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IP Journal of Diagnostic Pathology and Oncology

Journal homepage: https://www.jdpo.org/

Original Research Article

# Correlation of intraoperative squash smear and histopathology in central nervous system lesions: A 6-year retrospective study in a tertiary care hospital in Kerala

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ARTICLE INFO

*Article history:*
Received 19-07-2023
Accepted 27-09-2023
Available online 05-12-2023

*Keywords:*
Cytology
Histopathology
Intraoperative
Squash smear
Tumors

ABSTRACT

**Background:** Intraoperative squash smear cytology is considered to be of great value in intraoperative consultation of CNS pathology. It is a simple and reliable tool for rapid intraoperative diagnosis of CNS tumors.

**Objectives:** The study was undertaken to assess the accuracy of intraoperative squash smear in the diagnosis of CNS lesions and to correlate the squash cytology diagnosis with histopathological diagnosis taking the latter as the gold standard.

**Materials and Methods:** A retrospective study of 38 cases of CNS lesions from which samples were collected by surgery or biopsy for intraoperative consultations and histopathological diagnosis. Radiologically confirmed cases were only included in the study. Smears were prepared from the biopsy sample obtained at the time of operation and were stained with hematoxylin and eosin. Remaining tissue submitted for histopathology was later correlated with intraoperative cytology diagnosis.

**Results:** Out of 38 cases, histopathological diagnosis was compatible with cytological diagnosis in 36 cases, two cases showed discrepancies. The diagnostic accuracy of squash cytology was 94.73%. Even though two cases were diagnosed differently in cytology, it was proved to be a malignant tumor both in squash cytology and histopathological diagnosis. Hence sensitivity, specificity, positive predictive value and negative predictive value in detecting the neoplastic condition on cytology was 100% respectively.

**Conclusion:** Squash smear is a rapid, self-sufficient and cost effective method for the intraoperative diagnosis of CNS tumors. It can be considered as a mirror image to histopathological diagnosis and is of great value in intraoperative consultation of CNS pathology.



## 1. Introduction

Intracranial tumors are a diverse group of malignancies that are broadly classified as primary or secondary (metastatic). The current World Health Organization (WHO) classification describes more than 130 different central nervous system (CNS) tumors, entities, or variants. Although molecular markers have been integrated with morphology in the revised 2016 World Health Organization classification of brain tumors,[1] precise morphological assessment still remains the foundation for the diagnosis and rapid intraoperative assessment of morphological details is equally critical and rewarding. The squash smear preparation is a fairly accurate and reliable tool in the rapid intraoperative diagnosis of CNS tumors. The accuracy of this technique is nearly as good as that of frozen section.[2] It is mostly used for the rapid diagnosis of tumors, to differentiate between primary and secondary lesions in the

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https://doi.org/10.18231/j.jdpo.2023.047

CNS and for evaluating the extent of CNS tumors.

The squash smear technique was introduced into the intra-operative neurosurgical diagnosis by Eisenhardt and Cushing in 1930 for rapid examination of neurosurgical specimens to guide the surgeon during procedures.[3] Both squash cytology and frozen section are important diagnostic tools for targeting the lesion and optimizing the extent of surgery. Although histopathological appearance of a lesion is the final arbiter of the diagnosis, the clinical and therapeutic decisions often need to be made before the histological diagnosis is proven. The fact that small specimens are difficult to section on a cryostat has resulted in an increase in the popularity of squash cytology in the rapid intraoperative diagnosis of CNS tumors. A good cytological preparation of brain tissue displays high cellularity, crisp nucleo-cytoplasmic detail and occasionally the tissue architecture in the background. Cytological evaluation has the advantage of being inexpensive and simpler than frozen section technique.[4] The frozen section technique is more reliable and superior in terms of characterization of architectural details and cytomorphology. It has the limitation of difficult section cutting due to soft consistency of tissue, costly equipment, and freezing artifacts due to ice crystal formation.[5]

With the advent of stereotactic biopsy which provides an excellent opportunity to access deep seated CNS lesions, pathologists will have to equip themselves in evaluating very tiny tissues. They will also have to train themselves in relying entirely on cytological features for definitive diagnosis. In CNS stereotactic biopsies, a very limited tissue is available for evaluation and CNS cytology is the best method for checking the adequacy of the procedure.[6]

The present study was undertaken to assess the accuracy of intraoperative squash smear technique. It is a fast, easy and inexpensive method for the diagnosis of CNS tumors in a tertiary care hospital and studies have considered it as the best method to assist neurosurgeons in accurate intraoperative diagnosis. It has been seen to overcome the limitation of difficult section cutting, expensive equipment and freezing artifacts of frozen sections. It also does not require the time needed for histopathological diagnosis making it a fast and reliable method to guide surgeons during surgery. Also, with the introduction of new technology for finding deep seated lesions, pathologists should train themselves in cytomorphological interpretation of scanty material making smear preparation the best available method. The study aims to compare the diagnostic accuracy while taking histopathological diagnosis as the gold standard.

## 2. Objectives

1. To assess the accuracy of intraoperative squash smear in the diagnosis of central nervous system (CNS) lesions.
2. To correlate squash/crush cytology diagnosis with histopathology and to assess the validity of squash cytology.

## 3. Materials and Methods

The present study was conducted by the Department of Pathology of Jubilee Mission Medical College and Research Institute, Thrissur. It is a six-year retrospective study from 09/01/17 to 28/02/23. The study included 38 patients with central nervous system lesions, from whom samples were collected by surgery or biopsy. The decision to do surgical procedure was made on clinical demand and not for the sake of participation in the study. Ethical approval was obtained from the Institutional ethical committee prior to the study. The inclusion criteria included all the cases of CNS lesions confirmed by CT scan or MRI scan. Specimens were collected in two bottles one without any fixative and other one with 10% phosphate buffered formalin respectively. The material in empty bottle was used for making squash preparations, while that in formalin was routinely processed and paraffin embedded for histopathological examination. Each specimen was cut into tiny pieces, examined and representative tissue chosen. Smears were made by taking a tiny fragment of tissue which was then placed on a clean glass slide and subsequently smeared with another slide without exerting too much pressure.[7] The smear was immediately fixed in 80% isopropanol to avoid drying artifacts and stained with Hematoxylin and Eosin. Paraffin sections were made from the formalin fixed tissue samples and stained by Hematoxylin and Eosin. The squash cytology diagnosis was compared with the gold standard final histopathological diagnosis. Immunohistochemistry and special stains like PAS were used wherever required. The data was coded in MS Excel and was analyzed using SPSS software and was interpreted.

## 4. Results

The patients' age with CNS tumors included in the study ranges from 10 to 77 years with the mean age being 45.89 years. Six CNS tumors were seen in patients of age less than 20 years. Majority of the CNS tumors (n=12) were seen in adults of age more than 60 years. There were 20 male and 18 female patients [Table 1].

Histopathological and intraoperative squash diagnosis are summarized in [Table 2]. Out of 38 cases studied, histopathological diagnosis was compatible with the cytological diagnosis in 36 cases. Remaining two cases showed discrepancies which are depicted in [Table 3]. These two cases were diagnosed as glioblastoma grade IV on squash cytology and was subsequently diagnosed as anaplastic ependymoma grade III and diffuse astrocytoma grade II on histological diagnosis.

**Table 1:** Demographic profile of patients with CNS lesions

| Variable | Frequency |
|---|---|
| **Age** | |
| < 20 years | 6 |
| 21-30 years | 3 |
| 31-40 years | 4 |
| 41-50 years | 5 |
| 51-60 years | 8 |
| > 60 years | 12 |
| **Sex** | |
| Male | 20 |
| Female | 18 |

**Table 2:** Histological andcytological diagnosis of all the cases studied

| Histological diagnosis | Total cases | Percentage | Correct cytological diagnosis | Accuracy (%) |
|---|---|---|---|---|
| Glioblastoma grade IV | 7 | 18.4 | 7 | 100 |
| Granulomatous inflammation | 4 | 10.5 | 4 | 100 |
| Lymphoma | 4 | 10.5 | 4 | 100 |
| Anaplastic ependymoma grade III | 1 | 2.6 | 0 | 0 |
| Brain abscess | 1 | 2.6 | 1 | 100 |
| Malignant small round cell tumor | 1 | 2.6 | 1 | 100 |
| Meningioma | 4 | 10.5 | 4 | 100 |
| Metastatic adenocarcinoma | 4 | 10.5 | 4 | 100 |
| Schwannoma | 4 | 10.5 | 4 | 100 |
| Hemangioma | 2 | 5.3 | 2 | 100 |
| Chronic inflammation of spine | 1 | 2.6 | 1 | 100 |
| Diffuse astrocytoma grade II | 1 | 2.6 | 0 | 0 |
| Ganglioglioma | 1 | 2.6 | 1 | 100 |
| Pilocytic astrocytoma grade I | 1 | 2.6 | 1 | 100 |
| Medulloblastoma | 1 | 2.6 | 1 | 100 |
| Ependymoma grade II | 1 | 2.6 | 1 | 100 |
| Total | 38 | 100 | 36 | 94.73 |

**Table 3:** Discrepant cases (Histopathologyvs Cytology)

| Histopathological diagnosis | Cytological diagnosis |
|---|---|
| Anaplastic ependymoma grade III | Glioblastoma grade IV |
| Diffuse Astrocytoma grade II | Glioblastoma grade IV |

The cytology features of type and grade of lesions were correlated with histopathological examination and the diagnostic accuracy of squash cytology was found to be 94.73% (36/38) [Table 2]. Benign lesions were diagnosed in 16 cases, which include meningioma, schwannoma, hemangioma, chronic inflammation of spine etc. Malignant lesions were diagnosed in 22 cases, which include glioblastoma grade IV, lymphoma, anaplastic ependymoma grade III, malignant small round cell tumor etc. Even though two cases were diagnosed differently in cytology, it was proved to be a malignant tumor both in squash cytology and histopathological diagnosis. Hence sensitivity, specificity, positive predictive value and negative predictive value in detecting the neoplastic condition on cytology were 100% respectively. This indicates that intraoperative squash cytology is as effective as histopathological diagnosis.

The radiological diagnosis was correlated with the histopathological diagnosis of all the cases. In most of the cases of CNS lesions radiological diagnosis were nonspecific and offered differential diagnosis. However the histopathological diagnosis was the same as that of the differential diagnosis offered on radiological investigation.

Morphological features of some squash smears and their corresponding histopathology are shown in Figures 1 and 2.

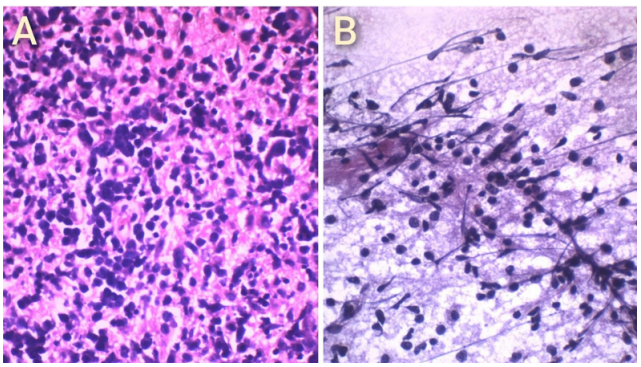


**Figure 1:** Lymphoma: **(A)** Histopathology [H&E, 40x]. **(B)** Squash cytology [H&E, 40x].

## 5. Discussion

The intraoperative diagnosis of CNS tumors by smearing the tissue can provide guidance in modifying the surgical approach by the neurosurgeons.[8,9] This is mainly because of the soft and friable nature of CNS tumors which can be stained quickly and give better cellular details. The exact location, clinical presentation of the patient and radiological findings will help the pathologist in making accurate cytological diagnosis. The ease of cytological preparation depends on the consistency of tumors. This is exhibited in tumors like gliomas, medulloblastomas and metastatic tumors. Whereas some tumors are difficult to

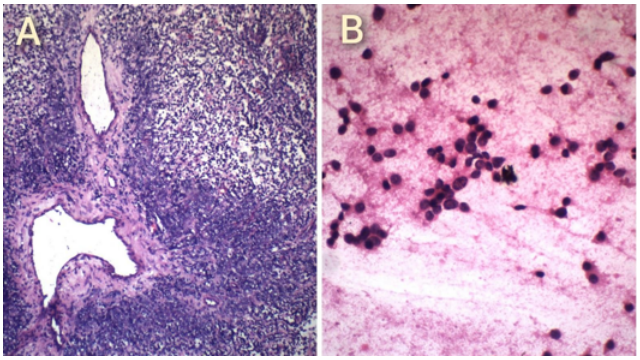


**Figure 2:** Malignant small round cell tumor: **(A)** Histopathology [H&E, 10x]. **(B)** Squash cytology [H&E, 40x].

smear due to its fibrous stroma which include nerve sheath tumors, epidermoid cyst and some meningiomas.[10]

In the present study, the CNS lesions ranged from 10 to 77 years of age. Clustering of cases was seen in the fifth decade (mean = 45.89 years) which was comparable with BN Kumarguru et al.[11] in which the mean age for CNS lesions was 42.7 years. Majority of the CNS tumors were seen in adults of age more than 60 years. There were 20 male and 18 female patients. This was also comparable with the study by SA Philip et al.,[12] which showed male preponderance of 102 cases (53.68%) among 190 total cases.

The diagnostic accuracy of squash cytology in the present study was found to be 94.73%. The diagnostic accuracy results for squash cytology was in concordance with those of other studies [Table 4]. In previous studies, the cytomorphological accuracy of diagnosis has varied from 74%-95%.[4] In a study by Kadiyala P et al[13] the diagnostic accuracy of squash cytology was 82% when only complete correlation was considered, whereas it was 91% when both complete and partial correlations were considered.

**Table 4:** The diagnostic accuracy of squash cytology for intraoperative diagnosis of intracranial lesions from various studies.

| Authors (year) | No. of cases | Diagnostic Accuracy (%) |
|---|---|---|
| Agrawal M et al (2014) | 41 | 95 |
| Samal S et al (2017) | 63 | 93.44 |
| Jindal A et al (2017) | 150 | 94 |
| P Kadiyala et al (2022) | 64 | 91 |
| Jain S et al (2022) | 53 | 90.57 |
| Present Study (2023) | 38 | 94.73 |

There were discrepancies in two cases in the present study [Table 3]. These two cases were diagnosed as glioblastoma grade IV on squash cytology and was subsequently diagnosed as anaplastic ependymoma grade III and diffuse astrocytoma grade II on histopathological diagnosis. Here the WHO grading was misinterpreted. The reason behind this misinterpretation could be attributed to regional variation of tissue in squash preparation. These variations in squash cytology and histopathological diagnosis were comparable with other studies. It is a known fact that astrocytomas may have different grades in different areas within the tumor that can lead to faulty grading of gliomas.[7]

The sensitivity, specificity, positive predictive value and negative predictive value of squash cytology in detecting whether the tumor is benign or malignant were 100% respectively which were comparable with some other studies. In the study by Bhardwaj et al[14] the sensitivity and specificity in detecting neoplastic lesions were 97.2% and 100% respectively. Similarly J Swasti et al.[4] reported that the sensitivity, specificity, positive predictive value, and negative predictive value in detecting neoplastic lesions on cytology were 94.4%, 85.7%, 98.07%, and 66.67% respectively.

## 6. Conclusion

Squash smear is a simple, rapid, reliable, self-sufficient and cost effective method for the intraoperative diagnosis of CNS tumors with overall high accuracy in comparison with histopathological diagnosis[4,15,16]. Squash cytology can be considered as a mirror image to histopathological diagnosis.[11] The soft consistency of the CNS lesions makes it easy to obtain intraoperative samples that are best suitable for CNS squash preparations rather than frozen section preparation. A good understanding with the cytological features of central nervous system lesions along with the correlation of clinical and radiological findings can further improve diagnostic accuracy of intraoperative squash cytology. Hence, CNS squash preparations can be used for rapid and reliable intraoperative diagnosis for CNS lesions.

## 7. Source of Funding

None.

## 8. Conflict of Interest

None.

## References

1. Louis DN, Ohgaki H, Wiestler OD. 2016.
2. Shah AB, Muzumdar GA, Chitale AR, Bhagwati SN. Squash preparation and frozen section in intraoperative diagnosis of central nervous system tumors. *Acta cytologica*. 1998;42(5):1149–1154.
3. Bajaj D, Ramchandani L, Ansari S, Yadav N, Bajaj J, Ratre S, et al. Intraoperative Squash Cytology and Histopathological Correlation of Glial Tumors at a Tertiary Care Hospital. *Indian J Neurosurg*. 2023;12(2):163–7.
4. Jain S, Kaushal M, Choudhary A, Bhardwaj M. Comparative evaluation of squash smear and frozen section in the intraoperative diagnosis of central nervous system tumours. *Cytopathology: J Br Soc Clin Cytol*. 2022;33(1):107–13.

5. Samal S, Kaira R, Sharma J, Singh I, Panda D, Ralli M, et al. Comparison of squash smears and frozen sections versus paraffin sections in intraoperative diagnosis of central nervous system lesions. *Oncol J India*. 2017;1(2):25–30.
6. 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(4):205–9.
7. Nalinimohan C, Padmaja P, Shailaja P, Deshpande AK. Intraoperative Squash Cytology of Central Nervous System Lesions: Review of an Eight-Year Experience. *Indian J Pathol: Res Pract*. 2018;7(10):1095–8.
8. 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.
9. Karre S, Sunkara A, Parigi M, Sreelakshmi I, Devojee M, Shanmugam6 C, et al. A 5 Year retrospective studies of cns tumours and correlation of squash cytology and histopathology. *Int J Acad Med Pharm*. 2023;5(3):2119–4.
10. Agrawal M, Chandrakar SK, Lokwani D, Purohit MR. 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.
11. Kumarguru BN, Santhipriya G, Kumar SK, Kumar RR, Ramaswamy AS, Janakiraman P, et al. 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.
12. Philip SA, Bai EL, 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.
13. Kadiyala P, Nannapaneni S, Murari A, Vallabhaneni KC, Damera NC, Diddi RR, et al. Study of intraoperative squash cytology and frozen section diagnosis of central nervous system lesions with histopathological correlation. *J NTR Univ Health Sci*. 2022;11(3):181–5.
14. Bhardwaj K, Kriplani D, Bhake A, Bhardwaj K. Study of intraoperative squash cytology of intracranial and spinal cord tumors. *Int J Res Med Sci*. 2015;3(11):3101–8.
15. Sethuraman JK, Singaravelu M, Ravishankar S, Mascarenhas J. Our institutional experience is use of "squash" cytology as an intraoperative guide in neurosurgical practice. *Int J Acad Med Pharm*. 2023;5(2):248–52.
16. Jaiswal S, Vij M, Jaiswal AK, Behari S. Intraoperative squash cytology of central nervous system lesions: A single center study of 326 cases. *Diagn Cytopathology*. 2012;40(2):104–12.

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**Cite this article:** Ayisha Hasna KV, Sebastian AA, Mathew J, Nair V, Chimmen JV. Correlation of intraoperative squash smear and histopathology in central nervous system lesions: A 6-year retrospective study in a tertiary care hospital in Kerala. *IP J Diagn Pathol Oncol* 2023;8(4):199-203.