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Original Research Article

Intraoperative Diagnosis of Central Nervous System Gliomas by Squash Smears Cytology

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Conflict of interest: Nil

Abstract:

Aim of the Study: To study the validity of rapid intra operative diagnosis of glial tumors by examining the squash cytologic smears.

Material & Methods: The study was performed over a period of 2 years from November 2019 to October 2021 at Tertiary Care Hospital, Telangana State. We screened 256 inpatients with central nervous system lesions.

Results: The present study total of 60 Glial lesion biopsies reported. Out of 60 cases, 39 were consisting of low grade gliomas and 16 were consisting of high grade gliomas. In our study low grade gliomas comprising of 65% and high grade gliomas were 16 cases (26.6%), non-neoplastic lesions were 3 cases(reactive gliosis) (5%), secondary tumors (metastatic deposits) were 2 cases (3.3%).

Conclusion: Intraoperative squash smear is simple, rapid, reliable and cost effective in the diagnosis of central nervous system tumors. The difficulty on squash smear can be overcome by histopathology and Immuno histochemistry. At present understanding of molecular genetic basis of diseases and application of molecular biology techniques to cell smears offering new approaches for diagnosis, prevention and treatment of central nervous system lesions.

Keywords: Central Nervous System Gliomas; Squash Smears Cytology.

Introduction

Smear cytology has become increasingly popular as an alternative approach or an adjunct to frozen sections for rapid intra operative diagnosis in neuropathology in the recent years, because the technique is very simple but accurate. Squash (smear) preparation consume very little tissue, allow better preservation of nuclear details,(compared to frozen sections) demonstrate glial processes well, can be prepared more rapidly and do not require special equipment hence economical [1].The smear technique for brain biopsy is a simple method whereby a diagnosis of normal or abnormal tissue can be made within minutes of the biopsy reaching the laboratory. If it is normal the neurosurgeon probably wishes to undertake further biopsy. Even if the abnormal tissue is not tumor, an immediate diagnosis facilities management decisions [2].

The role of intraoperative pathological diagnosis is crucial in neurosurgery. Besides rapid decision making during neurosurgical procedures it is also to be ensured that minimum injury is caused to the normal brain structures surrounding the intracranial neoplasms. [3]The neuropathologist has two principle techniques at his disposal for establishing a rapid tissue diagnosis- frozen's and smears. There is a place for frozen sections if the tissue is of firm consistency, otherwise there are several advantages of the smear techniques when compared to frozen sections.[4] The soft texture however, aids in smear preparation, often revealing exquisite cytological details [5].

The smears preserve the degree of vascularization, endothelial proliferation and the relationship of the tumor cells to blood vessels, thus it helps to assess the tumor. It is not wise to attempt interpreting a smear with no clinical correlation. Smears should be looked at intelligently in relation to the site of biopsy, the age and sex of the patient, the clinical history and the neuroradiological findings. A brief conversation with the neurosurgeon can often make a difficult situation much easier for the neuropathologist. Preoperative diagnosis based on the age and sex of the patient and the location and

appearance of the lesion on neuro imaging techniques are accurate in 75 to 80% of cases only.

Aim of the Study

1. To compare squash smears with the histopathological (HPE) diagnosis with immunohistochemistry (IHC) whenever required.
2. To compare present study with other studies.

Materials and Methods

A retrospective and prospective study of 60 patients with central nervous system lesions was done from November 2019 to October 2021 at Tertiary Care Hospital, Telangana State. The biopsies of CNS lesions which were surgically excised by craniotomy or by burr hole was subjected for cytological examination along with clinical and radiological details like age, gender, location, imaging studies and clinical differential diagnosis. The tissue was sent to the laboratory unfixed kept in awet gauze piece for intraoperative diagnosis. Smears were stained with toluidine blue and rapid haemotoxylin and eosin. After the smears were reported the remaining tissue was fixed in formalin and processed routinely with paraffin embedding and stained with haemotoxylin and eosin.

Preparation and Staining of Squash Smears: From the fresh tissue sent to the laboratory areas of interest are selected (usually 2-3 areas are used depending on the amount of tissue received) by naked eye examination. A small sample of the tissue 1-2mm cube is dissected out with a scalpel blade placed on one end of a lebelled glass slide. The second slide is kept on the first one and pressed over the dissected tissue. Optimal pressure is applied, and then slides the second slide towards the other end of the first slide to make a smear. Pressure applied depends on the consistency of tissue. Excessive pressure will cause crush artifacts. The squash smeared slides are immediately placed into absolute alcohol in a coplin jar for fixation. Two to four smears are made from each biopsy. Two slides were stained with aqueous toluidine blue and two with hemotoxylin and eosin.

Rapid Haemotoxylin and Eosin Method: The smears are immediately wet fixed in absolute alcohol for 1-2 minutes then rehydrated and stained with Harri's hemotoxylin for 1 min, differentiated in acid alcohols. Counter stained with 0.5% eosin 2 dips. Dehydrate rapidly through graded series of alcohol, cleared in xylene and mounted. The entire procedure took 3-5 minutes.

Stained smears were studied by light microscopy and subsequently correlated with the histopathologic appearance.

IHC staining Procedure

- The sections were de paraffinised, dehydrated & endogenous peroxidase Quenching done with 3% H2O2.
- Antigen retrieval done by Pressure cooker (HIER, heat induced epitope retrival) with Tris buffer (1.21 g of Tris Hydroxymethyl methylamine and 3.75 mg of EDTA in 1000 ml distilled water).
- Incubated with Primary antibody (GFAP and S100) which is ready to use, at room temperature in a humidifier chamber for 30 minutes.
- The sections were washed with TBS buffer (9.6 g of Tris Hydroxymethyl methylamine and 8.6 g of Nacl in 1000 ml distilled water).
- Chromogen DAB (DAKO labeled) used for detection of enzymatic activity.
- Counter staining was done with haematoxylin.
- Dehydrate in alcohol and xylene.
- Mount with DPX.
- Glial tissue itself is the Positive control.
- Non-glial tumors are the Negative control.

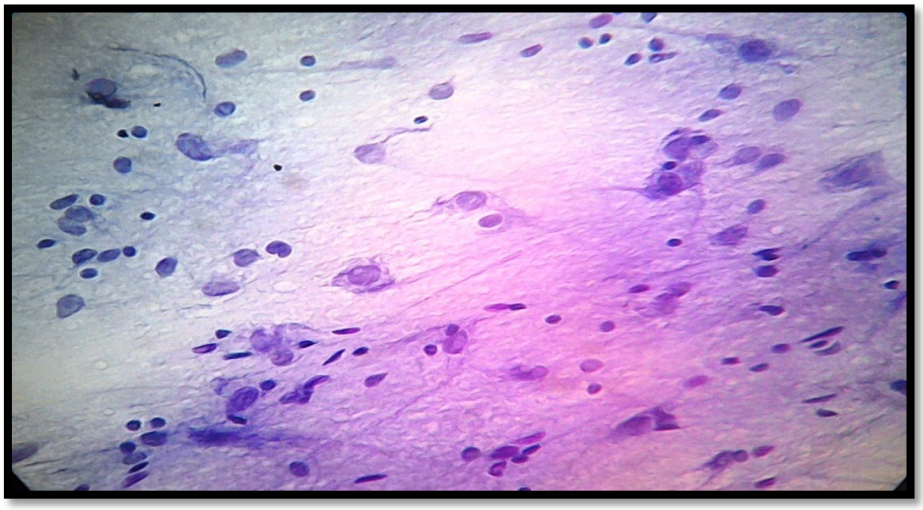


Figure 1: Reactive gliosis 40x TB

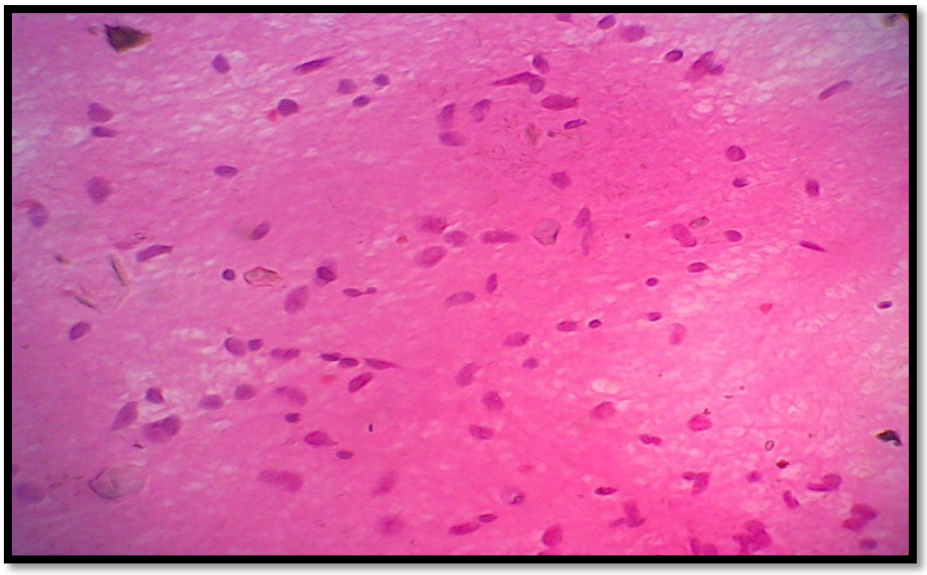


Figure 2: Low grade astrocytomas (grade I & II)

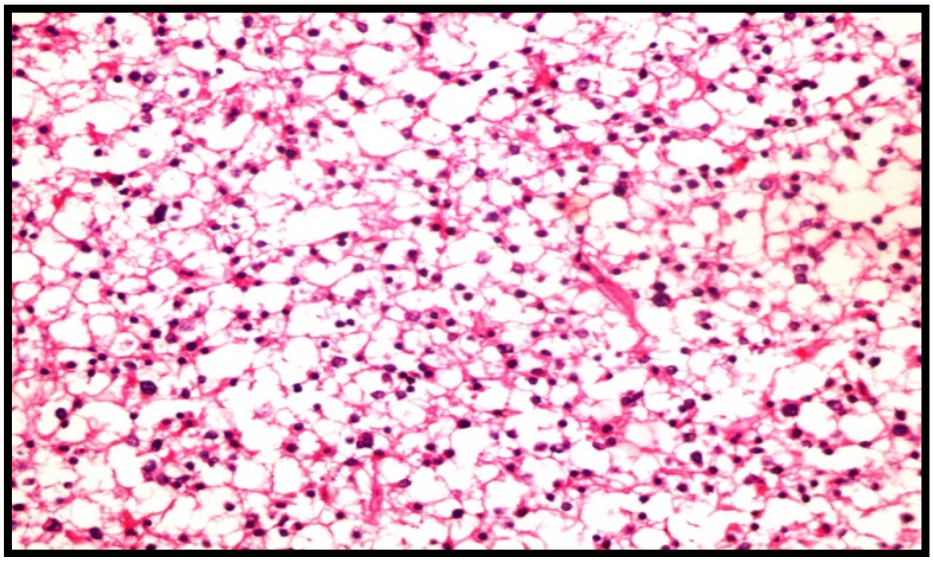


Figure 3: Glioma grade I 40x H&E

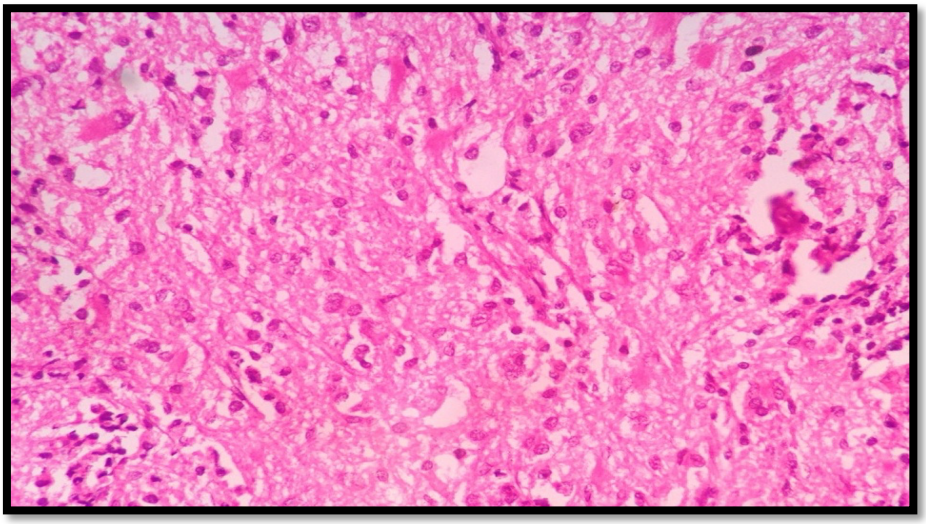


Figure 4: Glioma grade I H&E

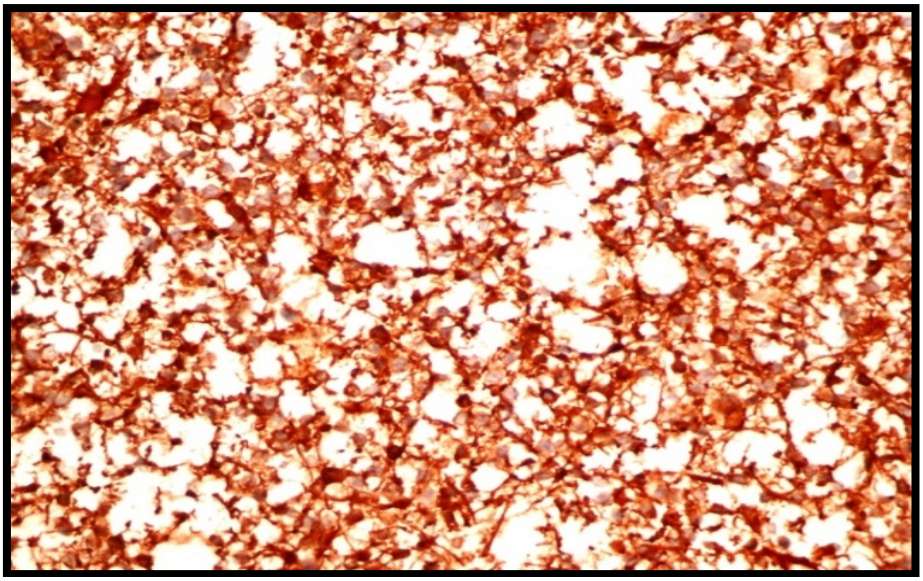


Figure 5: Glioma grade II H&E 40x

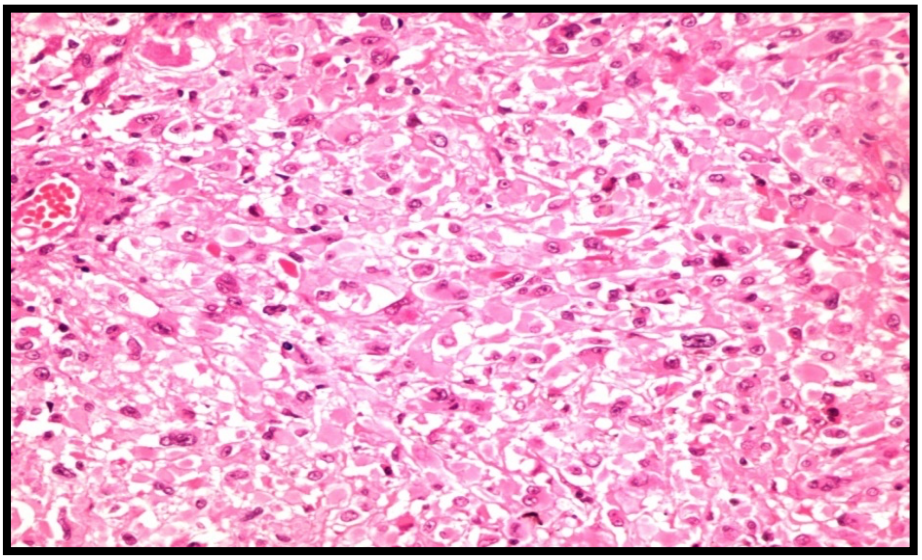


Figure 6: High grade astrocytoma (Glioma grade III)

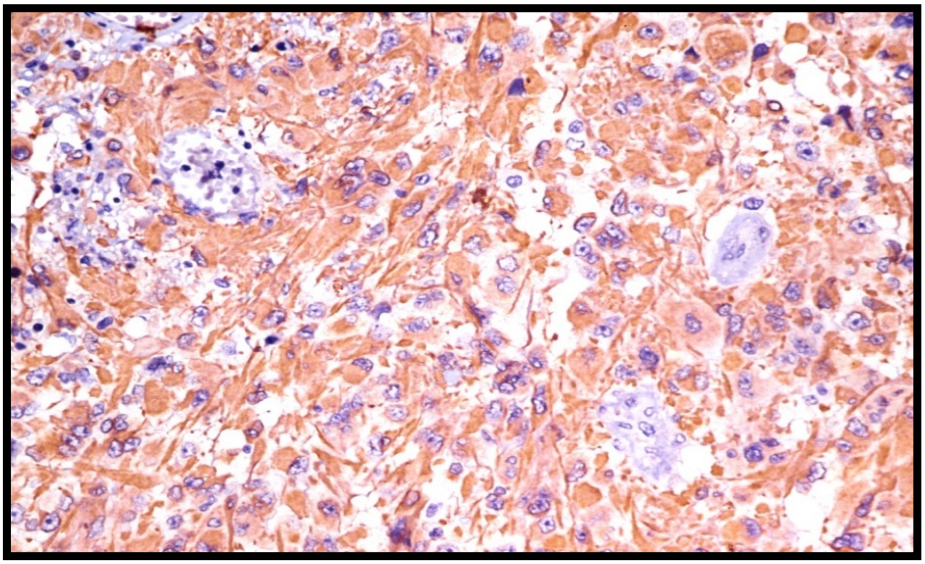


Figure 7: GFAP positive 40X

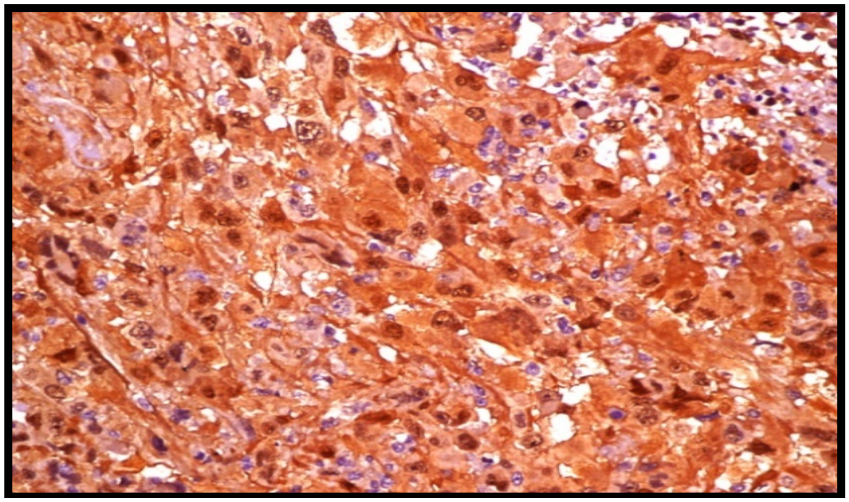


Figure 8: S100 positive-high grade glioma III 40X

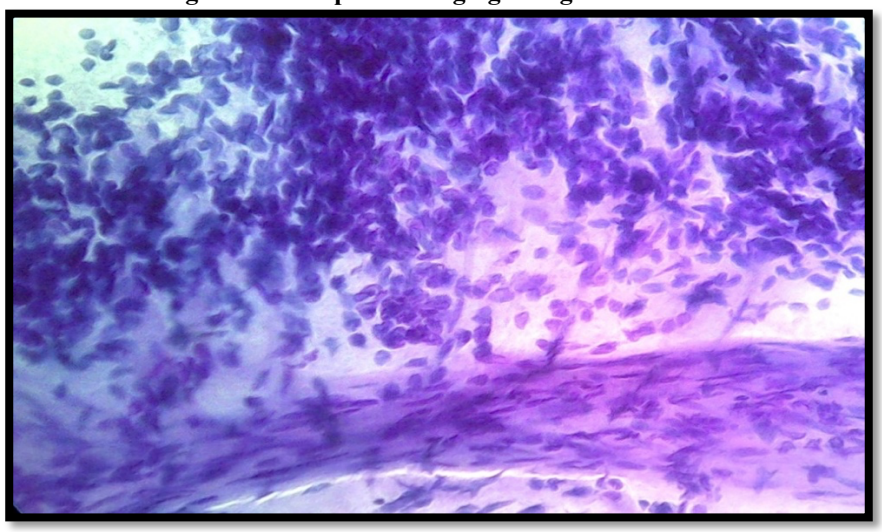


Figure 9: Squash glioma grade IV 40x

Results:

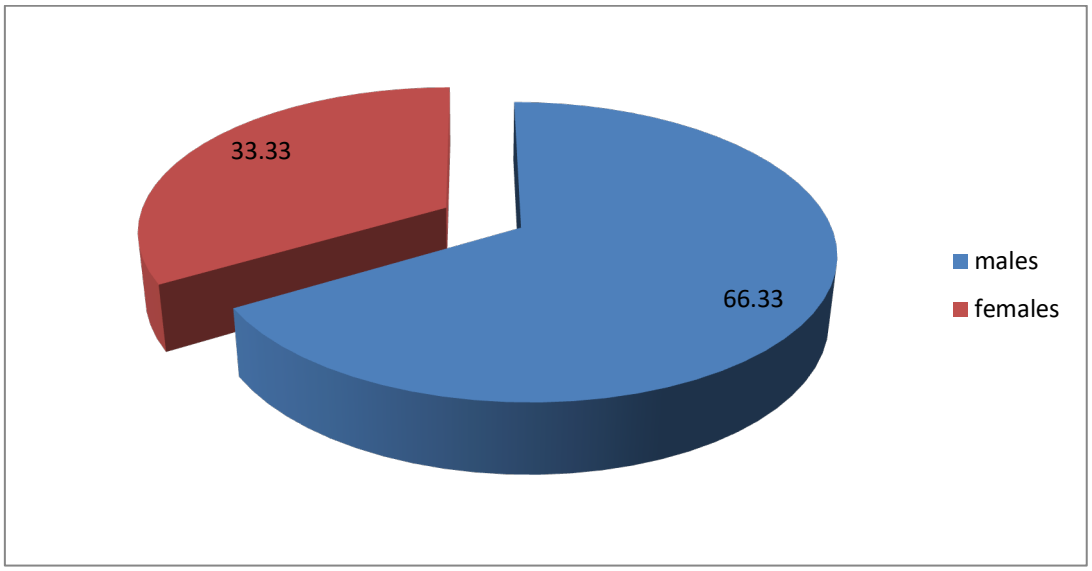
The present study of "Intraoperative diagnosis of central nervous system gliomas by squash smear cytology" consists of a total of 60 glial lesion biopsies reported during the period of 2 years from November 2019 to October 2021 at Tertiary Care Hospital, Telangana State. We screened 256 in patients with central nervous system lesions.
They were categorized as below(table.1).

Table1: Distribution of glial tumors:

Low grade gliomas	39 (65%)
High grade gliomas	16 (26.6%)
Non-neoplastic/reactive	03 (5%)
Secondary tumors	02 (3%)
Total	**60**

Table 2: Age related incidence of GLIAL Tumors

Age	No. of cases	Percentage
<10	08	13.3%
11-20	07	11.6%
21-30	03	5%
31-40	12	20%
41-50	22	36.6%
51-60	04	6.6%
>60	04	6.6%

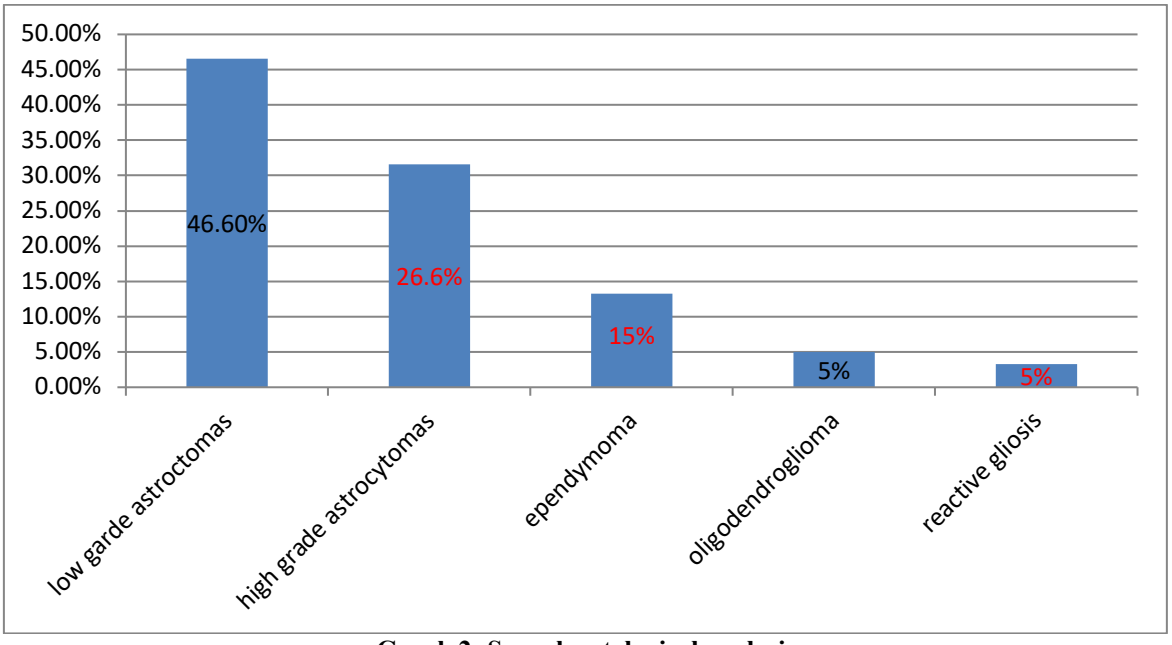


Graph 1: Sex incidence

Male: Female=1.7:1

Table 3: site wise distribution of glial tumors

Site	No. of lesions	Percentage
Frontal	30	50%
Frontoperietal	13	21.6%
Temporo parietal	08	13.3%
Occipito-parietal	02	3.3%
Base of the ventricles	02	3.3%
Supra sellar	02	3.3%
Corpus callosum	02	3.3%
Posterior fossa	01	1.6%



Graph 2: Squash cytological analysis

Table 4: Comparative analysis of cytology and histopathology

Diagnosis	On cytology	On histopathology	Accuracy
Astrocytoma low grade (I&II)	28	27	96.42%
Astrocytoma high grade (III&IV)	19	16	84.2%
Ependymoma	08	08	100%
Oligodendroglioma	03	03	100%
Reactive gliosis	02	02	100%
Total	60	56	93.3%

Table 5: List of discordant cases

Cytology diagnosis	Histopathology diagnosis
1 case of low-grade astrocytoma	1.reactive gliosis
3 cases of high grade astrocytomas	1. Adeno carcinoma Mets. 2. Lymphoma Mets. 3.ependymoma

Discussion

Squash smear cytology is a rapid and accurate technique to diagnose CNS lesions. This method can be used for needle biopsy, material at craniotomy or stereotactic brain biopsy samples.

A study of CNS lesions by squash smear technique gave a diagnostic accuracy of 87% by Asha et al 1989. [6] Bone dust is the commonest artifact from the drill hole in the skull and is mistaken for necrosis and true calcification in the lesion. Biopsy from the edge of the tumor shows mixture of tumor cells and reactive cells or taken from necrotic areas in the tumor results in correct diagnosis. Correlation of clinical CT, MRI scan features increases the diagnostic accuracy of the squash smear cytology.

Overall accuracy (including complete and partial agreement) for squash smear diagnosis of 60 cases was 93.3%.[16] WHO grade II also referred to as well differentiated showed irregular clusters of neoplastic astrocytes with ill-defined margins, some loosely arranged around thin walled blood vessels forming a vague papillary pattern. These results are consistent with study by N. Krishnani, N [7]. Kumari, S. Behari C. Rana, P. Gupta

In our study 1 case of low grade astrocytomas turned out to be a case of reactive gliosis.

3 cases of high grade astrocytoma were diagnosed as 1 case of adenocarcinoma mets,1 case of lymphoma mets. and 1 case of ependymoma.

Glioblastoma must be distinguished from metastatic neoplasms. Progressive multifocal leukoencephalopathy (PML) has to be differentiated from glioblastoma as bizarre astrocytes can be observed in both. The presence of low overall cellularity foamy histiocytes and enlarged nuclei of oligodendrocytes favours PML. These results are consistent with study by Velasco ME, Dahl D, Roessmann V, Gambetti P [8]

Other differential diagnosis of glioblastoma on smears includes pleomorphic xanthoastrocytoma and subependymal giant cell astrocytoma. In both the tumors pleomorphic tumor cells are the features but mitotic figures are rare or absent. Clinical features should be taken into account.

In our study one case of GBM was misinterpreted as pleomorphic xanthoastrocytoma due to diversity of cells. There was one case in our study, where only necrosis was seen on smears but histopathological examination showed typical features of GBM.

Among the various lesions gliomas offered more diagnostic problems in central nervous system tumors. In our study 56 cases were diagnosed correctly and 4 cases were incorrect.

Reactive gliosis commonly has a cellular heterogeneity that is not present in smears of well differentiated astrocytoma. Reactive glial proliferation is most commonly encountered in biopsies taken from the periphery of non-neoplastic cystic and enhancing CT scan lesions in which well differentiated astrocytoma is not a consideration. These results are consistent with study by Sundaram et al [9]

In our study out of 19 cases of high grade astrocytomas on cytosmear, 3 cases were turned out to be adenocarcinoma metastasis from GIT, lymphoma of unknown primary and ependymoma respectively on histology.

In our study (1 case) of pilocytic astrocytoma was encountered in a male, 8 years old, tumor was located in the cerebellar region. Smears showed cells with round or elongated nuclei which tend to be of low grade with unique bipolar long hair like (piloid) processes. Rosenthal fibers and eosinophilic granular bodies are frequent (Kleihues P, Cavenee WK 2000). Nuclear pleomorphism with microvascular proliferation is considered a sign of high grade of malignancy, is often seen and

presumed to represent degenerative atypia (Shankar SK 2000).

In our study there were 5.0% (3 cases) of oligodendroglicoma diagnosed on cytosmears and all of the cases of oligodendroglioma diagnosed correctly on histopathology.

Oligodendroglioma smears easily and may show a gritty sensation of calcification. In smears it appears as a high cellular neoplasm composed of discohesive uniform small cells with round nuclei having speckled chromatin. Calcification may be present. Numerous fine capillaries are present. The typical perinuclear halo “fried egg” appearance in tissue sections may not be seen on smears.

There were 3.48% (9 cases) of mixed glioma in our study and all nine could be reported correctly only on paraffin sections. They were diagnosed as low grade astrocytoma, oligodendroglioma and high grade astrocytomas. Restropective examination of smears however showed mixture of two distinct neoplastic cells, oligodendroglial cells and astrocytes (Adams 1981). The mixture of these two neoplasms could be with distinct components in two different areas as encountered in our study (Park JY, Suh YL, and Han J) [10].

Mixed gliomas caused a marked drop in diagnostic accuracy due to predominance of one histological type and partly due to sampling error.

In our study there were 15% (8 cases) of ependymomas, two were at the base of the ventricles and others were intracranial. All of them were diagnosed as same on histopathology. One case of high grade astrocytoma on cytosmears turned out to be ependymoma on histopathology. These results are consistent with study by 2005 Reyaz N, Tayyab M, Khan SA et al [11].The differential diagnosis includes choroid plexus tumors and PNET. Ependymal cells stain weakly or negatively with GFAP. Ultra-structural features of ependymal cells may be diagnostic.

In our study we misinterpreted 2 cases of metastatic deposits as high grade gliomas on cytosmears due to extensive necrosis and pleomorphic cells. In our study we encountered 2 cases (3.33%) of metastatic neoplasms in which 1 case was adenocarcinoma and 1 case of lymphoma on histopathology. Metastatic neoplasms are usually metastatic carcinomas. They are mostly multifocal, but can be present as solitary mass lesion.(chandrosoma 1989). The lung is the commonest primary carcinoma with brain metastasis followed by breast carcinoma. (Rubinstein LJ, 1977 [13]). According to a study done by Kleihues P, Cavenee WK 2000, the annual incidence of CNS metastasis is given as follows. Annual incidence of metastasis given as follows. Annual incidence of metastatic tumors (per 100,000 population) CNS metastasis 4.1-11

Smears of metastatic carcinoma show cohesive groups of large cells that are distinguished from malignant astrocytes by the absence of cytoplasmic processes. [2] If biopsy is from an area of the brain infiltrated by carcinoma the background of malignant epithelial cells may be reactive brain tissue. Cytomorphological features of the metastatic neoplasms rarely predict the primary site. Occasional smears may suggest renal or breast origin (Surg Pak. 2005) [14].

On analysis of 60 cases, 4 cases had discrepancies. They were preanalytical errors and analytical errors.Pre analytical errors were mostly seen in gliomas due to sampling error.

Analytical errors occurred mostly due to misinterpretation of small round cell tumors, pleomorphic cell tumors and in grading gliomas. Errors were more common where only necrosis, monomorphic cell pattern and pleomorphic cell pattern was seen.

Immunohistochemical assessment of GFAP status is an essential component of the evaluation of neuroepihelial tumors of CNS. Although GFAP status provides prognostic information, currently the major clinical value of determining GFAP status is to assess the likelihood of the patient's response to chemotherapy. In the current study, the GFAP expression was found to be positive in all cases of glial tumors.

This study revealed that the frequency of GFAP expression is highest in glial tumors. This result clarifies the importance of GFAP in differentiating glial from non-glial tumors.

Generally, astrocytoma reveals intense GFAP staining when compared to other gliomas especially at the fibrillary processes. These characteristics of astrocytic neoplasm were high lightened more by the GFAP stain than S100. High expression of GFAP was shown in astrocytic tumors with P value < 0.05. S 100 positive cells seen in all astrocytic tumors. Regarding ependymoma, all cases showed positive GFAP expression, this agrees with that reported by Miettinen et al. in 1986, Maruno M in 1987 [15] The expression of GFAP and S100 was strong mainly in ependymal rosettes and perivascular. This fact has been established by others. The increase of GFAP expression and intensity in both low grade astrocytoma and ependymoma appears to be related to the development of fibrillary processes and GFAP may have a similar function in these two types of cells. Another explanation of increased GFAP expression in low grade astrocytoma is the presence of Rosenthal fibers which contain heavy inclusions of GFAP&B-crystalline.

Regarding oligodendroglioma, all cases were positive to both GFAP and S100 (both cytoplasmic

and nuclear positivity) and this agrees with that reported by Vyberg et al [16]. in 2006.The expression of GFAP in oligodendroglioma was less intense and the pattern was ring like around the nucleus only with occasional short cytoplasmic processes and this is because the cells of this tumor usually contain a low level of GFAP and most cells which stain positively in oligodendroglioma are reactive astrocytes.

In this study, there was a significant inverse relationship between the grade of glioma and the proportional score with P value< 0.05. This result is consistent with those reported by others.

Conclusion

1. Squash smear technique is a economical, reliable, intraoperative rapid diagnostic method of diagnosis of central nervous lesions and has a place in determining the immediate management. The capability of examining a small bit of tissue obtained from a biopsy needle and being confident within five minutes is impossible or impractical with frozen sections.
2. The recent advent of stereotactic biopsy of small intracranial lesions suggests that in the future, smears will be utilized more often. Automation and laminar flow can be put into use to avoid contact with the biohazardous fresh brain tissue.
3. Experience and expertise of the pathologists in neurocytopathology can improve the diagnostic accuracy.
4. At present understanding of molecular genetic basis of diseases and application of molecular biology techniques to cell smears offering new approaches for diagnosis, prevention and treatment of central nervous system lesions.
5. Immunohistochemical Assessment of GFAP status is an essential component of the evaluation of neuroepihelial tumors of CNS. Glial fibrillary acidic protein (GFAP) and S-100 protein (S100P) showed almost equal distribution of both proteins in most of glial tumors.
6. GFAP was helpful in the diagnosis of neuroepithelial tumors and their differentiation from neuroglial tumors.

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