Original Research Article

Diagnostic Utility of Squash Cytology and Frozen Section in Central Nervous System Lesions at a tertiary care center

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Abstract

Introduction: Intra-operative pathological evaluation of central nervous system (CNS) lesions is essential for guiding neurosurgeons regarding the extent of resection and the need for additional sampling. Squash cytology and frozen section are the two most widely used techniques, each offering unique advantages and limitations.

Aim: To compare the diagnostic accuracy of squash cytology and frozen section in intra-operative diagnosis of CNS lesions and determine situations where one technique is superior.

Materials and methods: A cross-sectional study was conducted on 50 neurosurgical biopsies received for intra-operative consultation over one year. Squash smears and frozen sections were prepared, stained with hematoxylin and eosin, and compared with final paraffin-section diagnosis. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated.

Results: Among 50 cases, glial tumors formed the major proportion (n=22), with glioblastoma as the most common high-grade lesion (n=9). The diagnostic accuracy of squash cytology was 90%, sensitivity 92%, and specificity 78%. Frozen section demonstrated higher sensitivity (96%), specificity (88%), and overall diagnostic accuracy (96%). Frozen section was superior in architectural

assessment, whereas squash smear demonstrated better nuclear detail. Combined interpretation provided the highest accuracy.

Conclusion: Frozen section had marginally better diagnostic performance than squash cytology; however, both techniques complement each other. Using both methods simultaneously increases diagnostic accuracy and enhances intra-operative decision-making.

Key words

Squash cytology, Frozen section, CNS tumors, Intra-operative diagnosis, Neurosurgical pathology.

Introduction

Intra-operative diagnosis plays a pivotal role in neurosurgery as it helps guide the extent of tumor resection, assess tissue adequacy, and determine the need for additional biopsies. Although radiology offers significant preoperative insights, histopathology remains indispensable for confirming the nature of central nervous system (CNS) lesions during surgery [1, 2]. Squash cytology and frozen section are the two principal techniques used during intra-operative consultation. Squash cytology is particularly suitable for soft CNS tissues due to its ease of preparation, rapid turnaround time, and minimal tissue requirement. Nuclear and cellular details are often better appreciated on squash smears. Frozen section, in contrast, preserves tissue architecture and is particularly useful in identifying features such as necrosis, microvascular proliferation, whorls, and infiltrative patterns. However, brain tissue is prone to freezing artifacts due to its high water content, which can occasionally hinder interpretation [3, 4]. With the increasing emphasis on molecular classification in the WHO CNS tumor framework, optimal intra-operative tissue triaging has become essential. Understanding the strengths and limitations of squash cytology and frozen section can significantly improve diagnostic efficiency, especially when tissue is scanty or lesions are heterogeneous [5, 6]. This study aims to evaluate and compare the diagnostic accuracy of squash cytology and frozen section in the intra-operative assessment of CNS lesions in a sample of 50 cases.

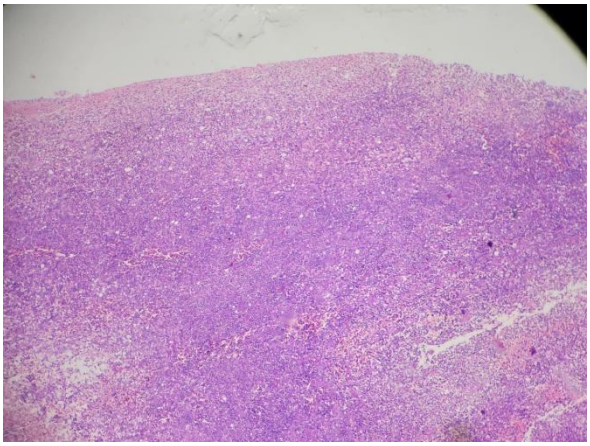
Materials and methods

This cross-sectional study was carried out in the Department of Pathology, SBKS MI & RC, a tertiary care teaching hospital over a period of one year. All neurosurgical specimens submitted for intra-operative consultation during the study period were screened for eligibility. Prior approval for the study was obtained from the Institutional Ethics Committee, and all procedures were performed in accordance with standard hospital protocols for diagnostic neuropathology. A total of 50 Central Nervous System (CNS) biopsies were included in the study. These comprised brain and spinal cord lesions that required rapid intra-operative evaluation to guide neurosurgical decision-making. Only those cases in which sufficient tissue was available for preparation of both squash cytology smears and frozen sections were considered. Lesions arising predominantly from bone, samples that were too scanty for technical processing, and specimens consisting mainly of blood clots or necrotic material without viable tissue were excluded to avoid misinterpretation and technical artifacts.

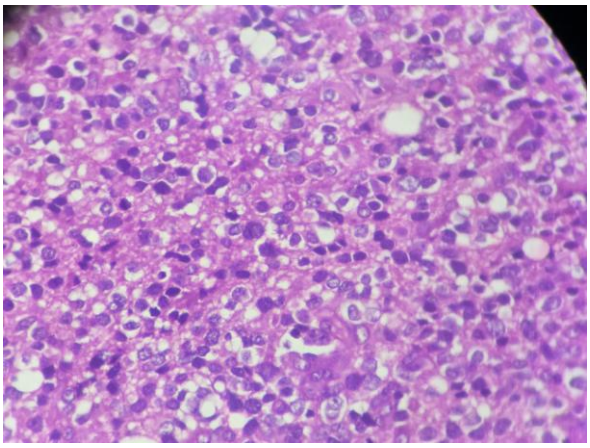
For squash cytology, a small portion of the tissue measuring approximately 0.5–1 mm^2 was selected from the biopsy sample. The tissue fragment was placed between two clean glass slides and gently compressed to spread the material evenly. Care was taken to avoid excessive pressure, which can distort nuclear morphology. The smears were immediately fixed in an ether–alcohol fixative to preserve cellular and nuclear details. After fixation, the slides were stained using the rapid hematoxylin and eosin (H&E) staining technique, allowing assessment of cytomorphology within minutes. A

separate portion of the biopsy specimen was processed for frozen section preparation. The tissue was quickly embedded in optimal cutting temperature (OCT) compound and mounted on a cryostat chuck. Sections were cut at a temperature of approximately –15°C, which is generally suitable for most CNS tissues. The thin frozen sections were then stained with routine H&E for immediate microscopic evaluation. This method allowed preservation of tissue architecture, enabling identification of patterns such as papillary structures, whorls, infiltrative margins, necrosis, and microvascular proliferation. Following intra-operative consultation, all specimens were subjected to routine processing. Formalin-fixed, paraffin-embedded (FFPE) sections stained with H&E served as the reference ("gold standard") diagnosis for all cases. Whenever necessary, additional levels and ancillary tests were performed to confirm the final diagnosis. The diagnostic impressions obtained from squash cytology and frozen section was compared independently with the final paraffin-section diagnosis. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy were calculated for both modalities. Standard definitions and statistical formulas for diagnostic test evaluation were applied to assess the performance of each technique (**Photo – 1 to 4**).

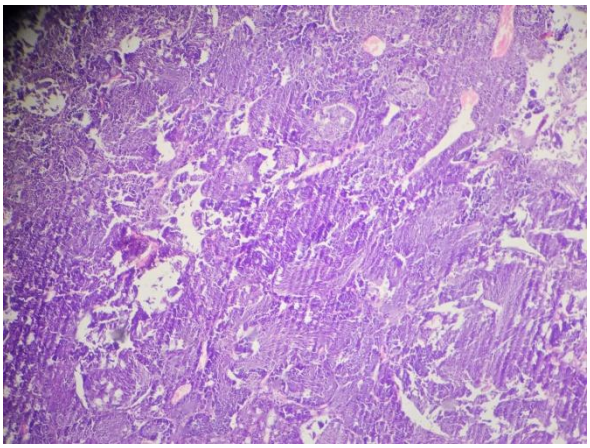
Photograph – 1: Low-power view showing a diffusely infiltrating glial neoplasm composed of monotonous oligodendroglial cells (H & E stain, 4X).



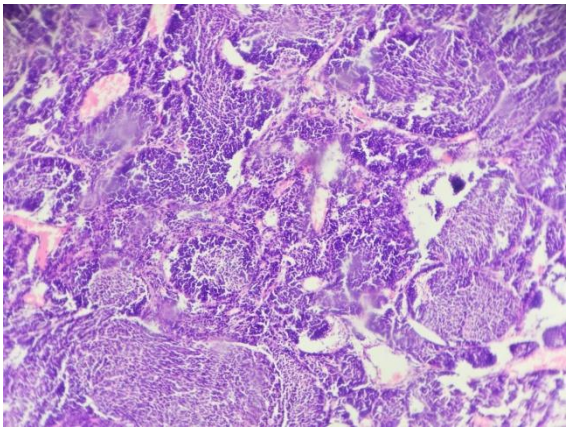
Photograph – 2: High-power view showing uniform round neoplastic cells with distinct cell borders and clear perinuclear halos, imparting the characteristic "fried-egg" appearance. (H & E stain, 40X)



Photograph – 3: Section shows a densely cellular tumor composed of sheets of small round to oval cells giving a classic "small round blue cell" appearance.(H & E stain, 4X).



Photograph – 4: Section shows Areas of nuclear molding with occasional Homer Wright rosettes (H & E stain, 10X).



Results

A total of 50 cases were included in the present study. The age of the patients ranged from 0 to 75 years, with the majority belonging to the 31–50 years age group (n=22, 44%). This was followed by patients aged 21–30 years (n=10, 20%). There was a distinct male predominance with a male-to-female ratio of 1.8:1. Out of the 50 cases evaluated, glial tumors constituted the largest group (n=22, 44%). This was followed by meningiomas (n=6, 12%), schwannomas (n=4, 8%), and metastatic lesions (n=4, 8%). The detailed distribution of cases as per intra-operative and final diagnosis is shown in **Table - 1**.

Table – 1: Distribution of cases as per intra-operative and final diagnosis.

Diagnosis	**Number of Cases (n=50)**
Glial tumours	22
Meningioma	6
Schwannoma	4
Metastatic tumours	4
Ependymoma	3
Medulloblastoma	3
Pituitary adenoma	2
Inflammatory / Granulomatous lesion	4
Others (Cysts, Demyelination etc.)	2

Both squash cytology and frozen sections were compared with the final paraffin-section diagnosis. Squash cytology rendered correct diagnosis in 45 cases, whereas frozen section diagnosis was correct in 48 cases. The remaining cases showed discrepancy due to sampling issues, thick smears, or freezing artefacts. A detailed comparison is shown in **Table - 2**.

Frozen section was more helpful than squash cytology in 18 cases, particularly in identifying architectural patterns in meningiomas, metastatic deposits, and high-grade glial tumors. Squash cytology was superior in 10 cases, especially in soft glial tumors where nuclear details were more prominent. Both techniques were equally useful in 20 cases. Diagnosis could be confidently given only when both methods were interpreted together in 5 cases. This comparison is summarized in **Table - 3**.

The diagnostic performance of squash cytology and frozen section was evaluated by comparing the intra-operative impressions with the final paraffin-embedded histopathological diagnosis, which served as the gold standard. Squash cytology demonstrated a diagnostic accuracy of 90%, correctly identifying 45 out of 50 cases. It showed a sensitivity of 92%, indicating a high ability to correctly identify true positive cases, while the specificity was 78%, reflecting a moderate ability to correctly rule out non-neoplastic or benign lesions. The positive predictive value (PPV) was 93%, suggesting that the majority of lesions diagnosed as positive on squash cytology were confirmed on final diagnosis. The negative predictive value (NPV) was 75%, indicating that a portion of negative cases on squash cytology were upgraded on paraffin sections, primarily due to sampling limitations or thick smears.

In comparison, frozen section demonstrated superior performance, with a diagnostic accuracy of 96% and correct diagnosis in 48 out of 50 cases. The technique showed a sensitivity of 96% and specificity of 88%, indicating better reliability in distinguishing between neoplastic and non-neoplastic lesions. The PPV and NPV were both high at 96% and 88%, respectively, reflecting greater consistency between intra-operative frozen section interpretation and final histopathological diagnosis. Overall, frozen section outperformed squash cytology across all diagnostic parameters, although both techniques were highly effective when used in conjunction. These are summarized in **Table – 4.**

Discussion

Intra-operative consultation plays a crucial role in neurosurgical practice by providing rapid and reliable diagnostic guidance to the operating

surgeon. Both squash cytology and frozen section techniques are essential tools in this setting, each contributing unique diagnostic advantages [7, 8]. In the present study of 50 CNS lesions, both modalities demonstrated high diagnostic performance; however, frozen section showed a slightly superior overall accuracy.

Table - 2: Comparison of Squash Cytology, Frozen Section and Final Diagnosis (n=50).

Lesion Type	Correct on Squash	Correct on Frozen	Correct on Histopathology (Gold Standard)
Glial tumours (n=22)	18	20	22
Meningioma (n=6)	4	6	6
Schwannoma (n=4)	3	4	4
Metastasis (n=4)	2	3	4
Ependymoma (n=3)	3	3	3
Medulloblastoma (n=3)	3	3	3
Pituitary adenoma (n=2)	2	2	2
Inflammatory/ Granulomatous (n=4)	4	4	4
Others (n=2)	1	1	2
Total Correct	**45/50**	**48/50**	**50/50**

Table - 3: Comparative Utility of Squash Cytology and Frozen Section (n=50).

Category	Number of Cases
Squash better than frozen	10
Frozen better than squash	18
Both equally useful	20
Diagnosis only when both interpreted together	5
Inconclusive on either method	2

Table - 4: Diagnostic Accuracy of Squash Cytology and Frozen Section.

Parameter	Squash Cytology	Frozen Section
Sensitivity	92%	96%
Specificity	78%	88%
PPV	93%	96%
NPV	75%	88%
Diagnostic Accuracy	90%	96%

Squash cytology correctly diagnosed 45 out of 50 cases (90%), whereas frozen section provided accurate diagnosis in 48 cases (96%). This is consistent with previous studies that have shown comparable accuracy for squash cytology (87–95%) and frozen section (90–97%). The slightly better performance of frozen section in our study can be attributed to its superior ability to demonstrate architectural patterns, which are essential for diagnosing tumors such as meningiomas, metastatic lesions, and glioblastomas. In contrast, squash cytology proved more effective in assessing soft, friable CNS lesions, particularly low-grade and intermediate-grade glial tumors, due to its ability to preserve nuclear morphology and cytoplasmic details.

Squash cytology was particularly useful in glial tumors, where nuclear atypia, fibrillary background, and cellular pleomorphism were more readily appreciated. The technique requires

minimal tissue and is simple, rapid, and valuable in cases where the biopsy specimen is limited [9, 10, 11]. In 10 cases, squash cytology provided a better diagnostic impression than frozen section. These were predominantly soft tissue lesions where architecture is less informative than cytological detail. However, squash smears may be suboptimal when the tissue is firm (e.g., meningioma, schwannoma), leading to thick or poorly spread smears, which can obscure critical diagnostic features. Additionally, crush artefacts may cause distortion of lymphocytes, leading to overdiagnosis of round-cell tumors. Frozen section offered significant advantages in evaluating architectural patterns, especially in meningiomas, metastatic tumors, and glioblastomas [12, 13]. In our study, frozen section was superior in 18 cases, particularly where features such as whorls, palisading necrosis, microvascular proliferation, or infiltrative borders were necessary for accurate diagnosis. Frozen section was more reliable in distinguishing infiltrative gliomas from reactive gliosis, as the latter demonstrates preserved architecture and absence of tumor infiltration. Similarly, the identification of necrosis in glioblastomas was more evident on frozen sections, aiding in the correct grading of the tumor.

However, frozen sections may show freezing artefacts, especially in soft brain tissue, which can obscure nuclear details [14, 15]. Nevertheless, in the present study, frozen section still maintained superior diagnostic accuracy. In 5 cases, a definitive diagnosis could only be reached when both squash cytology and frozen section were interpreted together. This highlights the complementary nature of the two techniques. Squash cytology provides excellent nuclear detail, while frozen sections preserve tissue architecture, making their combined use particularly valuable in heterogeneous tumors [16, 17].

Table - 5: Comparison of Diagnostic Performance of Squash Cytology and Frozen Section With Other Studies.

Study	Diagnostic Accuracy (%)	Sensitivity (%)	Specificity (%)
Savargaonkar, et al. (2001) [6]	Frozen: 94% Squash: 89%	Frozen: 96% Squash: 90%	Frozen: 88% Squash: 76%
Samal, et al. (2018) [18]	Frozen: 85.7% Squash: 75%	Frozen: 96.1% Squash: 94.4%	Frozen: 75% Squash: 85.7%
Biswal, et al. (2019) [19]	Frozen: 81.88% Squash: 78.88%	Frozen: 78.89% Squash: 81.11%	Frozen: 98.95% Squash: 98.89%
Present Study (2025)	**Frozen: 96%** **Squash: 90%**	**Frozen: 96%** **Squash: 92%**	**Frozen: 88%** **Squash: 78%**

Diagnostic discrepancies observed in the present study could largely be explained by a combination of sampling issues, technical limitations, and the nature of the tissue obtained during intra-operative consultation. One of the primary factors was sampling error, particularly in gliomas, which are known for their marked histological heterogeneity. When only low-grade or less representative areas were sampled during surgery, the intra-operative diagnosis tended to underestimate the true tumor grade, leading to discordance with the final paraffin-section findings. Technical constraints associated with both techniques also contributed to misinterpretation in several cases. Thick or inadequately spread squash smears occasionally obscured cytomorphological details and produced artefacts resembling round-cell tumors, thereby complicating accurate evaluation. Conversely, freezing artefacts in frozen sections sometimes resulted in distortion of tissue architecture and nuclear morphology, especially in soft brain tissue, making interpretation more challenging. Another important source of error

was the presence of necrotic or non-representative tissue. In two cases, the initial biopsy yielded predominantly necrotic material, which masked diagnostic features on squash cytology and led to incorrect intra-operative impressions. Only subsequent paraffin-embedded sections, prepared from more viable areas of the specimen, established the correct diagnosis. These patterns of diagnostic discordance have also been highlighted in previous studies, underscoring the critical importance of proper tissue sampling, awareness of technique-related artefacts, and correlation with clinical and radiological findings to improve accuracy during intra-operative neuropathological assessment. The results of the present study align with previously published literature, which shows slightly higher diagnostic accuracy for frozen sections as compared to squash smears. Studies by Savargaonkar, et al.; Samal, et al.; and Biswal, et al. have reported comparable findings, with frozen section providing greater reliability in lesions where architecture is critical [6, 18, 19]. (**Table – 5**) At the same time, the high diagnostic performance of squash cytology reinforces its value as an adjunct technique, particularly in resource-limited settings where access to cryostats may be restricted.

Conclusion

The present study supports the conclusion that both squash cytology and frozen section are indispensable tools for intra-operative diagnosis of CNS lesions. Frozen section demonstrates marginally higher diagnostic accuracy, but squash cytology remains essential due to its rapidity, minimal tissue requirement, and superior preservation of cytological detail. The integration of both techniques results in the highest diagnostic confidence and should be the preferred approach in routine neurosurgical pathology.

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