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# COMPARATIVE STUDY OF SQUASH CYTOLOGY AND HISTOPATHOLOGY IN GLIOBLASTOMA AND ITS VARIANTS

Pathology

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## ABSTRACT

**Introduction** Glioblastoma is the most aggressive and deadly of all brain tumors. It accounts for 12-15% of all intracranial neoplasm and 50-60% of all astrocytic tumours [(1)] Even though Glioblastoma is a quite rare tumor with a global incidence rate of less than 10 per 100,000 it significantly impacts the life of the affected patients due to its poor prognosis with a median survival time of only 14-15 months from the time of diagnosis[(2)]. **Materials And Methods** A total of 21 cases of Glioblastomas were studied over a period of 5yrs from 2017 to 2022. Intraoperative squash smears were prepared, stained with toluidine blue, "Hematoxylin and Eosin" and examined. The residual tissue samples were processed for paraffin section and stained by Hematoxylin and Eosin and IHC was done wherever necessary. Histopathological examination was carried out for confirmative diagnosis and squash smear cytology diagnoses were correlated with final histopathological findings. **Results** Out of 21 cases, 12 were Glioblastoma, 7 were Gliosarcoma, one each of Epithelioid Glioblastoma and Gemistocytic Glioblastoma. Male to Female Ratio is 2.5:1. Most common clinical presentations were Headache, giddiness, vomiting and weakness/paresis in lower limbs. Intraoperative Squash smear cytology could diagnose high grade gliomas with 100 % accuracy which was concordant with histopathological diagnosis. **Conclusion** Squash smear cytology findings, if interpreted with clinical picture and radio-imaging findings, will help to reach an accurate and rapid diagnosis of intracranial tumor, but histopathology remains gold standard for confirmation of diagnosis and differentiating the variants.

## KEYWORDS

Glioblastoma, Gliosarcoma, Epithelioid Glioblastoma, Gemistocytic Glioblastoma,

## INTRODUCTION

Glioblastoma is the most malignant neoplasm among the astrocytic tumors and is designated as WHO grade IV [3]. They typically affect adults and are preferentially localized to cerebral hemispheres. Glioblastomas manifest at all ages, but preferentially affect adults, with a peak incidence between 40 and 70 years[4]. Primary glioblastomas typically develop in older patients (mean age 62 years), whereas secondary glioblastomas manifest in younger patients (mean age approximately 45 years.). The majority of glioblastoma are found in the supratentorial brain (frontal, temporal, parietal, and occipital lobes), with rare occurrence in the cerebellum, brain stem and the spinal cord[2]. There is Marked diversity in the clinicopathological characteristics of glioblastomas and recent studies suggest presence of a cancer stem cell population which may account for this heterogeneity, tumour recurrence and therapeutic resistance[13]

## MATERIALS AND METHODS

This study was conducted over a period of 5years in the department of pathology collaborating with department of Neurosurgery in a tertiary care hospital. A total of 21 cases of Glioblastoma were included in this study. A small portion of the biopsy sample was crushed between two slides and smear prepared.

The smears were immediately put in 95% ethyl alcohol for 2 minutes for wet fixation and stained with rapid H and E stain and air-dried smears with toluidine blue. Remaining tissue processed for paraffin sections for histopathological report and immunohistochemistry was done wherever necessary. Final diagnosis was done on Histopathology and compared with initial cytological diagnosis. Anatomical location of the tumor was based on radiological imaging and or operative findings. Glioblastoma was diagnosed and classified as per the 2021 WHO

## RESULTS

In the 5 years study period from March 2017 to August 2022, there were 260 cases of central nervous system space occupying lesions in our institution, among them 34 cases were of astrocytic tumors out of which 21 were diagnosed as Glioblastoma. The overall incidence of Glioblastoma in our institution is 8.07%. The maximum number of cases were diagnosed between 5th and 6th decade (Table 1). In our study, a male predominance was found (Male: female=2.5:1) (Table2). The youngest patient was 2 years old while the oldest one was 72 years old. GBM was found to be more common in male in the 41-70 years age group and in females in the 41-60 years age group. Headache, giddiness, vomiting and weakness in lower limbs were the commonest symptoms patient presented with.

Majority of the patients had duration of symptoms from 3 to 6 months. 95 % (20 cases) had supratentorial Glioblastoma and only 5% (1 case) had infra tentorial location. 8 cases in frontal lobe, 5 cases in parietal lobe, 4 cases in occipital lobe, 2 cases in temporal lobe, one case in cerebellum, and one case in corpus callosum. The most common location of the tumor was frontal lobe followed by Parietal and occipital lobe. (Table 3). Glioblastoma was seen in the age group of 50 to 70 years. Gliosarcoma was seen in the age group of 40 to 72 years, and single case of Epithelioid Glioblastoma was seen in young girl of 16 yrs, and single case of Gemistocytic Glioblastoma was seen in middle aged women of 40 yrs.

**Table 1: Age Wise Distribution of Cases**

| Age | Glioblastoma | Gliosarcoma | Epithelioid Glioblastoma | Gemistocytic Glioblastoma | Percentage |
|---|---|---|---|---|---|
| 1-20yrs | 1 | 2 | 1 | - | 19.05% |
| 21-40yrs | - | | | 1 | 4.76% |
| 41-60yrs | 8 | 3 | - | - | 52.38% |
| 61-80yrs | 3 | 2 | - | - | 23.81% |
| Total | 12 | 7 | 1 | 1 | 100% |

In our study Glioblastoma was most common between 41 to 60yrs of age.

**Table 2: Gender Wise Distribution of tumours**

| Gender | No of Cases | Percentage |
|---|---|---|
| Males | 15 | 71.4% |
| Females | 06 | 28.6% |
| Total | 21 | 100% |

In this study it was noted that Glioblastoma is more common in men than women, Male to Female Ratio: M: F=2.1:1

**Table 3: Site Wise Distribution of Tumours**

| S.NO | Site | NO. Of Cases | Percentage |
|---|---|---|---|
| 1 | Frontal | 08 | 38.10% |
| 2 | Parietal | 05 | 23.81% |
| 3 | Occipital | 04 | 19.05% |
| 4 | Temporal | 02 | 9.52% |
| 5 | Cerebellum | 01 | 4.76% |
| 6 | Corpus Callosum | 01 | 4.76% |
| | Total | 21 | 100% |

Our Study Showed most common site of High-Grade Gliomas is Frontal lobe

**Table 4: Spectrum of Glioblastomas, Correlation of Histopathological Diagnosis Versus Cytological Diagnosis**.

| Histopathological Diagnosis | WHO Grading | Cytological Diagnosis | No 0f Cases | Percentage |
|---|---|---|---|---|
| Glioblastoma | Grade-IV | High Grade glioma | 12 | 57.14% |
| Gliosarcoma | Grade-IV | High Grade glioma | 07 | 33.3% |
| Epithelioid Glioblastoma | Grade-IV | High Grade glioma | 01 | 4.76% |
| Gemistocytic Glioblastoma | Grade-IV | High Grade glioma | 01 | 4.76% |
| Total | | | 21 | 100% |

Our Study showed commonest type of High-Grade Glioma is Glioblastoma (57.1%)

**Table 5: IHC Markers done in this study**

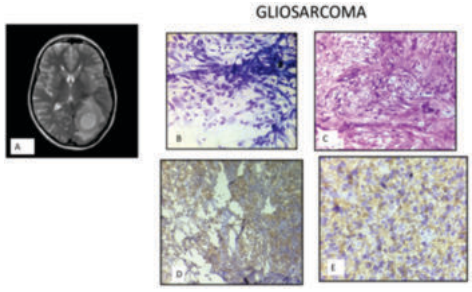
| IHC Marker | Glioblastoma | Gliosarcoma | Epithelioid glioblastoma | Gemestocytic glioblastoma |
|---|---|---|---|---|
| GFAP | + | + | + | + |
| Vimentin | - | + | - | - |
| EMA | - | - | + | - |
| INI 1 | - | - | + | - |

**Table 6: Radiological and histopathological correlation**

| Age of Patient/Sex | Radiological Diagnosis | Histopathology diagnosis |
|---|---|---|
| 58yrs/M | High grade Glioma | Glioblastoma |
| 49yrs/M | Lesion in Parietal lobe | Glioblastoma |
| 64yrs/M | Space occupying lesion in frontal lobe, ? metastasis | Glioblastoma |
| 52yrs/F | High Grade Glioma, ? meningioma | Glioblastoma |
| 06ysr/M | Pilocytic astrocytoma | Glioblastoma |
| 72yrs/M | Cystic Glioma | Glioblastoma |
| 40yrs/M | High Grade Glioma | Glioblastoma |
| 55yrsF | Space occupying lesion, | Glioblastoma |
| 46yrs/F | High Grade Glioma | Glioblastoma |
| 51yrs/M | Lesion in frontal lobe | Glioblastoma |
| 58yrs/M | Metastasis | Glioblastoma |
| pp41yrs/M | High Grade Glioma | Glioblastoma |
| 02yrs/F | Cystic lesion in cerebellar region | Gliosarcoma |
| 70yrs/M | Lesion in Corpus Callosum region | Gliosarcoma |
| 65yrs/M | High grade glioma | Gliosarcoma |
| 14yrs/M | High grade glioma | Gliosarcoma |
| 42yrs/M | Mass lesion, High grade Glioma | Gliosarcoma |
| 55yrs/M | SOL.? Metastasis | Gliosarcoma |
| | | |
| 50yrs/M | High Grade glioma | Gliosarcoma |
| 16yrs/F | SOL? Rhabdoid tumour | Epithelioid Glioblastoma |
| 40yrs/F | Tuberculoma | Gemistocytic Glioblastoma |

**Gliosarcoma**

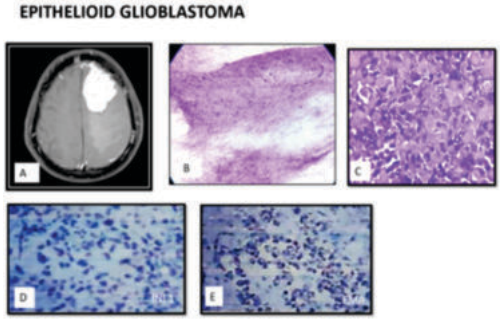
Is one of the primary tumours of central nervous system with biphasic component composed of malignant glial and sarcomatous elements. The incidence of Gliosarcoma is about 1 to 8% of all Glioblastomas[10]. In our study we had 7 cases Gliosarcomas with most common clinical presentation of headache, giddiness, vomiting and paresis of lower limbs.




**(Figure-1)A:** Irregular Space occupying Lesion in Occipital region, B: Squash smear cytology shows endothelial proliferation and large, bizarre nuclei, arborizing vessels, C: Biphasic component: glial component with pleomorphic astrocytic cells with nuclear atypia, mitosis, and microvascular proliferation. Sarcomatous component with densely packed, spindle shaped cells with nuclear Atypia. D: GFAP Positive, E: Vimentin Positive

**Epithelioid glioblastoma**:

Epithelioid Glioblastoma is a relatively recently recognized and a rare highly malignant variant of glioblastoma which has predilection for younger age group and children[11]. In our study we had a single Case of Epithelioid Glioblastoma seen in 16year old young girl, with clinical presentation of weakness in both lower limbs and vomiting.

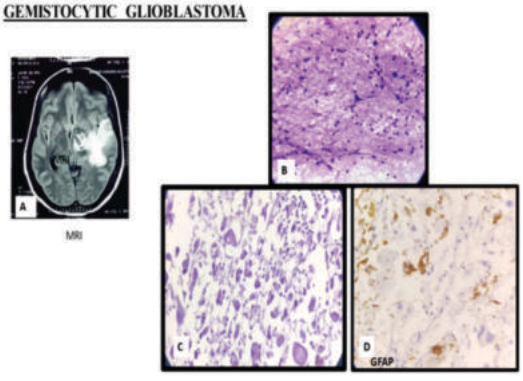



**(Figure-2)A:** Irregularly enhancing mass in Parietal lobe, B: Squash smear showing marked cellularity, moderate to marked nuclear pleomorphism with fine fibrillar glial processes, C: Large pleomorphic astrocytic cells with plump processes and massive accumulation of glial fibrillary protein: D: GFAP: positive

A: Homogeneously enhancing mass in the left frontal lobe. B: Squash smear cytology showing marked cellularity, Nuclear Hyperchromasia, moderate to markedly pleomorphic cells with fine fibrillar glial processes C: Histopathological Examination: Shows Uniform population of large epithelioid cells with distinct cell border, eosinophilic cytoplasm and laterally placed nuclei with prominent nucleoli. D:INI: Positive, E: EMA Positive.

**Gemistocytic Glioblastoma**:

Exact incidence of Gemistocytic Glioblastoma is not known[12]. In our study we had a single case of Gemistocytic Glioblastoma with clinical presentation of Headache and vomiting with paresis in lower limbs.




**(Figure-3)** A: Irregularly enhancing mass in Parietal lobe, B: Squash

smear cytology showing marked cellularity, moderate to marked nuclear pleomorphism with fine fibrillar glial processes, C: Large pleomorphic astrocytic cells with plump processes and massive accumulation of glial fibrillary protein, D: GFAP; Positive.

## DISCUSSION

Despite decades of research, Glioblastoma still remains one of the deadliest tumours of central nervous system. It is grade IV astrocytoma (WHO Classification) and is most frequent of all primary brain tumours[3].With an incidence rate of 3.19 per 100,000 persons, it is rare in children. The incidence is 1.6 times higher in males compared to females and 2.0 times higher in Caucasians compared to Africans and Afro-Americans, with lower incidence in Asians and American Indians[9]. Incidence rate of Glioblastoma at our institute is 8.07% which is similar to the incidence stated by Ghosh et al who reported an incidence of 7.9% [6]. In our study, Glioblastoma accounted for 61.76% of all astrocytic tumours,52% of Glioblastomas were encountered in the fourth to sixth decade (Table 1) which is similar to study conducted by Asha Shenoy et al who reported 45% of tumours in 4th to 6th decade [7]. The mean age was 57 years. The youngest patient was 2-year-old while the oldest one was 72-year-old. Male: female ratio in our study was found to be 2.5:1 (Table 2). which is similar to study conducted by Khanna M et al who reported male to female ratio of 2.38:1[1]. Childhood glioblastomas are extremely rare compared to their adult counterparts [8]. In our study we encountered 4 cases of pediatric Glioblastomas, among which 2 were of Gliosarcoma, one was primary Gliosarcoma in 2 yr old female child and other was a secondary Gliosarcoma in 14 yr old male child, who was operated for Glioma 3 years back, one case of Epithelioid Glioblastoma seen in 16 yr old, one case of Glioblastoma in 6 yr old male child.

The duration of symptoms was predominantly between 3 months to 6 months in all primary Glioblastomas, one case of secondary Gliosarcoma presented with above symptoms since 3years. According to the literature, Glioblastomas are commonly located in supratentorial region, in our study too 95% (20 cases) had supra tentorial location and only 5% (one case) had infra tentorial location. In this study, 4 types of Glioblastomas were encountered (Table-4). All of these 21 cases of Glioblastomas were analyzed by intraoperative squash cytology. Almost all cases (21 out of 21 cases) were correctly diagnosed as high-grade gliomas on squash cytology. In 21 out of 21cases, Squash cytology was concordant with Histopathology report accounting for 100% accuracy (Table 5), which is nearly similar to other studies done by Iqbal et al[17] and Garima et al[16]. (Table-7)

The Final histopathological diagnosis was also correlated with radiological diagnosis (Table 6).

**Table 7: Comparison of various studies on diagnostic accuracy of intra operative squash smears**

| Various studies | Diagnostic accuracy |
|---|---|
| Nigam S, et al[14] | 89.3% |
| Torres L.F, et al[15] | 92.2% |
| Garima P, et al[16] | 93.13% |
| Iqbal. M, et al[17] | 95.3% |
| Our study | 100% |

## CONCLUSION

Preoperative neuroimaging studies cannot confidently distinguish different types of lesions in brain. Squash smear cytology proved to be a simple, inexpensive, rapid intraoperative neuropathological technique which help the surgeons plan the extent of surgery. Histopathology is gold standard for confirmation of diagnosis and to differentiate the types of Glioblastomas. Newer technique like immunohistochemistry and molecular analysis has great prognostic significance and help to decide the line of treatment.

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