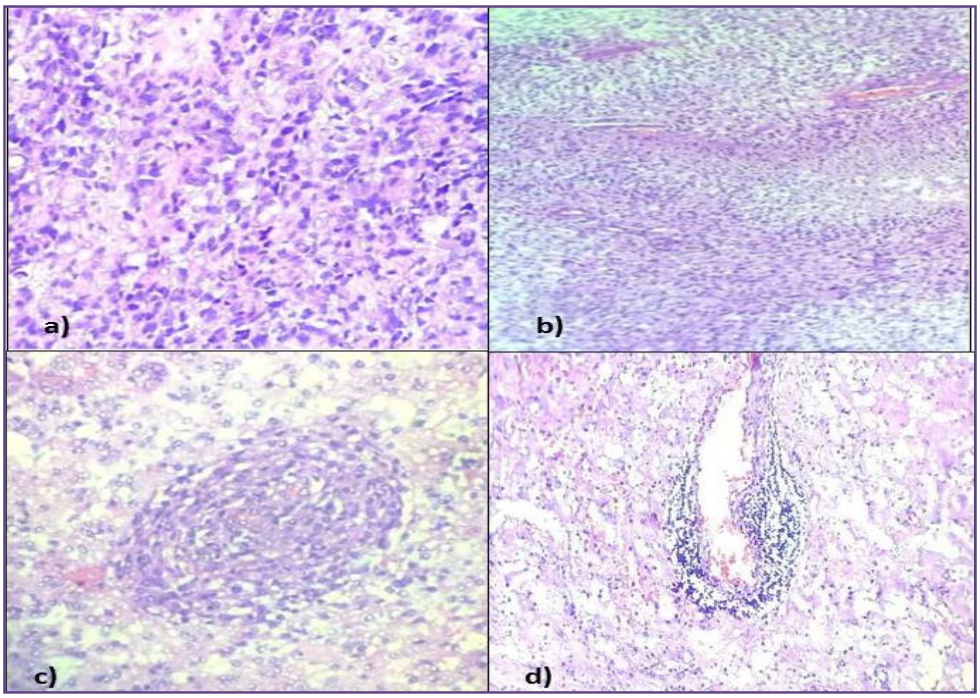

Fig. 2: GBM Histopathology: (a) GBM showing marked pleomorphism and gemistocyte (HE 400X) (b) GBM showing pseudopalisaded necrosis (HE 400X) (c) GBM showing glomeruloid appearance of microvascular proliferation. (HE 400X) (d) GBM showing perivascular lymphocytes . (HE 400X).

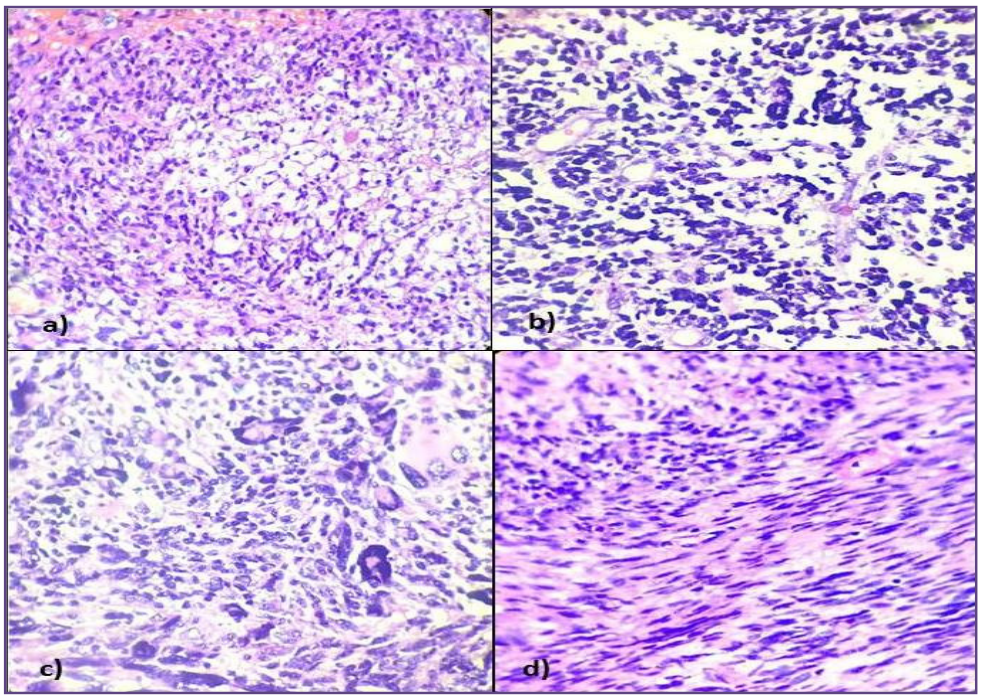

Fig. 3: GBM Variants :(a) GBMO showing Oligodendroglial component on right side (HE 400X) (b) GBM PNET showing small cells in sheet. (HE 400X) (c) Giant cell GBM showing multinucleated giant cells (HE 400X) (d) Gliosarcoma showing spindle cells in fascicles in lower part of section. (HE 400X).

Discussion

GBM or astrocytoma grade IV (WHO classification) is the most aggressive and the most frequent of all primary brain tumors.[4] Incidence rate of GBM at our institute was 9.2% which is almost similar to the incidence stated by Ghosh et al who reported an incidence of 7.9% for GBM. WHO (2007) states an incidence of around 12-15% for GBM of all intracranial neoplasms[5].

In our study, 45 % of CNS tumors were encountered in the fourth to sixth decade. The mean age was 46.4 years. The youngest patient was 5 year old while the oldest one was 73year old. The finding is similar to study done by Manisha Khanna et al, who found majority of the tumors in age group of 51-60 years [6]. Childhood glioblastomas are extremely rare compared to their adult counterparts [4].Of the 40 cases, 27 were male and 13 were female. The male: female ratio in our study was found to be 2.07:1 which was comparable with Manisha Khanna et al [6] (2.38:1). Ghosh et al. observed a male/female ratio of 3.9:1[5].

Although the duration of symptoms was more than 3 months in 11 cases, there were no histological proven or past clinical history of high grade gliomas. Hence no case of secondary GBM was found in the study.

According to the literature, GBM is preferentially located supratentorially [4]. In our study too about 95 % of cases were supratentorial and 5% cases showed infratentorial GBM.

In our study, the commonest site of GBM was frontal region and temporo-parietal region (17.5% each). These findings were comparable to study by Manisha Khanna et al [6]. In our study, 10% tumors were present in frontal lobe involving corpus callosum but none extended to other lobe or across the midline. In literature [7] it is said that the corpus callosum is relatively resistant to infiltration by edema or infection. Any lesion seen extending across the midline in this way, whether symmetric or asymmetric, should always be suspected of being a diffuse astrocytoma.

MRI Brain mainly revealed extensive white matter edema (97.5%) noted with significant midline shift. Non enhancing areas corresponded to haemorrhage and necrosis on histopathology were seen in 100 % of the cases. These findings is in accordance to study by Gabriel Iacob et al [4].

In our study, morphological analysis included the degree of cellularity, pleomorphism, mitosis, type of necrosis and vascular proliferation. 29 cases of GBM (72.55%) were markedly cellular. Moderate cellularity was seen in 11 cases (27.5%). 29 cases (72.5%) of the tumors showed cells of small size. Non palisading necrosis was present in majority of the cases (55%) whereas 35% of the cases showed palisading necrosis and in 10 % both type of necrosis was

present. Variable numbers of mitotic figures were seen in almost all cases (100%) Microvascular proliferation was seen in all cases (100%) .

Of the 40 cases, 34 cases were analyzed on the frozen section .All these were given the diagnosis of high grade glioma on the basis of micro vascular proliferation and necrosis. On further histopathological examination, these were given the final diagnosis as Conventional GBM (26 cases) or GBMO (9 cases).

All the cases of glioblastoma diagnosed on squash smear and frozen section showed significantly increased population of glial cells in a fibrillary background with mitoses with microvascular proliferation and necrosis.

The study by Chandrasoma PT *et al* provides evidence that, with careful target placement, stereotactic biopsy can provide biopsy material that represents the entire lesion with an accuracy that is sufficient for clinical management [8].

In present study, we had 26 cases (65%) of Conventional GBM. It was prevalent in 3rd to 5th decade (69.3%). Out of 26 cases of Conventional GBM , 20 were found in males and 6 in females. The ratio in Conventional GBM was 3.3:1. Headache and paresis was the main presenting symptom in most cases of conventional GBM. Most frequent site affected in patients with Conventional GBM was frontal.

Majority of the cases showed marked cellularity (69.2%), moderate pleomorphism (92.3%), presence of small cells (73.1%), non-palisading necrosis (61.5%), microvascular proliferation (92.3%) and presence of gemistocytes (30.7%). Mitosis was present in all the cases. Giant cells were seen in 7.7% of the cases. These correlates with findings mentioned in literature[9,10].

Clinical and pathological studies of GBMO are currently scarce. Its exact incidence is thus largely unknown and has ranged from 4% to 27% of all GBMs in previous studies[9, 11-13] with an incidence of 22.5% (9 cases) in the current study.

In present study, it was noted that GBMO was prevalent in elderly patient's i.e.in age group of 60-70 years. The youngest patient of GBMO was a 16 year old male and the oldest patient was also a male of 72 years. Out of 9 cases of GBMO , 6 were found in males and 3 in females. So GBMO was more common in male just as Conventional GBM, but the M: F ratio of GBMO was 2:1, compared to 3.3:1 in Conventional GBM. Patients with GBMO (33%) more commonly presented with convulsion than Conventional GBM (7.7%). Sites commonly affected by GBMO were frontotemporal, temporo-parietal, and parieto-occipital (22.2%).

In a study of Yongzhi Wang et al., 40 (18.3%) of the 219 primary GBMs selected fulfilled the criteria for GBMO. Fourteen patients (35%) had seizure attacks as presenting symptoms. The patients with GBMO were more likely to present with seizures than were patients with conventional GBM. There were no significant differences in sex, tumor location, tumor-related seizures were more frequent in the GBMO patients (35%) than in conventional GBM patients (19.7%).

Microscopic examination of GBM-O showed Oligodendroglial component intermingled with or in different foci of glioblastomatous tissues (figure-19). Majority of the cases showed marked cellularity (66.6%), moderate pleomorphism (88.9%), and presence of small cells (88.9%), palisading necrosis (66.7%) and thrombosed blood vessels (22.2%). All the cases showed microvascular proliferation (100%) and Mitosis (100%). These findings were similar to findings mentioned in literature[14, 15]

Giant cell GBM is a rare variant of GBM thought to encompass 2-5% of GBM diagnoses [1], while in our study incidence is about 7.5% which is slightly higher. In the present study, patients with Giant cell GBM presented mainly with headache (66.7%).This finding was almost similar to Conventional GBM (69.2%). The other symptoms were loss of consciousness, aphasia and paresis (33.3 %) each. In contrast to the Conventional GBM, majority of the cases (66.7%) presented with duration of symptoms of more than 3 months. This finding is in contrast with the findings of Valle et al. where they found the duration of symptoms to be short (and similar to Classical GBM)[16]. The difference noted may be due to small sample of the gcGBM in the present study.

As the name implies, the tumor cells are markedly enlarged and bizarre, often appearing multinucleated. All the cases showed marked cellularity, moderate pleomorphism , Giant cells , palisading, non-palisading and pseudo palisading necrosis were found in 33.3% respectively. All cases showed mitosis and microvascular proliferation. Gemistocytes were seen in 66.7% and 33.3% showed perivascular lymphocytes.

In this study, patients with Conventional GBM and gcGBM showed similar gender and racial distributions as well as insignificant tumour size and location differences. However, age at diagnosis was significantly younger in gcGBM vs. GBM (51 vs. 62 years) and gcGBMs were more likely to undergo complete resection. [17]

In the present study gliosarcoma accounted for 2.5% of all GBM case (1/40). In various series, the incidence of GS has been reported to vary from 2% to 8 % [18]. This corroborates with our study findings.

In the present study, the only case of Gliosarcoma, a 55 year old female presented with psychiatric symptoms since 8 days. Tumour was located in the left fronto-parietal region. Study by Morantz [19]and colleague described that great percentage of patients with GS were older than 60 years of age and in study by Manisha et al [6] most of the patients were in the age group of 51 to 70 years and found GS most commonly in the temporal region, followed by frontal and parietal. Common site of occurrence was cerebrum and the common clinical symptoms were muscle weakness, headache and mental changes.

Microscopic examination of GS showed sarcomatous tissue intermingled with or in different foci of pre-existing glioblastomatous tissues . The case showed marked cellularity, moderate pleomorphism, small cells, non-palisading necrosis, mitosis, microvascular proliferation and presence of secondary structure of Scherer. These findings were similar to Manisha khanna et al[6]. Narendra Kumar et al.[20] reviewed 27 gliosarcoma patients and found that all the tumors were having the biphasic histologic pattern consisting of gliomatous and sarcomatous components which is in accordance to our study.

In our study we found 1 case of GBM-PNET in a 26 year old female who presented with convulsions since 15 days. The tumor histo-morphologically consisted of undifferentiated cellular areas alongside classic GBM areas. The undifferentiated cellular areas composed of small undifferentiated cells with scant cytoplasm and oval round hyper chromatic nuclei. Immunohistochemistry revealed astrocytic component of the tumor strongly positive for GFAP and undifferentiated area stained strongly for synaptophysin. This concluded the diagnosis of GBM-PNET. [Table6]

As the clinical and histopathological properties of those tumors have been described only recently, the number of cases reported in the literature is limited. The largest series published on these tumors belong to Varlet et al[21](n=40) and Perry et al. (22) (n=53). These studies highlighted the clinical, radiological, and histopathological differences of GB-PNET from classic GBM. They are encountered more commonly among adults and 52.5% of tumors are localized in the temporal lobe, whereas they are rarely seen with infratentorial localization. Having a well circumscribed character facilitates the surgical excision.

Conclusion

The most common histological findings of GBM included marked cellularity, moderate pleomorphism, microvascular proliferation, non-palisading necrosis and mitosis. Clinical and histopathology remains the main tool for differentiating the variants as radiological differentiation is not possible due to similar appearing features. Newer diagnostic technique like immunohistochemistry and molecular analysis has great prognostic significance and help to decide the line treatment.

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