Original Article

Intraoperative Squash Smear in Central Nervous System Lesions and Its Correlation with Histopathology

Vijayalaxmi S Patil*, S.B. Hippargi, Mamatha K, Sneha Jawalkar, Sai Chulki

Department of Pathology, BLDE(DU) Shri B.M. Patil Medical College, Hospital & Research Centre, Vijayapura, Karnataka, India

DOI: 10.21276/APALM.3395

***Corresponding Author**:
Dr Vijayalaxmi S Patil
vijayalaxmi.patil@bldedu.ac.in

Submitted: 28-Jun-2024
Final Revision: 19-Sep-2024
Acceptance: 28-Sep-2024
Publication: 06-Oct-2024

Abstract

Background: Space-occupying lesions (SOLs) of the central nervous system (CNS) are a significant cause of neurological morbidity and mortality. Intraoperative squash smear cytology is a simple and reliable technique for the rapid intraoperative diagnosis of neurosurgical specimens. It has proven particularly valuable in diagnosing CNS tumors and aids the surgeon in planning the extent of surgery. This study was designed to assess the accuracy of intraoperative squash smear in diagnosing central nervous system (CNS) space-occupying lesions.

Methods: This study was a retrospective observational study consisting of 41 patients diagnosed with space-occupying lesions in the CNS. Smears were prepared from the biopsy sample obtained at the time of operation and were stained with rapid hematoxylin and eosin, Papanicolaou stain. The cytological diagnosis offered intraoperatively was compared with the histopathological diagnosis. WHO classification of CNS tumors 2021 was used to classify tumors.

Results: Among the forty-one cases studied, thirty-four cases showed complete correlation with histopathology, five cases showed partial correlation, and two cases were discrepant. The diagnostic accuracy was 82.9% by complete concordance and increased to 95% on applying partial concordance criteria.

Conclusion: Intraoperative squash smear cytology is a simple, rapid, cost-effective method and provides diagnosis with fair accuracy in brain lesions, especially brain tumors, and is of great value in intraoperative consultation.

Keywords:

Central Nervous System Lesions, Intraoperative Diagnosis, Squash Cytology, Histopathology.

Introduction

Space-occupying lesions (SOLs) in the central nervous system (CNS) are significant due to their potential to cause substantial neurological morbidity and mortality. These lesions can be tumors, abscesses, hematomas, cysts, or any other abnormal masses that occupy space within the cranial or spinal cavities [1]. Intracranial lesions occur at a rate of 10 to 17 cases per 100,000 people annually [2].

The clinical features of intracranial lesions are typically diverse and often resemble those of many other diseases, frequently

leading to diagnostic delays. Preoperative diagnostic tools, such as computed tomography (CT) and magnetic resonance imaging (MRI), are crucial for detecting CNS lesions. However, the radiological diagnosis of a space-occupying lesion (SOL) must be confirmed by histological examination of a tissue biopsy [3].

The concept of intraoperative cytology in the evaluation of central nervous system (CNS) lesions was first introduced by Eisenhardt and Cushing in the 1920s [4]. Russel et al. further elaborated on crush cytology and introduced the "wet film technique" for diagnosing CNS lesions [5].

Crush cytology and frozen section are two important diagnostic tools for intraoperative CNS consultation, but both show variable results due to the lack of experience of pathologists in the interpretation of neurosurgical specimens. While a few studies in the literature have demonstrated a correlation between frozen section and cytology in the intraoperative evaluation of CNS lesions, cytology is preferred over frozen section due to the formation of ice crystal artifacts on frozen sections, the limited availability of frozen section machines in our country, and the difficulty in their maintenance [6].

CNS tumors are very soft and gel-like in consistency due to scant connective tissue, and this feature is exploited in squash smears as cytological details are easily seen in smears. This method has been shown to play an important role, especially in the diagnosis of CNS tumors, by avoiding unnecessary extensive craniotomies and helping the neurosurgeon plan and modify the extent of surgery [2].

The study was designed to assess the accuracy of intraoperative squash smear in the diagnosis of central nervous system (CNS) space-occupying lesions.

Materials and Methods

The present study was conducted in the Department of Pathology, BLDE (Deemed to be University), Shri B.M. Patil Medical College, Hospital & Research Centre, Vijayapura. It was a retrospective observational study carried out after obtaining institutional ethical clearance, from June 2019 to June 2022.

All cases presenting with CNS lesions, in which intraoperative cytopathological evaluation and subsequent histopathological evaluation were performed, were included in the study. Cases in which cytology smears were inadequate for interpretation were excluded from the study.

Intraoperatively, tissue samples sent in isotonic saline were utilized to make smears for cytological evaluation using the squash technique. Smears were prepared according to standard operating procedures. Alcohol-fixed smears were stained routinely by hematoxylin and eosin (H&E) and Papanicolaou (PAP) methods.

After the completion of the operative procedure, the resected biopsy samples received in 10% formalin were examined grossly and processed. Paraffin-embedded sections were prepared and routinely stained with H&E. Special stains (ZN) were used in selected cases. After examining the H&E-stained formalin-fixed paraffin-embedded (FFPE) tissue sections, a final diagnosis was given. Intraoperative cytological diagnosis was compared to histopathological diagnosis, which is considered the gold standard, and the results were calculated. The WHO classification of CNS tumors (2021) was used to classify the tumors.

Results

Forty-one patients with intracranial lesions were enrolled in the study. They underwent surgery, and their specimens were

examined. There were 23 females and 18 males in the study. The study included 4 pediatric patients and 37 adults, with the mean age of the adults being 49 years, and an age range of 22 to 75 years.

Among the 41 cases studied, 4 were non-neoplastic lesions, and 37 were neoplastic. Non-neoplastic lesions included 2 cases of granulomatous inflammation (Fig. 1 & 2), 1 epidermal cyst, and 1 case of mucormycosis. Mucormycosis was misinterpreted on cytology as high-grade glioma due to the detection of only necrotic material.

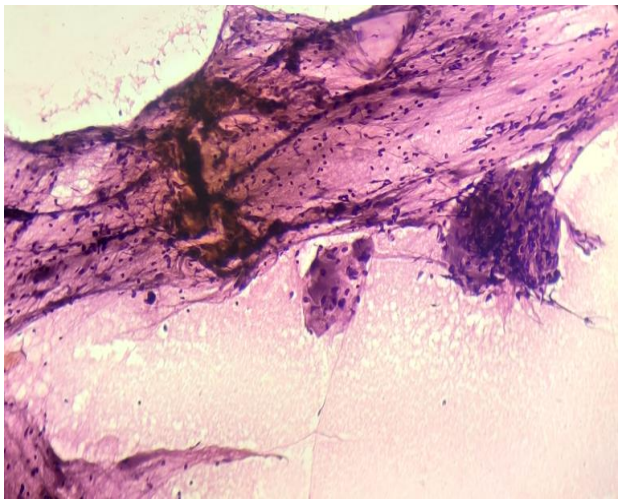

Figure 1: Photomicrograph of Squash Smear showing necrotising granuloma (H&E stain,10 x)

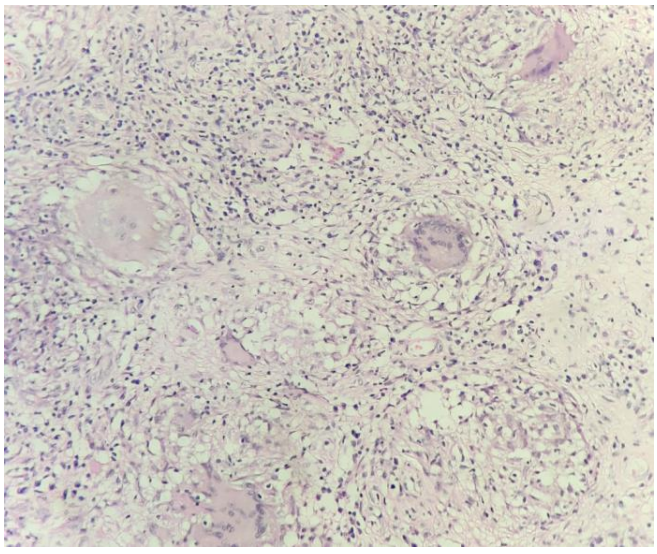

Figure 2: Photomicrograph of tissue section showing necrotising granuloma (H&E stain,10 x)

Of all the neoplastic lesions in our study, glial tumors were the most common, comprising about 60.9%, among which grade 2 astrocytoma was the most common (29.2%, n = 12). Five cases of meningioma (Fig. 3), two cases of medulloblastoma, and one case each of ependymoma (Fig. 4), pituitary adenoma, neurocytoma, schwannoma, and metastatic carcinoma were noted in our study. The distribution of tumors according to the final histopathological diagnosis and the accuracy of squash cytology is tabulated in [Table 1].

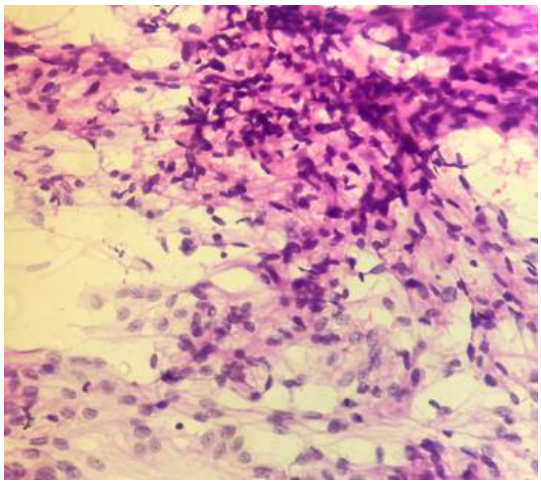

Figure 3: Photomicrograph of Squash Smear of meningioma showing spindle shaped meningothelial cells (H&E stain, 40x)

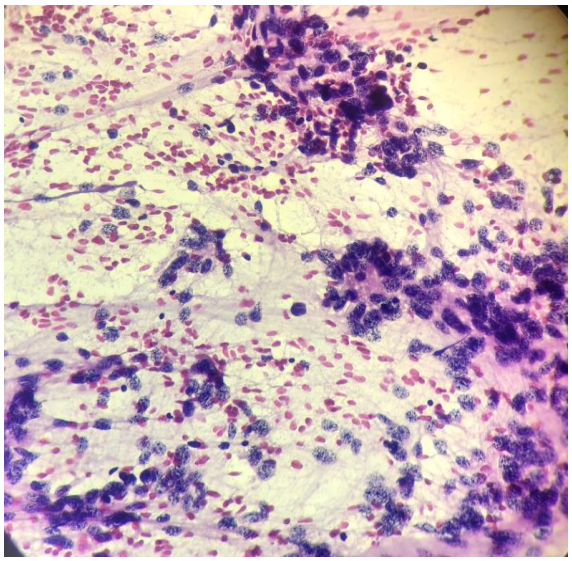

Figure 4: Photomicrograph of Squash Smear of ependymoma showing small round cells and rosettes (H&E stain, 40 x)

Table 1: Accuracy of squash cytology in diagnosis of different CNS lesions

Sl No	Histopathological diagnosis	Correct cytopathological diagnosis	Total cases	Accuracy
1	Granulomatous inflammatory lesions	02	02	100%
2	Epidermal cyst	01	01	100%
3	Mucormycosis	00	01	0
4	Astrocytoma Grade 2	8	12	66.7%
5	Astrocytoma Grade 3	3	5	60%
6	Glioblastoma	7	8	87.5%
7	Meningioma	5	5	100%
8	Ependymoma	1	1	100%
9	Medulloblastoma	1	2	50%
10	Neurocytoma	1	1	100%
11	Pituitary adenoma	1	1	100%
12	Schwannoma	1	1	100%
13	Metastatic carcinoma	1	1	100%

Cases where the cytology and histopathology diagnoses and grades matched were considered to have complete correlation. Cases where the intraoperative cytological diagnosis did not match the histological examination were categorized as discrepant. Instances where the tumor grade was lower on squash smear cytology compared to histopathology were classified as partially correlated.

Complete concordance of cytology with histopathology was noted in 34 cases, while 5 cases showed partial correlation with histopathology. In this study, two cases of grade 2 astrocytomas were overgraded as grade 3 astrocytomas on cytology, while two cases of grade 3 astrocytomas were undergraded as grade 2 on cytology. There were eight cases of glioblastoma, and one case among them was undergraded as grade 3 astrocytoma.

In the present study, two cases were discrepant. One case of mucormycosis was misinterpreted as a high-grade glioma, and one case of medulloblastoma was misdiagnosed as ependymoma on squash cytology.

The diagnostic accuracy was 82.9% by complete concordance and increased to 95% when applying partial concordance criteria.

Discussion

The difficulty in easily accessing the contents of the cranial cavity makes diagnosing and managing CNS tumors more challenging compared to other visceral tumors. Advances in neuroimaging have revolutionized the diagnosis and treatment of these tumors. The advent of stereotactic biopsy and endoscopic approaches has made it possible to access previously inaccessible lesions. These techniques have increased the responsibility of pathologists, who now play a crucial role in the evaluation, diagnosis, and management of these lesions [7].

The importance of intraoperative diagnosis in CNS tumors cannot be overstated. It aids in targeting lesions and provides guidance to neurosurgeons in adjusting and monitoring the surgical approach. Additionally, it helps assess margins for obtaining biopsies, ensures the adequacy of stereotactic biopsies, and facilitates the collection of samples for investigations such as cultures. The choice of method for intraoperative diagnosis depends on the available technology, the type of neurosurgical procedure being performed (craniotomy or stereotactic biopsy), and the amount of tissue available [7].

The strength of squash cytology lies in its simplicity, speed, robustness, and ability to provide detailed cellular information while preserving tissue for paraffin embedding. It requires minimal equipment and technical skill, making it accessible at the operative site. One of its key advantages is that even very small specimens are suitable for smear preparation, which is crucial for surgical procedures involving intracranial lesions in functionally important areas of the brain. Additionally, it offers excellent teaching material for cytopathologists [7].

The accuracy of cytological diagnosis relies heavily on the quality of cytological preparation and staining of the smear. Smearing ease is influenced by the consistency of the tumor tissue. Soft and friable tissues found in CNS tumors are easier to smear, yielding better cellular details. This characteristic is particularly evident in gliomas, medulloblastomas, pituitary adenomas, most meningiomas, and metastatic tumors. Conversely, tumors like nerve sheath tumors, epidermoid cysts, and certain fibroblastic types of meningiomas are relatively tougher to smear [8, 9, 10].

Although smears provide good cytological details, a few diagnostic difficulties were encountered due to misrepresentation of the tumor tissue. There were two discrepant cases in our study. Tissue from unrepresentative areas led to erroneous diagnosis in discrepant cases. One case of mucormycosis was misinterpreted as high-grade glioma as only necrotic tissue was obtained, and the radiological diagnosis suggested high-grade glioma. A case of medulloblastoma was misdiagnosed as ependymoma on squash

due to the presence of a perivascular architecture of cells forming pseudo-rosettes.

In the present study, there were five partially correlated cases that varied in grade on cytology and histopathology. They were either under-graded or over-graded on squash smear cytology. The grade of astrocytomas varies within a single tumor, and hence, grading of astrocytoma on cytology occasionally poses problems. One case of glioblastoma was under-graded as anaplastic astrocytoma due to the absence of necrosis, and no thick-walled vessels could be appreciated. Two cases of anaplastic astrocytoma were misgraded as low-grade astrocytoma due to only occasional mitoses in the smear sampled. Two cases of grade 2 astrocytoma were over-graded as anaplastic astrocytoma as the sampled smear showed increased cellularity and microvascular proliferation.

The overall diagnostic accuracy of squash smear cytology was found to be 82.9%. The overall diagnostic accuracy studied by various authors varied from 76% to 96% [1-16].

Conclusion

Squash smear cytology is an accurate, reliable, rapid, safe, and cost-effective intraoperative diagnostic method that provides excellent nuclear and cytoplasmic details of intracranial lesions, especially in resource-limited settings. Familiarity with the cytomorphological features of CNS lesions, combined with clinical and radiological correlation, can enhance the diagnostic accuracy of intraoperative squash cytology.

Acknowledgements: None

Funding: None

Competing Interests: None

References

1. Kumarguru BN, Santhipriya G, Janakiraman P. A comparative study of squash smear cytology diagnosis and radiological diagnosis with histopathology in central nervous system lesions. J Cytol. 2022;39(1):1–8.
2. Ramchandani L, Bajaj D, VineethKumar RK, Bajaj J. Diagnostic utility of intraoperative squash smear cytology of intra-cranial lesions in a resource-limited setup of central India. World Neurosurg X. 2024;22:100311.
3. Jindal A, Diwan H, Kaur K, Sinha VD. Intraoperative squash smear in central nervous system tumors and its correlation with histopathology: 1-year study at a tertiary care centre. J Neurosci Rural Pract. 2017;8(2):221–4.
4. Philip SA, Lakshmi Bai E, Vani Padmaja GJ, Kumari S. Analysis of intraoperative squash cytology of central nervous system lesions and its correlation with immunohistopathology and radiology. J Cytol. 2023;40(1):1–4.
5. Yadav KK, Bhatti R, Moorchung N, Mutreja D, Carvalho AS. Is crush cytology of central nervous system lesions relevant in surgical practice today? J Neurosci Rural Pract. 2019;10:34–8.
6. Samal S, Kalra R, Sharma J, Singh I, Panda D, Ralli M. Comparison between crush/squash cytology and frozen section preparation in intraoperative diagnosis of central nervous system lesions. Oncol J India. 2017;1:25–30.
7. Patil SS, Kudrimoti JK, Agarwal RD, Jadhav MV, Chuge A. Utility of squash smear cytology in intraoperative diagnosis of central nervous system tumors. J Cytol. 2016;33:205–9.
8. Goel D, Sundaram C, Paul TR, Uppin SG, Prayaga AK, Panigrahi MK, et al. Intraoperative cytology (squash smear) in neurosurgical practice – pitfalls in diagnosis: experience based on 3057 samples from a single institution. Cytopathology. 2007;18:300–8.
9. Jaiswal S, Vij M, Jaiswal A, Behari S. Intraoperative squash cytology of central nervous system lesions: a single-center study of 326 cases. Diagn Cytopathol. 2012;40(2):104–12.
10. Iqbal M, Shah A, Wani MA, Kirmani A, Ramzani A. Cytopathology of the central nervous system. Part I. Utility of crush smear cytology in intraoperative diagnosis of CNS lesions. Acta Cytol. 2006;50(6):608–16.
11. Shukla K, Parikh B, Shukla J, Trivedi P, Shah B. Accuracy of cytological diagnosis of central nervous system tumours in crush preparation. Indian J Pathol Microbiol. 2006;49:483–6.

12. Agrawal M, Chandrakar S, Lokwani D, Purohit M. Squash cytology in neurosurgical practice: a useful method in resource-limited setting with lack of frozen section facility. J Clin Diagn Res. 2014;8(10):9–12.
13. Roessler K, Dietrich W, Kitz K. High diagnostic accuracy of cytologic smears of central nervous system tumors. A 15-year experience based on 4,172 patients. Acta Cytol. 2002;46:667–74.
14. Savargaonkar P, Farmer PM. Utility of intra-operative consultations for the diagnosis of central nervous system lesions. Ann Clin Lab Sci. 2001;31:133–9.
15. Sidawy MK, Jannotta FS. Intraoperative cytologic diagnosis of lesions of the central nervous system. Am J Clin Pathol. 1997;108(4 Suppl 1)–66.
16. Powell SZ. Intraoperative consultation, cytologic preparations, and frozen section in the central nervous system. Arch Pathol Lab Med. 2005;129:1635–52.