

# Evaluation of the Squash Smear Technique in the Rapid Diagnosis of Central Nervous System Tumors: A Cytomorphological Study

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## Abstract

**Objectives:** To compare the squash smear technique with the histopathological examination in the diagnosis of Central Nervous System (CNS) gliomas and to assess the utility of the squash smear technique for the rapid diagnosis in the neurosurgical biopsies for real time intraoperative consultation. **Methodology:** This was a prospective study conducted in the Department of Neuropathology of Sir J.J. hospital for a period of 6 months. Fifty cases of radiologically suspected CNS gliomas were sent for intraoperative smear cytology and subjected to both histopathological examination and squash smear cytology. The two techniques were then compared for their ability to diagnose as well as grade the tumor. Appropriate statistical analysis methods were then applied to the findings. **Results:** Smear cytology and histopathology diagnoses were compared. The following values were obtained: accuracy of 88%, sensitivity of 91.6%, specificity and positive predictive value of 100%. **Conclusion:** Intraoperative cytological diagnosis is fairly accurate, safe, simple, reliable and cost effective tool for rapid diagnosis of CNS gliomas and is of great value in intraoperative consultation of CNS tumors.

## INTRODUCTION

The intraoperative cytology preparation was first introduced by Eisenhardt and Cushing in early 1930 and by Badt in 1937.<sup>(1,2)</sup> This technique was further championed and documented by Russell et al., in 1937.<sup>(3)</sup> The present technique was introduced by her along with Sir Hugh Cairns in 1930s. The technique has recently gained importance because of advent of CT and MRI guided stereotactic biopsies. In the neurosurgical practice, a rapid pathological diagnosis of the space occupying lesion of the nervous system helps the surgeon to plan the extent of surgery and modify it accordingly.<sup>(4)</sup> Squash smear technique in neuropathology is now well established and continues to gain momentum. High resolution and specialized neuroimaging techniques combined with the use of stereotactic biopsies commonly require the rapid and definitive intraoperative diagnosis on minute and diminutive tissue specimens. The intraoperative smear cytology (squash preparation) is fairly accurate, simple and reliable tool for rapid intraoperative diagnosis of neurosurgical biopsies.<sup>(5)</sup> The current study was undertaken to assess the utility of intraoperative consultations for rapid cytomorphological

diagnosis by squash smear technique and correlate with histopathological diagnosis of CNS gliomas.

## METHODOLOGY

The present study was conducted over a period of 6 months. It was conducted in the Department of Neuropathology, Grant Medical College and Sir J.J. Hospital, Mumbai, India. It included 50 cases of CNS neoplasms which were subjected to intraoperative smear cytology. The biopsy samples obtained at the time of surgery were transported immediately to the neuropathology laboratory in isotonic saline for processing. A smear slide was prepared by taking 1-2 millimeters (mm) of the biopsy material with the scalpel blade, placing the material on a slide and crushing with another slide with just enough pressure to spread the tissue into a thin film. It was then fixed in 95% alcohol and stained by Hematoxylin and Eosin (H & E). Further, paraffin sections were prepared by the residual tissue and stained by H & E. Relevant clinical and radiological data were noted. Smear cytology diagnosis was correlated with the histopathological findings. The tumours were classified according to the World Health Organization classification of

CNS neoplasm 2007.<sup>(6)</sup> The observations were then subjected to appropriate statistical analysis methods.

## RESULTS

The cases where the intraoperative cytological diagnosis was same as the histological diagnosis including the grade of the malignant tumor were considered as the complete correlation. Of the total number of cases two cases were inconclusive i.e. they were neither diagnosed by histopathological examination (HPE) nor by squash smear cytology. Forty-eight cases gave a definitive finding on HPE. Of these, complete correlation with squash smear cytology was obtained in forty-four cases. Further, the grading of four cases was misinterpreted by smear technique. Thus, on statistical analysis the overall diagnostic accuracy obtained in our study was 88%. The sensitivity obtained was 91.6% while, the specificity and positive predictive value obtained were 100%. The results obtained on subjecting the cases to both histopathological and cytological techniques are shown in table 1.

**Figure 1**

Table: 1 Histopathology and Squash smear cytology

Total number of cases (n)	n=50
Correct Histopathological diagnosis	48 (96%)
Correct Cytological diagnosis	44 (88%)
Cases misinterpreted on Cytology	04
Cases inconclusive	02

A significant drop was observed in the diagnostic accuracy of oligodendroglioma, distinguishing between pure astrocytoma, oligodendroglioma and mixed glioma (Table: 2).

**Figure 2**

Table 2: Correlation of Histopathological and Cytological diagnosis

Histopathological diagnosis	Total cases n=50 (%)	Correct Cytological diagnosis	Accuracy
Astrocytoma I & II	25 (50%)	24	96%
Astrocytoma III & IV (GBM)	11 (22%)	10	90.9%
Oligodendroglioma and Mixed Glioma	08 (16%)	06	75%
Ependymoma	04 (8%)	04	100%

Four cases were misinterpreted on cytological examination. But they showed partial correlation, comprising 3 cases of discrepancy of grading and 1 case where one of the components had missed during intraoperative cytological

diagnosis. (Table 3)

**Figure 3**

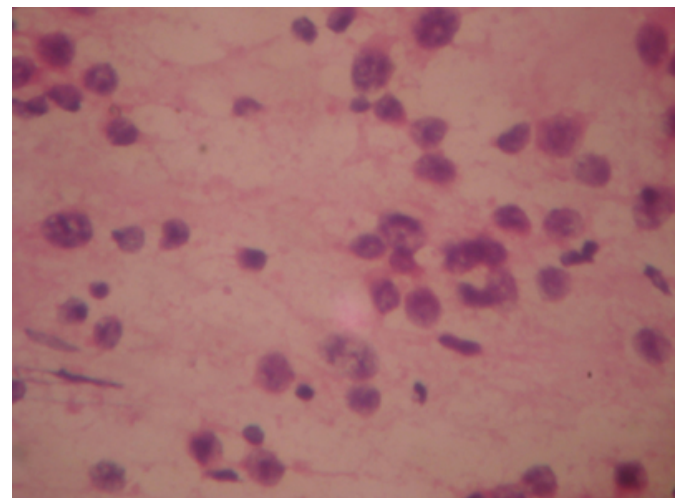
Table 3: Detail of the cases misinterpreted on Cytology, Total cases- 04

Final histopathological diagnosis	Cytological diagnosis	Cases misinterpreted on cytology
Mixed glioma	Oligodendroglioma	01
	Astrocytoma(low grade)	01
Anaplastic Astrocytoma	Low grade Glioma	01
Glioblastoma Multiforme(GBM)	Anaplastic Astrocytoma	01

The most common tumors in our study were Astrocytomas I & II (low grade), which contributed to 50% of the cases. These moderately cellular neoplasms showed minimal anisocytosis with fine to coarse granular chromatin and inconspicuous nucleoli, the cytoplasm was scanty and showed variable processes with fibrillary background (Fig 1).

**Figure 4**

Fig 1: (H & E 40X) Smear of a low grade Astrocytoma showing moderate cellularity against a fibrillary background.

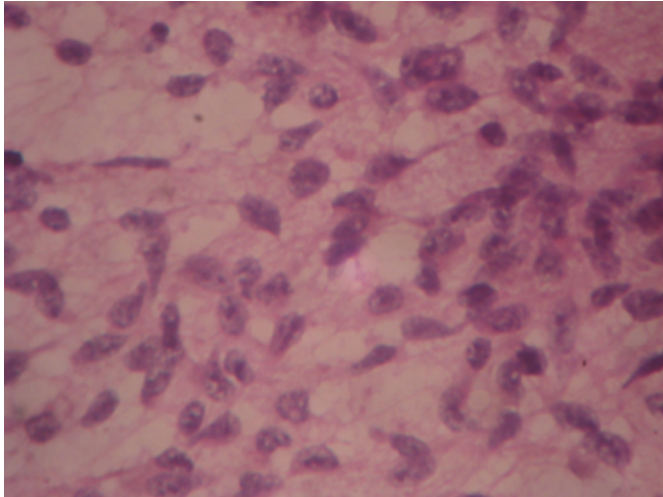


Astrocytoma III & IV (Glioblastoma Multiforme) contributed to 22% of cases. The evenly spread smears of glioblastomas showed cytological heterogeneity with high cellularity. The cells were closely related to blood vessels in a 'perivascular gradient pattern' along with endothelial cell proliferation, glomeruloid bodies, marked cellular pleomorphism, atypia, multinucleation, mitotic figures and necrosis (Fig 2). Anaplastic astrocytoma (Grade III), in addition showed distinct papillary pattern with masses of closely packed cells having hyperchromatic and pleomorphic nuclei, coarse chromatin and prominent nucleoli. Mitotic

figures and increased vascular proliferation were also noted.

### Figure 5

Fig 2: (H & E 40X) Smear from a glioblastoma showing dense cellularity with numerous mitotic figures. (High grade glioma)



Oligodendrogliomas (8%) smeared well and showed moderate cellularity comprised of uniform round cells with well-defined cell margins in a non-cohesive pattern. Cytoplasm was clear with fine granular background, which lacked fibrillary material. Few cases showed prominent thin walled vessels and foci of calcification.

Ependymomas (8%) were highly cellular tumors and showed cells in papillary pattern along the blood stroma and many layers of cells lying on it. Perivascular pseudorosettes were prominent. The cells had scanty cytoplasm and round to oval nuclei with fine granular chromatin and conspicuous nucleoli.

### DISCUSSION

In our study, the overall diagnostic accuracy obtained on using squash smear technique was 88%. A similar diagnostic accuracy was obtained by a study done in France,<sup>(10)</sup> while, other studies have reported a diagnostic accuracy varying from 86% to 97.3%.<sup>(4, 5, 7, 8, 10, 11, 12)</sup> Further, in our study a significant reduction in the diagnostic accuracy of oligodendroglioma was seen. This finding correlated with the findings obtained by a study done in Austria.<sup>(4)</sup> The probable reason for this could be sampling error or the dense fibrillary background of astrocytic component.<sup>(13)</sup>

The squash smear techniques reported a sensitivity of 91.6%. A similar study done in Brazil reported this finding as 97.9%.<sup>(11)</sup> As stated by some studies the reason cited for this

low value could be significant variance observed in grading different areas of smears of astrocytomas. Some authors have also mentioned that in the undergraded cases there are areas of both less and more aggressive astrocytomas and the cytological sampling fails to show the anaplastic component. Further, literature states that small biopsies are not suitable for grading of gliomas.<sup>(7, 11, 12)</sup> A study done in France concludes that multiple biopsies from different area might lessen the false positivity and false negativity.<sup>(10)</sup> The specificity and positive predictive value obtained was 100%. This correlated with the findings obtained in a study done in Brazil which reported a specificity and positive predictive value of 95% and 99.1% respectively.<sup>(11)</sup>

In our study, final diagnosis of two cases failed to show concordance with the final diagnosis. This could be due to the causes like biopsy taken from cyst wall, increased fibrous component, lack of architecture on cytology, morphology obscured by necrosis and inflammation, reactive changes and resistance to desegregation.

### CONCLUSION

Intraoperative smear cytological diagnosis is fairly accurate, safe, simple reliable and cost effective tool for rapid diagnosis of CNS gliomas. It is a preferred method because it offers great details of cellular morphology avoiding distortion and ice artifacts often introduced by frozen section technique. Intraoperative smears permit rapid and reliable diagnosis of CNS gliomas, which helps the surgeon to monitor and modify the approach of surgery. The newer investigating modalities and approaches have placed the neuropathologist in a key role in the diagnosis, further management and treatment of patients with CNS lesions. Nevertheless, it is very essential to review the clinical data as well as radiological, CT and MRI findings before intraoperative evaluation of squash imprint of brain lesions.

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