

Diagnostic Utility of Squash Smear Cytology in Intraoperative Evaluation of CNS Tumors: A Cyto-Histopathological Correlation Study

Sapna Negi¹, Kavita Mardi², Gian Chand³

¹Junior Resident, Department of Pathology, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India. ²Professor, Department of Pathology, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India. ³Associate Professor and Head, Department of Neurosurgery, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India

Abstract

Background: Central nervous system (CNS) tumors require rapid intraoperative diagnosis to guide surgical management. Squash smear cytology is a simple, rapid, and cost-effective technique widely used for this purpose. This study evaluates its diagnostic accuracy by correlating cytological findings with final histopathology and immunohistochemistry (IHC). **Material and Methods:** This cross-sectional analytical study included 50 patients underwent neurosurgical procedures for intracranial space-occupying lesions at IGMCM Shimla. Fresh tissue was used to prepare squash smears stained with Leishman and rapid H&E, while remaining tissue underwent routine histopathology with selective IHC. Cytological findings were correlated with histopathology, the gold standard, and diagnostic indices were calculated using Epi Info 7.2.5.0. **Results:** Patients ranged from 12–86 years (mean 46.3 years) with slight male predominance (54%). Cytologically, meningioma (40%) and high-grade astrocytoma (38%) were most common. Histopathology confirmed meningioma in 40% and high-grade gliomas in 34% of cases, with a wide spectrum of additional tumors. Overall cyto-histological concordance was 90%, with 100% accuracy for high-grade gliomas, oligodendroglioma, SEGA, hemangioblastoma, metastatic carcinoma, eosinophilic granuloma, and choroid plexus carcinoma. Meningioma accuracy was 95%. Discordance (10%) resulted from hemorrhagic smears, poor spreading, spindle-cell mimics, or inadequate samples. Sensitivity was 92%, PPV 100%, and overall diagnostic accuracy 90%. **Conclusion:** Squash smear cytology is a rapid, accurate, and dependable intraoperative diagnostic tool for CNS tumors, demonstrating excellent concordance with histopathology and strong diagnostic performance across a wide tumor spectrum.

Keywords: Squash Smear Cytology, CNS Tumors, Intraoperative Diagnosis, Histopathology, Immunohistochemistry.

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INTRODUCTION

Central nervous system (CNS) tumors, although relatively uncommon compared with other malignancies, remain a major cause of neurological disability and death. The clinical presentation of CNS tumors largely depends on their location and growth pattern, with symptoms such as headache, vomiting, seizures, visual disturbances, motor deficits, or gait instability.^[1] Due to their non-specific nature, imaging modalities like CT and MRI form the cornerstone for initial evaluation. However, radiology alone is insufficient for definitive diagnosis. Histopathology remains the gold standard for accurate classification, grading, and prognostication, particularly with the inclusion of molecular markers in the WHO 2021 CNS tumor classification.^[2]

Intraoperative diagnosis plays a crucial role in neurosurgical practice, helping surgeons determine the nature of the lesion and decide the appropriate extent of surgical excision. Most CNS tumors are soft, friable, and paucicellular in stroma, making them ideally suited for smear preparation.^[3] Squash cytology provides rapid, reliable assessment of cellular morphology, nuclear features, background matrix, and, in some cases, even architectural patterns. An added advantage is that it preserves most of the biopsy sample for subsequent histopathology, immunohistochemistry, and ancillary

molecular tests.^[4]

Several studies have demonstrated high diagnostic accuracy for squash smear cytology, typically ranging from 85% to 95% when correlated with final histopathological diagnosis.^[5] Jindal et al.,^[6] reported an accuracy of 94% in a series of 150 cases, while Khuroo et al documented 90.56% accuracy in 106 cases. These findings underscore the reliability of squash cytology as a rapid intraoperative tool.^[7]

With this background, the present study was conducted at Indira Gandhi Medical College, Shimla, to evaluate the diagnostic utility of squash smear cytology in the intraoperative assessment of CNS tumors. The study aims to analyze the cytological, histopathological, and immunohistochemical features of CNS tumors, and to determine the diagnostic accuracy, sensitivity, and Positive predictive value (PPV) of the

Address for correspondence: Dr. Sapna Negi
Junior Resident, Department of Pathology, Indira Gandhi Medical College, Shimla,
Himachal Pradesh, India
E-mail: sapnanegi917@gmail.com

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squash smear technique by correlating smear findings with final histopathological diagnosis.

MATERIALS AND METHODS

Study design and Population: This hospital-based cross-sectional analytical study was conducted in the Department of Pathology at Indira Gandhi Medical College (IGMC), Shimla, in collaboration with the Department of Neurosurgery, over a period of one year after obtaining Institutional Ethics Committee approval. The study included patients undergoing neurosurgical procedures for intracranial space-occupying lesions (ICSOL) in whom intraoperative pathological evaluation was requested.

Sample Size: Sample size was calculated using Epi Info version 7.2.5.0 with 95% confidence, 97% sensitivity, 50% prevalence, and 7% margin of error, yielding 45 cases; however, 50 cases were included.

Specimen handling and Squash Smear Preparation: Fresh tissue samples obtained intraoperatively from all cases were subsequently subjected to squash cytology for rapid cytomorphological evaluation along with clinical and radiological details. Each specimen was grossly examined for consistency, necrosis, or hemorrhage. Small fragments (1–2 mm) were used to prepare squash smears, yielding 8–10 slides per case. Smears were stained with Leishman and rapid hematoxylin and eosin (H&E) stains for cytological assessment. The remaining tissue was fixed in 10% buffered formalin, processed routinely, and embedded in paraffin for histopathological examination. Formalin-fixed paraffin-embedded sections were stained with H&E, and immunohistochemistry (IHC) was performed in selected cases for diagnostic confirmation and subtyping using standard protocols, including antigen retrieval and HRP-DAB visualization, with appropriate controls. Cytological findings from squash smears were compared with

histopathological diagnoses, considered the gold standard. Concordance, discordance, and causes of discrepancies were systematically evaluated.

Statistical Analysis: Data were entered in Microsoft Excel and analyzed using Epi Info (version 7.2.5.0). Categorical variables were expressed as frequencies and percentages, and quantitative variables as mean $\pm$ standard deviation. Diagnostic performance (sensitivity, specificity, PPV, NPV, and accuracy) of squash smear cytology was calculated using histopathology as the reference standard.

RESULTS

This cross-sectional analytical study was conducted over one year in the Department of Pathology, IGMC Shimla, following Institutional Ethics Committee approval. Fifty patients undergoing surgery for intracranial space-occupying lesions were included. In line with the study objectives, each case was evaluated to determine the diagnostic utility of squash smear cytology and to correlate cytological findings with corresponding histopathological and immunohistochemical features. Immunohistochemical evaluation was performed using markers including SSTR2A, EMA, PR, and p53 in meningiomas, and IDH-1, ATRX, and Ki-67 in gliomas, aiding in tumor characterization, grading, mutation assessment, and WHO-based classification of CNS tumors.

[Table 1] presents the demographic and baseline clinical characteristics of the 50 patients. Most patients were aged 31–40 years (22%), followed by 41–50 (20%) and 51–60 years (18%), with a mean age of 46.3 years. A slight male predominance was observed (54%; male-to-female ratio 1.17:1). Anatomically, lesions most commonly involved the frontal lobe (20%) and fronto-parietal and vertebral levels (18% each), underscoring their frequent involvement in CNS neoplasia.

Table 1: Demographic and Baseline Clinical Characteristics of Patients with CNS Tumors (n = 50)

Characteristic	Category	Number (n)	Percentage (%)
Age Group (years)	11–20	2	4%
	21–30	2	4%
	31–40	11	22%
	41–50	10	20%
	51–60	9	18%
	61–70	8	16%
	71–80	7	14%
	>80	1	2%
Mean Age ($\pm$ SD)	—	46.3 $\pm$ 21.3 years	—
Sex	Male	27	54%
	Female	23	46%

[Table 2] highlights the cytological impressions rendered from squash smear preparations. Meningioma (40%) and high-grade astrocytoma (38%) were most common. Three smears (6%) were deemed unsatisfactory due to obscuring

hemorrhage, necrosis, or inadequate spreading. These findings highlight the broad diagnostic utility of squash cytology as a rapid intraoperative tool for CNS lesions.

Table 2: Spectrum of Cytological Diagnoses on Squash Smear Cytology (n = 50)

Cytological Diagnosis	Number of Cases (n)	Percentage (%)
Meningioma	20	40%
High-grade Astrocytoma	19	38%
Metastatic Carcinoma	2	4%
Choroid Plexus Carcinoma	1	2%

Fibroblastic Meningioma	1	2%
Hemangioblastoma	1	2%
Oligodendroglioma	1	2%
Subependymal Giant Cell Astrocytoma (SEGA)	1	2%
Eosinophilic Granuloma	1	2%
Unsatisfactory Smears	3	6%
Total	50	100%

[Table 3] summarizes the definitive histopathological diagnoses, highlighting the heterogeneous spectrum of CNS tumors. Meningiomas were the most common lesions, predominantly WHO Grade 1 variants, while high-grade gliomas formed the second largest group, mainly

glioblastoma IDH-wild type and grade 4 IDH-mutant astrocytomas. Other glioma subtypes and several rare tumors were also identified. Overall, the table underscores the essential role of histopathology in accurate tumor classification and grading.

Table 3: Spectrum of Histopathological Diagnoses in CNS Tumors (n = 50)

Histopathological Diagnosis	Number of Cases (n)	Percentage (%)
Meningioma (Overall)	20	40%
• Meningothelial meningioma, WHO Grade 1	4	20% of meningiomas
• Transitional meningioma, WHO Grade 1	4	20%
• Psammomatous meningioma, WHO Grade 1	2	10%
• Metaplastic meningioma, WHO Grade 1	2	10%
• Angiomatous meningioma, WHO Grade 1	1	5%
• Fibroblastic meningioma, WHO Grade 1	1	5%
• Lymphoplasmacytic meningioma, WHO Grade 1	1	5%
• Chordoid meningioma, WHO Grade 2	2	10%
• Atypical meningioma, WHO Grade 2	2	10%
• Anaplastic meningioma, WHO Grade 3	1	5%
Glioblastoma, IDH wild type, WHO Grade 4	10	20%
Astrocytoma, IDH mutant, WHO Grade 4	7	14%
Astrocytoma, IDH mutant, WHO Grade 3	1	2%
Astrocytoma, NOS, WHO Grade 2	1	2%
Pleomorphic xanthoastrocytoma, WHO Grade 3	1	2%
Oligodendroglioma, WHO Grade 3	1	2%
Subependymal giant cell astrocytoma, WHO Grade 1	1	2%
Choroid plexus carcinoma, WHO Grade 3	1	2%
Metastatic carcinoma	2	4%
Solitary fibrous tumor, WHO Grade 1	1	2%
Hemangioblastoma, WHO Grade 1	1	2%
Calvarial cavernous hemangioma	1	2%
Ancient schwannoma, WHO Grade 1	1	2%
Eosinophilic granuloma	1	2%
Total	50	100%

[Table 4] outlines the diagnostic agreement between squash smear cytology and final histopathology. Concordance was achieved in 90% of cases, demonstrating a strong overall performance of intraoperative cytology. The five discordant cases were attributable to hemorrhagic obscuration, spindle-

cell mimicry, insufficient material, or poor smear spreading—each highlighting inherent limitations of smear-based interpretation. Despite these challenges, the high rate of concordance underscores the reliability of squash cytology as an intraoperative diagnostic modality.

Table 4: Cyto-Histopathological Concordance, Discordance, and Error Analysis in CNS Tumors (n = 50)

Parameter Category	Details / Diagnosis	Number (n)	Percentage (%)	Comments / Notes
Overall Diagnostic Agreement	Complete concordance between cytology & histopathology	45	90%	High diagnostic reliability
	Discordant cases	5	10%	Explained below
Tumor Groups with 100% Concordance	High-grade astrocytoma / Glioblastoma	19	100%	All correctly predicted on cytology
	Choroid plexus carcinoma	1	—	Full agreement
	Subependymal giant cell astrocytoma	1	—	Full agreement
	Eosinophilic granuloma	1	—	Full agreement
	Metastatic carcinoma	2	—	Full agreement
	Hemangioblastoma	1	—	Full agreement
	Oligodendroglioma	1	—	Full agreement
Meningioma Concordance	Confirmed meningioma cases	19 / 20	95%	One misdiagnosed case detailed below
Discordant Case 1	Cytology: Fibroblastic meningioma	—	—	Final Dx: Solitary fibrous tumor; STAT6 positive; spindle-cell mimicry
Discordant Case 2	Cytology: Hemorrhage	—	—	Final Dx: Calvarial cavernous hemangioma; blood obscured tumor cells

Discordant Case 3	Cytology: Meningioma	—	—	Final Dx: Ancient schwannoma; S100/SOX10 positive; SSTR2 negative
Discordant Case 4	Cytology: Inadequate sample	—	—	Final Dx: Meningioma; insufficient material after smear
Discordant Case 5	Cytology: Poor spread	—	—	Final Dx: Pleomorphic xanthoastrocytoma; morphology lost
Unsatisfactory Smears	Non-diagnostic cytology	3	6%	Necrosis, hemorrhage, poor spreading
Reasons for Discordance	<ul style="list-style-type: none"> • Hemorrhagic/necrotic aspirates • Poor smear technique • Cytological mimicry (spindle-cell tumors) • Loss of architecture 	—	—	Key limitations of squash cytology

[Table 5] highlights the strong diagnostic performance of squash smear cytology, with a sensitivity of 92%, positive predictive value of 100%, and overall diagnostic accuracy of 90%. Although specificity and NPV could not be computed due to the absence of true-negative cases, the available metrics clearly reflect strong diagnostic capability.

Meningiomas showed 95% diagnostic accuracy, while high-grade astrocytomas were identified with 100% accuracy. The unsatisfactory smear rate was low (6%), mainly due to necrotic or hemorrhagic tissue, supporting squash cytology as a reliable intraoperative diagnostic tool.

Table 5: Diagnostic Performance Metrics of Squash Smear Cytology in CNS Tumors (n = 50).

Performance Parameter	Value	Percentage (%)	95% Confidence Interval	Comments / Notes
Total cases evaluated	50	100%	—	All confirmed CNS tumors
Sensitivity	—	92%	89.96% – 94.04%	High true-positive detection
Specificity	—	—	—	Not calculated (no true negatives in study)
Positive Predictive Value (PPV)	—	100%	—	All positive cytology results matched histology
Negative Predictive Value (NPV)	—	—	—	Cannot be computed (no negative cases)
Overall diagnostic accuracy	—	90%	87.5% – 92.5%	Strong concordance with final HPE
Unsatisfactory smear rate	3 cases	6%	—	Due to necrosis, hemorrhage, poor smearing
Meningioma accuracy	19 / 20 cases	95%	—	One SFT misinterpreted as fibroblastic meningioma
High-grade astrocytoma accuracy	19 / 19 cases	100%	—	Excellent cytological predictability
Accuracy in rare tumors	100%	—	—	SEGA, CPC, metastasis, EG, hemangioblastoma
Common causes of diagnostic error	—	—	—	Hemorrhage, limited sample, cytological mimicry

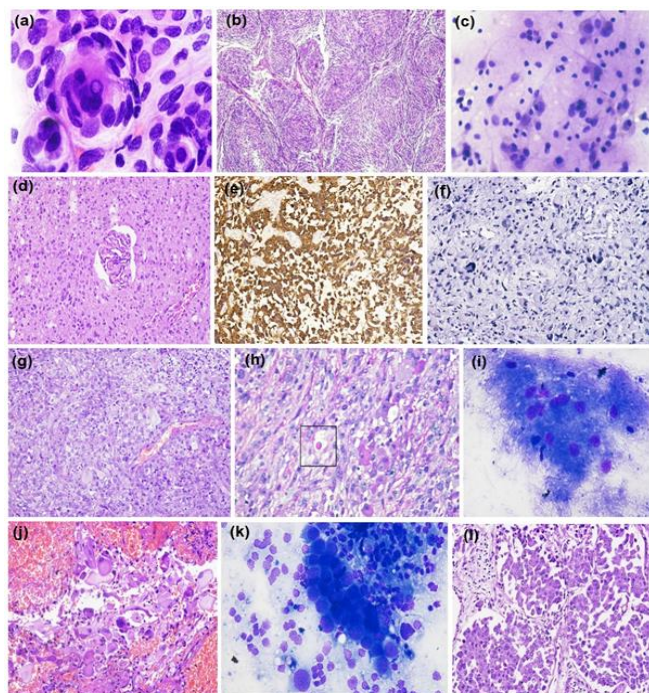


Figure 1: Representative squash smear, histopathology, and IHC findings in CNS tumors. (a) Meningothelial meningioma showing whorls with nuclear grooves and intranuclear inclusions (H&E, 40x). (b) Syncytial nests and whorls of meningothelial cells (H&E, 10x). (c) Grade 4 astrocytoma with

hyperchromatic round to ovoid nuclei (Leishman, 40x). (d) IDH mutant grade 4 astrocytoma showing microvascular proliferation and necrosis (Leishman, 40x). (e) IDH1 immunoreactivity (40x). (f) Loss of ATRX nuclear expression (40x). (g) Pleomorphic xanthoastrocytoma, CNS WHO grade 3, with sheets of xanthomatous cells (H&E, 20x). (h) Eosinophilic granular bodies (PAS, 40x). (i) Subependymal giant cell astrocytoma with a fibrillary background (Leishman, 40x). (j) Giant ganglion like tumor cells (H&E, 40x). (k) Metastatic carcinoma showing papillary clusters with vesicular chromatin, prominent nucleoli, and basophilic cytoplasm (Leishman, 40x). (l) Nests of epithelial tumor cells (H&E, 20x).

Representative cytological, histopathological, and immunohistochemical findings of selected CNS tumors are shown in [Figure 1]. Meningothelial meningioma demonstrated characteristic syncytial nests and whorls, with nuclear grooves and intranuclear inclusions. Grade 4 astrocytoma showed hyperchromatic nuclei, microvascular proliferation, and necrosis, with IDH1 immunoreactivity and loss of ATRX expression in the IDH mutant tumor. Pleomorphic xanthoastrocytoma showed sheets of xanthomatous cells and eosinophilic granular bodies. Subependymal giant cell astrocytoma demonstrated a fibrillary background with characteristic giant ganglion like tumor cells. Metastatic carcinoma showed papillary clusters and nests of epithelial tumor cells with vesicular chromatin and prominent nucleoli [Figure 1].

DISCUSSION

Accurate intraoperative diagnosis of central nervous system (CNS) tumors is essential for neurosurgical decision making because brain tissue is delicate, functionally important, and often available only in limited quantities during biopsy. Squash smear cytology provides rapid intraoperative assessment and is commonly used with crush and touch preparations.^[8] Its diagnostic performance depends on tissue consistency, smear quality, staining, and correlation with clinical and radiological findings.^[6] The present study evaluated the diagnostic accuracy of squash smear cytology by comparing intraoperative findings with final histopathology and immunohistochemistry (IHC).

The highest proportion of patients was observed in the 31 to 40 years (22%) and 41 to 50 years (20%) age groups, consistent with Prakasam G et al.,^[9] and Acharya S et al.,^[10] The mean age of 46.3 ± 21.3 years was comparable to that reported by Prakasam G et al.,^[4] (46.4 ± 10.06), whereas Ramchandani L et al.,^[5] and Tejaswi P et al.,^[11] reported younger cohorts. A slight male predominance (M:F = 1.17:1) was similar to previous studies.^[5,11] The frontal lobe was the most frequent site (20%), consistent with findings from Bajaj D et al.,^[12] and Yeganathan SB et al.^[13]

Meningiomas were the most common tumors (40%), with WHO Grade 1 lesions predominating, consistent with previous reports.^[9,14] Higher grade variants were also identified, emphasizing the importance of accurate intraoperative assessment. SSTR2A showed 100% positivity, while EMA and PR further supported the diagnosis. Variable p53 expression was observed. These findings support the diagnostic value of SSTR2A, EMA, and PR in differentiating meningiomas from spindle cell mimics.^[15-17]

Gliomas constituted the second largest group. IHC markers including IDH1, ATRX, p53, and Ki 67 assisted in distinguishing IDH mutant astrocytomas from IDH wild type glioblastomas and supported contemporary WHO classification.^[16-18] High grade astrocytomas showed increased Ki 67 proliferation indices, consistent with their biological behavior.

Overall squash cytology accuracy was 90%, within the range reported in previous studies (80% to 97.9%). Kumar Verma S et al.,^[19] reported 97.9% accuracy, whereas Ramchandani et al.,^[5] reported 89.7%. Sensitivity and PPV were both 93.6%, comparable to findings reported by Sanjeev K et al.,^[20] and Maity P et al.,^[18] Specificity and NPV could not be calculated because the study included only confirmed lesions, a limitation also reported in previous studies.

Meningiomas showed 95% diagnostic accuracy, comparable with Manda N et al.,^[14] (95.5%), Banerjee M et al.,^[21] (100%), Jain S et al.,^[22] (100%), and Prakasam G et al.,^[9] (94.4%). The single discordant case involved a solitary fibrous tumor interpreted as fibroblastic meningioma, reflecting the recognized cytological overlap among spindle cell tumors.

Squash cytology performed particularly well for glial tumors, with 100% accuracy for glioblastoma, 90% for

astrocytoma, and 100% for oligodendroglioma. These findings fall within previously reported ranges for astrocytoma, glioblastoma, and oligodendroglioma.^[8] High grade gliomas often smear readily, allowing better nuclear detail and facilitating cytological diagnosis. Rare tumors, including SEGA, hemangioblastoma, choroid plexus carcinoma, metastatic carcinoma, and eosinophilic granuloma, were also accurately diagnosed, supporting the value of clinical and radiological correlation.

Discordance occurred in 10% of cases and was mainly related to cytological mimicry and sampling limitations, consistent with previous reports. Hemorrhagic obscuration, poorly spread smears, and overlapping morphology contributed to diagnostic errors. Ancient schwannoma and calvarial hemangioma are recognized diagnostic challenges and have also been misclassified in previous studies. The unsatisfactory smear rate of 6% was comparable with reports by Jindal et al.,^[6] Shrestha et al.,^[23] and Maity P et al.,^[18] emphasizing the importance of proper tissue handling and communication between the neurosurgeon and pathologist.

The diagnostic accuracy observed was comparable with studies from India, Nepal, and Brazil, where reported accuracy ranged from 80% to 97.3%. Squash cytology also offers advantages in speed, nuclear detail, cost effectiveness, and tissue conservation compared with frozen section in selected settings.^[12, 23]

The major strength of this study was the comprehensive assessment of CNS tumors using cytology, histopathology, and IHC according to WHO CNS 2021 standards. The inclusion of diverse intracranial and spinal lesions with real time intraoperative sampling enhanced its clinical relevance. Limitations included the modest sample size, absence of true negative cases preventing calculation of specificity and NPV, technical difficulties with hemorrhagic or poorly spread smears, limited tissue availability, and inconsistent access to advanced molecular markers.

Overall, squash smear cytology demonstrated high diagnostic accuracy, sensitivity, and cytohistological concordance. Its performance was particularly strong for meningiomas and high-grade gliomas and remained useful for rare tumors. Routine use, supported by appropriate sampling, improved smear preparation, multidisciplinary communication, and complementary IHC, may further reduce diagnostic pitfalls. Future multicentric studies incorporating molecular profiling may help refine CNS tumor classification and improve intraoperative diagnostic precision.

CONCLUSION

Squash smear cytology is a rapid, reliable, and highly informative intraoperative diagnostic tool for CNS tumors, with an overall accuracy of 90% in this study. High-grade gliomas and meningiomas showed strong concordance, and several rare entities were accurately identified. Most discrepancies resulted from hemorrhagic or inadequate samples and cytological overlap rather than interpretive errors. Correlation with clinical, radiological, histopathological, and IHC findings further enhances its value, particularly in resource-limited settings lacking frozen section or advanced molecular diagnostic facilities.

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Conflicts of interest

There are no conflicts of interest.

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