Original Article

DOI: 10.7860/JCDR/2026/78158.22737

Pathology Section

Utility and Accuracy of Intraoperative Squash Smear Cytology for Intraspinal Lesions: A Cross-sectional Study

SHUBHANGI VINAYAK AGALE[1], SNEHAL VITTHAL CHAVHAN[2], SANJAY RAMKRUSHNA BIJWE[3], BUDDHESHWAR NARAYAN HIWALE[4], PRACHI RAOSAHEB SALVE[5], KSHITJA MANGALSINGH PAWARA[6]

ABSTRACT

Introduction: Intraoperative cytological diagnosis has gained importance because of its technical simplicity, ability to display abnormal cellularity, nuclear and cytoplasmic details. It helps the surgeon to plan the extent of surgery and modify it accordingly.

Aim: To study the cytomorphology of spinal lesions by intraoperative squash cytology.

Materials and Methods: The present retrospective cross-sectional study was conducted for three years from January 2021 to December 2023 at a tertiary care hospital, Mumbai, Maharashtra, India. Total of 72 cases of intraspinal lesions were included in the study. Intraoperative squash smears were made and were stained with rapid Haematoxylin & Eosin (H&E) stain. Paraffin H&E-stained sections were prepared from the residual tissue and additional tissue was sent for histopathology. Smear cytology diagnoses were correlated with histopathological findings. Statistical analysis was done, and diagnostic accuracy was calculated.

Results: The study included 72 cases with Male:Female (M:F)=1.8:1. The diagnostic accuracy for schwannoma was highest (21/72), followed by meningioma (19/72) and neurofibroma (9/72). By comparing the results, intraoperative squash smear cytology had a sensitivity of 98.5% and specificity of 75%. The accuracy of the study was 95.8% and three cases were discordant.

Conclusion: Intraoperative squash cytology is a fairly accurate, reliable and cost-effective method for rapid diagnosis of Intraspinal lesions. When correlated with histopathological findings, it demonstrates high diagnostic concordance, reinforcing its role as a supportive tool in the intraoperative setting. Its simplicity, rapid turnaround time, and minimal resource requirement make it especially beneficial in resource-limited settings.

Keywords: Abnormal cellularity, Histopathology, Smear cytology

INTRODUCTION

Cytological techniques have been used for diagnosis of brain lesions since 1930 when Eisenhardt L and Cushing H advocated the use of touch preparation for rapid diagnosis of tumours [1]. Squash smear provides a good cytological information with clear cytoplasmic and nuclear details with some characteristic cellular architecture of some lesions.

Importance of intraspinal squash smear is that it is fast, reliable, and cost-effective intraoperative diagnostic tool, requiring clinical and radiological context for accurate interpretation. Smear cytology offers great insight into the cellular morphology while simultaneously avoiding distortion and ice artefacts often introduced by frozen section technique. Thus, squash smear cytology of intraspinal lesions performed intraoperatively fulfils all the determinants of an excellent diagnostic modality [2].

Intraspinal tumours are relatively uncommon lesions with an incidence of 15% of primary central nervous system tumours. Intraspinal tumours may arise from the spinal cord, nerve roots, meninges, vessels, sympathetic chain, or vertebrae. They can be benign or malignant, primary or secondary, often causing severe morbidity. The location of the intraspinal lesions is important to arrive at a definitive clinical diagnosis [2].

Rapid cytological diagnosis for spinal lesions is also gaining importance nowadays [3]. Despite several pitfalls, intraoperative diagnosis is vital for determining optimal intraoperative management of spinal lesions without waiting for routine paraffin embedded sections [4,5].

The present study aimed to compare the study of cytomorphology of intraoperative squash cytology, with histopathological diagnosis. Also, to correlate it with histopathological diagnosis and to compare statistical data of squash cytology.

MATERIALS AND METHODS

The present retrospective cross-sectional study was conducted over a period of three years from January 2020 to December 2022 at the Department of Neuropathology, tertiary care hospital, Mumbai, Maharashtra, India. (IEC/Pharm/RP/194/June/2024). Patients of all age groups who were clinically and/or radiologically suspected or diagnosed with cases of intraspinal lesions and for which tissue samples were sent for intraoperative squash cytology were included.

Study Procedure

Previous slides and medical records of patients with intraspinal lesions of all the age groups from the Neuropathology Department were acquired. Stereotactically obtained biopsy sample received were accompanied by proper requisition form which included a detailed clinical history along with radiological findings related to the lesion. Specimen was squash with the help of two slides smeared. Slides were immediately fixed and stained rapid haematoxylin and eosin and mounted with Distyrene, Plasticizer, and Xylene (DPX) cytopathological findings were recorded. Remaining tissue as well as additional tissue sent for histopathological examination were processed for paraffin sections for histopathological reporting. Smear cytology diagnosis were correlated with histopathological findings.

Sensitivity, specificity and accuracy for squash smear cytology were calculated using the formulas-

- Sensitivity=True Positives/True Positives +False Negatives
- Specificity=True Negatives/True Negatives +False Positives
- Accuracy=(True Negative+True Positive)/(True Negative+True Positive+False Negative+False Positive)

The sensitivity, specificity and diagnostic accuracy were calculated. Lesions with accurate histopathological as well as cytopathological diagnosis were taken as true positive, which in the present study were 66 cases. True negative were the cases in which opinion was not possible on both intraoperative squash smears as well as histopathology due to inadequate sampling. False positive cases were those cases which were overestimated on squash smear cytology but on histopathological examination came out to be low-grade of the same or of another lesion of lower grade. And similarly false negative were those cases which were underestimated on the squash smear cytology but turned out to be high-grade following the histopathological examination.

STATISTICAL ANALYSIS

Descriptive statistics were used to analyse the data.

RESULTS

Age and sex-wise distribution: Out of total 72 cases, 39 (54.2 %) were male and 33 (45.8%) were female with sex ratio of 1.8:1 with a slight male preponderance. Intraspinal lesions were commonly seen in the 5th decade of life [Table/Fig-1].

S. No.	Age group (years)	Male	Female	No. of cases	Percentage (%)
1	0-10	3	2	5	6.9
2	11-20	4	3	7	9.7
3	21-30	6	5	11	15.2
4	31-40	4	6	10	13.8
5	41-50	8	8	16	22.2
6	51-60	7	2	9	12.6
7	61-70	5	4	9	12.6
8	71-80	2	3	5	6.9
	Total	39	33	72	100

[Table/Fig-1]: Age and sex wise distribution.

Distribution of cytodiagnosis: Intraspinal lesions were distributed into two broad categories: Neoplastic lesions and non-neoplastic lesions. Total 72 cases were studied out of which 63 cases were correctly diagnosed on squash smear cytology. It included 59 cases of neoplastic lesions (93.65 %) and four cases non-neoplastic lesions (6.34%). Most common Cytodiagnoses provided intraoperatively among the neoplastic lesions were schwannoma (20 cases, 31.74%) followed by meningioma (19 cases, 30.15%), neurofibroma (9 cases, 14.28%) and gliomas (4 cases, 6.34%) and among non-neoplastic lesions was epidermoid cyst (2 cases, 3.17%), dermoid cyst (1 case, 1.57%) and arachnoid cyst (1 case, 1.57%) [Table/Fig-2].

Distribution of lesions	Intraspinal lesions	Cases (%)
Neoplastic lesions	Schwannoma	21 (29.16)
	Meningioma	19 (26.38)
	Neurofibroma	9 (12.5)
	Gliomas (all grades)	6 (8.33)
	Malignant round cell tumour	2 (2.77)
	Metastasis in spine	2 (2.77)
	Hemangioblastoma	1 (1.38)
	Osteoblastoma	1 (1.38)
	Benign vascular lesion	1 (1.38)
Non-neoplastic lesions	Dermoid cyst	1 (1.38)
	Arachnoid cyst	1 (1.38)
	Epidermoid cyst	2 (2.77)
No opinion possible	-	6 (8.38)
Total		72 (100 %)

[Table/Fig-2]: Showing distribution of cytodiagnosis.

Concordance of histopathological and cytological diagnosis: Of total 72 cases, histopathological diagnosis was rendered in 66 cases. Cytohistopathological concordance was found in 63 cases. Six cases could not be diagnosed due to inadequate sampling.

Most common neoplastic lesion observed was schwannoma. A 100% diagnostic accuracy was observed in cases such as meningioma (9 cases), neurofibroma (9 cases), malignant round cell tumour (2 cases), haemangioma (1 cases), hemangioblastoma (1 cases), osteoblastoma (1 cases) and in all the non-neoplastic lesions which were epidermoid cyst (2 cases), arachnoid cyst (1 cases), dermoid cyst (1 cases). However, diagnostic accuracy for schwannoma was 95.23% and for gliomas (6 cases) was 66.66% which was less as compared to other lesions [Table/Fig-3].

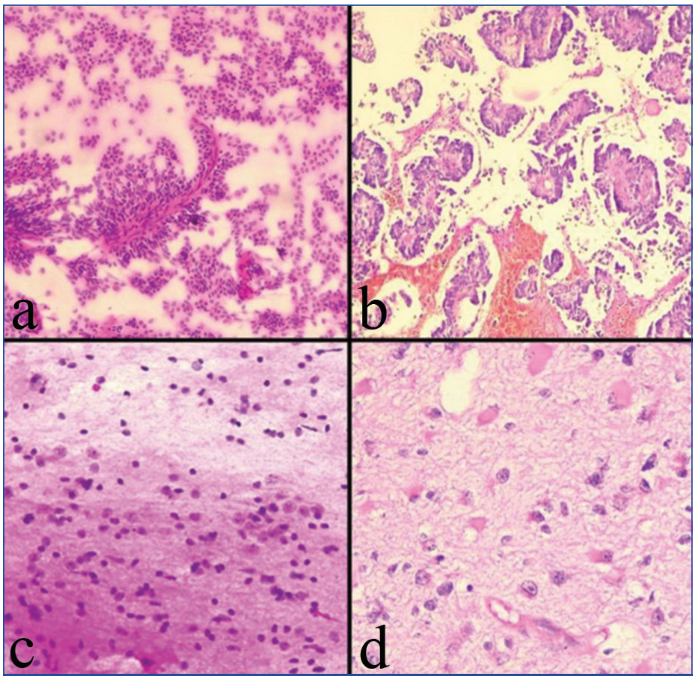
Intraspinal lesions	Histopathological diagnosis	Correct cytological diagnosis	Diagnostic accuracy by cytology (%)
Neoplastic lesions			
Schwannoma	21 (31.81)	20 (31.74)	95.23
Meningioma	19 (28.78)	19 (30.15)	100
Neurofibroma	9 (13.63)	9 (14.28)	100
Gliomas	6 (9.09)	4 (6.34)	66.66
Malignant round cell tumour	2 (3.03)	2 (3.17)	100
Metastasis in spine	2 (3.03)	2 (3.17)	100
Haemangioblastoma	1 (1.51)	1 (1.58)	100
Osteoblastoma	1 (1.51)	1 (1.58)	100
Haemangioma	1 (1.51)	1 (1.58)	100
Non-neoplastic lesions			
Epidermoid cyst	2 (3.03)	2 (3.17)	100
Dermoid cyst	1 (1.51)	1 (1.58)	100
Arachnoid cyst	1 (1.51)	1 (1.58)	100
Total	66	63	95.83%

[Table/Fig-3]: Concordance of histopathological versus cytological diagnosis.

The smear revealed a monomorphic population of oval to spindle-shaped tumour cells arranged concentrically around vascular cores, forming characteristic perivascular pseudorosettes, a key diagnostic feature of ependymoma [Table/Fig-4a]. Histological section highlights well-formed perivascular pseudorosettes, where tumour cells were radially arranged around blood vessels with intervening anuclear zones, confirming the cytological impression [Table/Fig-4b].

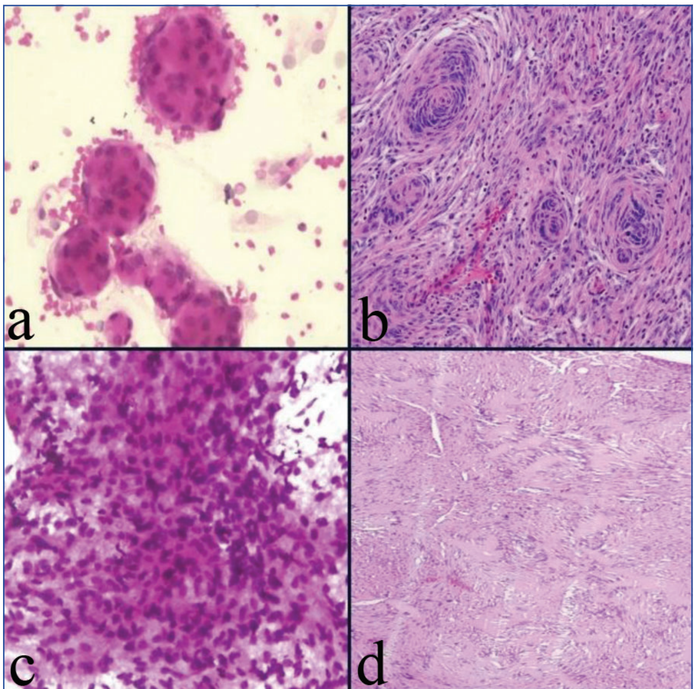
Smear showed scattered tumour cells with oval, hyperchromatic nuclei in a fibrillary background, consistent with a diffuse astrocytic origin. No necrosis or microvascular proliferation is seen [Table/Fig-4c]. Histological section revealed astrocytic cells with oval to elongated nuclei, variable cytoplasmic appearances, and fibrillar glial processes. Occasional gemistocytes with abundant eosinophilic cytoplasm were also observed [Table/Fig-4d].

Squash smear showed meningothelial cell nests with characteristic cellular whorls, a hallmark cytological feature of meningioma. The cells appear cohesive with indistinct cytoplasmic borders and round to oval nuclei [Table/Fig-5a]. Histological section confirmed the presence of meningothelial whorls, with tightly packed tumour cells forming concentric layers, supporting the cytological diagnosis of meningioma [Table/Fig-5b]. Smear displays spindled cells with indistinct cytoplasm and elongated nuclei with blunt, tapered ends, typical of schwannoma. The smear background might show fibrillary material. [Table/Fig-5c]. Section demonstrated alternating hypercellular Antoni A areas and hypocellular Antoni B areas, with



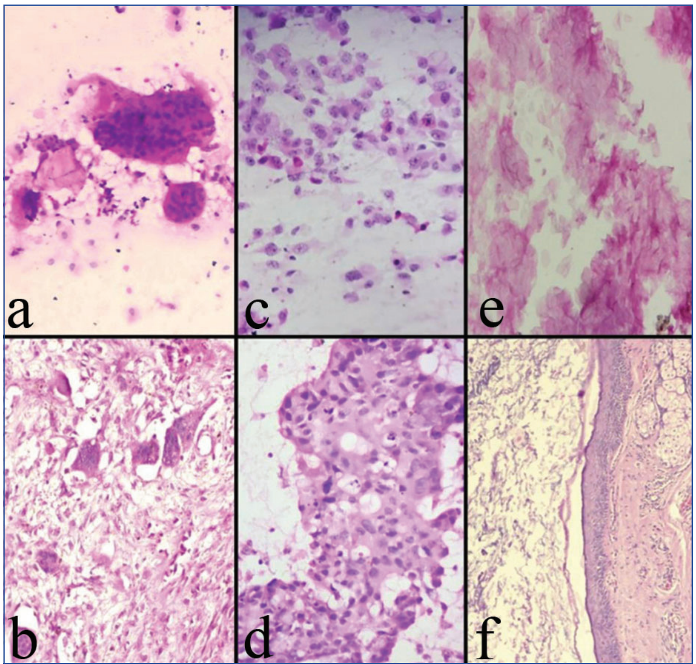
[Table/Fig-4]: a) Ependymoma- Smear showed monomorphic, oval to spindle cells arranged around vascular cores and perivascular pseudo rosette. (Rapid H&E, 200 x); b) Ependymoma- Section showing perivascular Pseudo-rossette (H&E, 200 x); c) Diffuse Astrocytoma-Smears revealed tumour cells with oval hyperchromatic nuclei in a fibrillary background. (Rapid H&E, 200 x); d) Diffuse Astrocytoma- Section revealed tumour cells with oval to elongated astrocytic nuclei and varying appearance of tumour cell cytoplasm and fibrillar glial processes and gemistocytes. (H&E, 200x).

nuclear palisading around fibrillary processes forming verocay bodies, diagnostic of schwannoma [Table/Fig-5d].



[Table/Fig-5]: a) Meningioma- (squash smear, x200) Meningothelial cell nest with characteristic cell whorls; b) Meningioma-(H&E, x200) Meningothelial cell nest with characteristic cell whorls; c) Schwanoma-(squash smear, x200) Spindled cells with indistinct cytoplasm and elongated nuclei with blunt pointed ends; d) Schwanoma-(H&E, x200) reveals hypercellular Antoni A areas and hypocellular Antoni B areas along with nuclear palisading around fibrillary process (Verocay bodies).

Smear showed mononuclear and binucleated osteoblasts dispersed throughout the sample, along with scattered osteoclastic giant cells, supporting a diagnosis of osteoblastoma [Table/Fig-6a]. Section revealed diffusely scattered osteoclast-type multinucleated giant cells, often in association with osteoid matrix and reactive bone, confirming the cytological impression [Table/Fig-6b]. Smear displayed pleomorphic tumour cells with round to oval shapes, moderate eosinophilic cytoplasm, and hyperchromatic nuclei, suggestive of a metastatic malignancy [Table/Fig-6c]. Section showed round to oval pleomorphic tumour cells with moderate eosinophilic cytoplasm and hyperchromatic, irregular nuclei, consistent with metastatic tumour deposits [Table/Fig-6d]. Smear revealed anucleated squames or "cell ghosts", indicative of keratinous debris, typical of dermoid cyst contents [Table/Fig-6e]. Section confirmed the presence of keratinising squamous epithelium along with skin adnexal structures such as hair follicles and sebaceous glands, diagnostic of dermoid cyst [Table/Fig-6f].



[Table/Fig-6]: a) Osteoblastoma- (squash smear, x200) Squash showed presence of mononuclear and binucleated osteoblasts, along with scattered osteoclastic giant cells; b) Osteoblastoma-(H&E, x400) showed diffusely scattered osteoclast type, multinucleated giant cells; c) Metastasis- (Squash smear, x200) Pleomorphic tumour cells round to oval with moderate amount of eosinophilic cytoplasm and hyperchromatic nuclei; d) Metastasis- (H&E, x200) Tumour cells round to oval with moderate amount of eosinophilic cytoplasm and hyperchromatic pleomorphic nuclei; e) Dermoid Cyst- (squash smear, 400 x) Detached anucleated squames or "cell ghost"; f) Dermoid Cyst- (H&E, x100) Keratinising squamous epithelium with skin adnexa.

DISCUSSION

Approach to the intraoperative squash smear cytology was started in 1930 by Eisenhardt L and Cushing H, followed by its evaluation by Bradt in 1937 [1,6]. The main edge of intraoperative squash cytology is to provide surgeons critical details about the lesion in less time, which subsequently assists in the planning of surgical and further therapeutic approach [7,8]. The management can also be modified in an individualised manner [9,10]. The use of intraoperative squash cytology in the diagnosis of brain lesions has been extensively narrated in literature, but its role in spinal tumours is still underutilised. Thus, the aim of the current study was mainly to assess its utility in intraspinal tumours.

In the present study, the participants age ranged from two to 72 years similar to a study by Sarkar S et al., [11]. There were 39 males and 33 females, with a male to female ratio of 1.8: 1. The maximum number of cases was seen in 5th decade of life. Distribution of lesions in the present study constituting neoplastic lesions (93.66%) and non-neoplastic lesions (6.34%) were comparable to observations made by Bhardwaj K et al., involving 92.85% neoplastic lesions [2]. Schwannoma was the most common lesion in this study (21/72 cases) with diagnostic accuracy of 95.23%. This finding was concordant with the findings of the studies done by Kar M et al., (92.3%) and Anita AM et al., (100%) [1,3]. Meningioma was the second most common lesion encountered (19/72 cases) with 100%diagnostic accuracy.

A 100% accuracy was also seen in many neoplastic lesions (neurofibroma, malignant round cell tumour, metastatic lesions, osteoblastoma, hemangioblastoma) and non-neoplastic lesions which were epidermoid cyst, dermoid cyst and arachnoid cyst. Out of the total 72 cases, three showed discordance. One of these was a case of fibrous meningioma which showed spindle shaped cells resembling fibroblast forming intersecting fascicles in a collagenous background on histopathology was given a diagnosis of schwannoma on cytopathology which was similar in the study of Kar M et al., with reason for misinterpretation stated as cell type error [1]. However, in the study conducted by and Bhardwaj K et al., Anita AM et al., such misinterpretation was not seen [2,3]. In the study conducted by Kobayashi S et al., interpreted that in schwannoma the nuclear length to breadth ratio was > 2 [12], whereas in the fibrous meningioma it is <2, which was also referred in the study conducted by Jain S et al., [13].

A low-grade glioma was misinterpreted as high-grade on squash smear. Final histopathological diagnosis was given as diffuse astrocytoma {World Health Organisation (WHO) Classification of Tumors of the Central Nervous System Grade 2}. Grading error in present study was similar to study conducted by Kar M et al., [1]. Also, cytology of low-grade tumour on histopathology and on immunohistochemistry follow up turned out to be a high-grade tumour. On histopathology, two differential diagnoses were given: a) Anaplastic astrocytoma (CNS WHO grade 3); and b) Pleomorphic Xantho-astrocytoma (WHO CNS grade 2) and on follow-up with immunohistochemistry diffuse midline Glioma, H3 K27- altered (CNS WHO grade IV) was given. Absence of pleomorphic cells was the reason for misdiagnosis on the cytology.

Most of the studies reviewed for the present study have reported high accuracy rate of intraoperative squash smear diagnosis of spinal cord tumour ranging from 80 to 99% [1,2,11]. Overall diagnostic accuracy in the present study was 95.8% which is in the accordance with all the literature reviewed for the present study [Table/Fig-7] [1,2,14].

S. No.	Name of study	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)
1	The present study	98.5	75	95.83
2	Kar M et al., 2018, Kolkata, West Bengal, India [1].	95.75	80	95.75
3	Bhardwaj K et al., 2015 Mumbai, India [2]	97.22	100	98.57
4	Bajaj NK et al., [14] 2016 Hyderabad, India	98.6	100	98.65

[Table/Fig-7]: Showing comparison with other studies [1,2,14].

Limitation(s)

A key limitation of the present study, however, is its relatively small sample size, which may affect the generalisability of the findings. Larger, multicentred studies are needed to validate these results further.

CONCLUSION(S)

The present study shows a great concordance of cytopathological and histopathological features thus spotlighting the importance of intraspinal squash smear making it as rapid, reliable, fairly accurate and cost-effective method for intraoperative diagnosis.Smear cytology offers great insight into the cellular morphology while simultaneously avoiding distortion and ice artefacts often introduced by frozen section technique making squash a better diagnostic modality for intraspinal lesions. The discrepancies mainly arose from improper sampling. A precise and pinpoint radio-imaging can counter this and improve the yield of the squash smear cytology. However, diagnostic errors are inevitable due to occasional complexities and some lesions may require further histopathological examination and/or immunohistochemical markers for a definitive diagnosis. Squash cytology does not require elaborate machinery and specific trained staff underscoring its importance in resource limiting setting and thus for this reason intraoperatively, cytology of the intraspinal lesions fulfils almost all the determinants of an excellent diagnostic modality.

REFERENCES

[1] Kar M, Sengupta M, Sarkar S, Bera S, Datta C, Chatterjee U, et al. Role of squash cytology in intraoperative diagnosis of spinal lesions. Journal of Cytology. 2018;35(3):139.

[2] Bhardwaj K, Kriplani D, Bhake A, Bhardwaj K. Study of intraoperative squash cytology of intracranial and spinal cord tumours. Int J Res Med Sci. 2015;3(11):3101-08.

[3] Anita AM, Jahan S, Ramdurg S, Melkundi S, Patil AG. Intraoperative squash cytology of spinal cord lesions with histopathological concordance. Thyroid. 2019;4:20-0.

[4] Tejaswi P, Shirisha K, Kumar D A. A study of squash smear cytology in intraoperative diagnosis of CNS tumours and its histopathological concordance. Indian Journal of Pathology and Oncology. 2020;7(3):441-46.

[5] Jha B, Patel V, Patel K, Agarwal A. Role of squash smear technique in intraoperative diagnosis of CNS tumours. Int J Med Sci Public Health. 2013;2(4):889-92.

[6] Eisenhardt L, Cushing H. Diagnosis of intracranial tumours by supravital technique. The American Journal of Pathology. 1930;6(5):541.

[7] Chimmen JV, Ayisha Hasna KV, Angela Ann Sebastian, Mathew J, Nair V. Concordance of intraoperative squash smear and histopathology in central nervous system lesions: A 6-year retrospective study in a tertiary care hospital in Kerala. IP Journal of Diagnostic Pathology and Oncology/IP Journal of Diagnostic Pathology and Oncology/Journal of Diagnostic Pathology and Oncology. 2023;8(4):199-203.

[8] Majumdar K, Saran RK, Tyagi I, Shankar R, Singh D. Role of intraoperative squash smear cytology as a diagnostic modality in lipoma of quadrigeminal cistern. Journal of Neurosciences in Rural Practice. 2013;04(01):59-62.

[9] Kumar Verma S, Kumar R, Srivani J, Jonathan J. Diagnostic accuracy of squash preparations in central nervous system tumours. Iranian Journal of Pathology. 2013;8(4):227-34.

[10] Mitra S, Kumar M, Sharma V, Mukhopadhyay D. Squash preparation: A reliable diagnostic tool in the intraoperative diagnosis of central nervous system tumours. J Cytol. 2010;27:81-85.

[11] Sarkar S, Sengupta M, Datta C, Chatterjee U, Ghosh SN. Evaluation of intraoperative cytological smears for diagnosis of brain tumours with special reference to immunohistochemistry. Indian Journal of Medical and Paediatric Oncology. 2017;38(03):296-301.

[12] Kobayashi S. Meningioma, neurilemmoma and astrocytoma specimens obtained with the squash method for cytodiagnosis. A cytologic and immunochemical study. Acta Cytol. 1993;37(6):913-22. PMID: 8249513.

[13] 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. 2022;33(1):107-13.

[14] Bajaj NK, Somalwar SB, Nagamuthu EA, Kotla SS. Study of intraoperative squash cytology of intracranial and spinal cord lesions with histopathological and IHC study. Journal of Evidence Based Medical Health. 2016;3(55):2820-25.

PARTICULARS OF CONTRIBUTORS:

1. Professor and Head, Department of Pathology, Grant Government Medical College and Sir JJ Hospital, Mumbai, Maharashtra, India.
2. Assistant Professor, Department of Pathology, Grant Government Medical College and Sir JJ Hospital, Mumbai, Maharashtra, India.
3. Professor, Department of Pathology, Grant Government Medical College and Sir JJ Hospital, Mumbai, Maharashtra, India.
4. Professor, Department of Pathology, Grant Government Medical College and Sir JJ Hospital, Mumbai, Maharashtra, India.
5. Junior Resident, Department of Pathology, Grant Government Medical College and Sir JJ Hospital, Mumbai, Maharashtra, India.
6. Junior Resident, Department of Pathology, Grant Government Medical College and Sir JJ Hospital, Mumbai, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Prachi Raosaheb Salve,
300 Resident Hostel, Grant Government Medical College and Sir JJ Group of Hospitals, Byculla Mumbai-400008, Maharashtra, India.
E-mail: prachisalve71@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 20, 2025
- Manual Googling: Jun 09, 2025
- iThenticate Software: Jun 11, 2025 (16%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Jan 19, 2025**
Date of Peer Review: **Apr 18, 2025**
Date of Acceptance: **Jun 13, 2025**
Date of Publishing: **Apr 01, 2026**