Indian Journal of Pathology and Oncology 2020;7(3):441–446

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Pathology and Oncology

Journal homepage: www.ipinnovative.com

Original Research Article

A study of squash smear cytology in intraoperative diagnosis of CNS tumors and its histopathological correlation

P Tejaswi[1,*], K Shirisha[1], Ashok Kumar D[1]

[1] *Dept. of Pathology, Kamineni Academy of Medical Sciences and Research Centre, Hyderabad, Telangana, India*

ARTICLE INFO

Article history:
Received 14-12-2019
Accepted 29-02-2020
Available online 19-08-2020

Keywords:
CNS tumors
Intraoperative diagnosis
Squash cytology

ABSTRACT

Background: Intra operative cytology is an important diagnostic modality especially central nervous system tumors. Squash smears most widely used cytological technique is rapid, accurate and relatively uses little tissue. It also helps the surgeon to plan the extent of surgery.
Aim: To assess the accuracy of squash smear by comparing with the histopathological diagnosis.
Materials and Methods: A study of 52 cases at our institute. Complete clinical, demographic and radiological findings were recorded. The smears were stained by rapid haematoxylin and eosin and toluidine blue stain. The cytomorphological features were studied and were compared with the tissue sections.
Results: Glial tumors formed the largest category of tumors constitutes 40.3%. A total 47 cases showed complete concordance with histodiagnosis with a diagnostic accuracy of 95.9%. Conclusion: Squash smear cytology proved to be a simple, inexpensive, rapid intraoperative neuropathological technique particularly useful in astrocytomas.

1. Introduction

Squash smear is the most widely used cytological technique in intraopertaive neuropathology. Although frozen section is superior to smear cytology for assessment of architectural features smear cytology allows better demonstration of nuclear morphology particularly in distinguishing astrocytic and oligodendroglial tumors. Squash smear cytology was first introduced by Eisenhardt and Cushing in early 1930.[1] This technique is simple, rapid, inexpensive, accurate intraoperative diagnostic modality[2] which provides a rapid pathological diagnosis of the space occupying lesions of the nervous system. It provides adequate material has been obtained and helps the neurosurgeon to plan the extent of surgery. The soft consistency of the central nervous system (CNS) tissue is best suited for squash cytology which in fact is a hindrance for frozen section.[3] The objective of this study is

1. To study the CNS tumors on squash smear cytology.
2. To correlate squash cytology with histopathology examination.

2. Materials and Methods

The study was done at our institute over a period of 2 yrs. A complete clinical and radiological findings were noted.[4] A total of 52 neurosurgical specimens were received intraoperatively for squash smear cytology.

Preparation of crush smear: Tissue was first grossly examined for necrosis and hemorrhage. Two or more tissue fragments measuring no more than 2 mm diameter were taken.[5] Tissue was placed on the Centre of a labeled glass slide. A second labeled slide was placed over the first slide and smeared with the second glass slide without exerting too much pressure.[6–8] Smears were fixed in 99.9% isopropyl alcohol and stained with rapid hematoxylin and eosin (H and E) stain and toluidine blue. Minimum of five to eight smears were made. Remaining tissue was submitted for

* *Corresponding author*.
E-mail address: tejaswi18reddy@gmail.com (P. Tejaswi).

https://doi.org/10.18231/j.ijpo.2020.087

histopathological examination.

3. Results

A total of 52 cases were received from the neurosurgical department intraoperatively for squash smear cytology. Most common clinical features being seizures and neurological deficits. Increased incidence was seen in 41-60 years age group. The youngest case being 1 yr old and the oldest being 68-year-old male. Slight male preponderance with Male to Female ratio of 1.6:1. Glial tumors were most common comprising about 40.38% (21 cases) followed by meningioma 15.38% (8 cases).

The cases with the same diagnosis and grade on cytology and histopathology were considered as complete correlation. Deviation of grade of tumor with lesser grade on squash smear cytology and the ones which did not correlate with histological examinations were categorized as discordant cases.

Out of the 52 cases, gliomas were most common[9,10] and medulloblastomas were common in children.[11] Gliomas which constituted 21 cases (40.38%), low grade astrocytoma's were 8/52 cases(15.38%), 11/52 (21.1%) were high grade gliomas and 2/52 (3.8%) were oligodendroglioma. Low grade glioma the cytological concordance was 87.5% discrepancy was seen in one case. High grade glioma concordance was seen in 72.7% of cases. The lineage of gliomas was correctly established in 84.21% of cases and discrepancy was seen more in grading of tumors most of the cases were under graded.

Other cases included 8 cases of meningioma, 6 cases of schwannoma, 4 cases of metastasis, 3 cases of anaplastic ependymoma, and 3 cases of lymphoma, 2 cases of medulloblastoma, one each case of embryonal tumor, DNET and pituitary adenoma where the concordance was 100%. The distribution of cases and cyto-histo correlation were given in Table 1. Also included two cases of non-neoplastic conditions which correlated histopathologically. A case of reactive gliosis which was reported as low grade glioma.

Out of 52 cases, 47cases showed concordance (90.3%) and 5 cases showed discordance (9.61%).

The sensitivity, specificity, positive predictive value and negative predictive value was found to be 97.83%, 66.67%, 97.3% and 66.6% respectively. The diagnostic accuracy was found to be 95.92%.

4. Discussion

Brain and other nervous system cancers account for 1.4% of all new cancer cases in the US.[12] The intraoperative neuropathological techniques used includes frozen section histology and smears or imprint cytology. Frozen section is superior to cytology for the assessment of architectural features, however squash smear cytology allows better nuclear morphology and provides rapid diagnosis and do not need any sophisticated instrumentation. The squash technique was introduced in as early as the 30's. However with the advent of stereotactic neurosurgical techniques that produce very small specimens that are difficult to section on the cryostat has resulted in increased popularity of the squash preparation in rapid diagnosis.[13] The advantages of squash smears are that it is easy to smear with good cellularity, can be done even when the sample is limited, and intraoperative diagnosis can be rendered within 15-20 min.[14]

The cytologic details well-illustrated on smears include astrocytic processes in astrocytomas, neuropil background in neurocytic tumors, oligodendroglial/neurocytic cells with round and regular (if not anaplastic) nuclei that are smaller than an astrocyte with dense chromatin, ganglion type neurons with large nuclei with prominent nucleoli, meningothelial cells plump nuclei with pseudo-inclusions and pituitary lesions with rounded nuclei with punctate 'salt and pepper' chromatin. However, interpretation of each of these entities is benefited by synchronous evaluation of histologic patterns which is better achieved on frozen section.A pit fall of using frozen sections is that, subsequent formalin-fixed paraffin wax embedded sections, show freezing artifact. Cytological technique are more rapid and require little tissue.[15]

Our study includes 52 cases the youngest patient being 1yr child and oldest being 68yr old. With slight male preponderance. Peak incidence of brain tumors was observed in 41-60 yrs of age.

Astrocytomas constituted largest category accounting for 40.38%. Of which high grade glioma were 11 cases and low grade gliomas were 8 cases. On cytology, the low grade glioma showed low to moderate cellularity with the tumor cells round, oval to slightly elongated normochromic nuclei rimmed by pale staining cytoplasmic processes. (Figure 1). Squash smears of high grade gliomas (55.5% cases) were highly cellular and showed tumor cells against a fibrillary background with necrotic debris and marked endothelial proliferation (Figure 2 a, b). Subsequent histopathology sections showed high cellularity with bizarre tumor cells, tumor giant cells, large areas of necrosis, mitosis, pseudo palisading around tumor necrosis and endothelial proliferation (Figure 2 c, d). Similar findings were reported by other authors.[16]

In our study discordance was seen mostly in case of gliomas. 3 cases of high-grade gliomas were reported as low grade gliomas on squash. Possibility of sampling errors or lack of representative sample and often necrosis doesn't stick to the slide. Similar difficulty was observed in other studies.[17,18]

A case of reactive gliosis on smear cytology reported as low-grade glioma. Reactive gliosis is often interpreted as low grade glioma because during intraoperative biopsy

Table 1: Distribution of cases

Histopathology diagnosis	No of cases	No of cases correctly diagnosed by squash	Discordant cases	Accuracy
Low grade astrocytoma	08	07	01	87.5%
High grade astrocytoma/ GBM	11	08	03	72%
Oligodendroglioma	02	01	01	50%
Anaplastic ependymoma	03	03	00	100%
Embryonal tumor	01	01	00	100%
Meningioma	08	07	00	100%
Schwannoma	06	06	00	100%
Metastasis	04	04	00	100%
Lymphoma	03	03	00	100%
DNET	01	01	00	100%
Medulloblastoma	02	01	00	100%
Pituitary adenoma	01	01	00	100%
Inflammation	02	02	00	100%
Total	52	47	05	90.38%

Table 2: Comparison with other studies

Author	No. of cases	Correlated with HPE	Accuracy
Malhotra et al	18	18	100%
Shukla et al	140	135	96.42%
Padma et al	11	9	81.8%‘
Brommeland et al	100	95	95%
Present study	53	47	95.9%

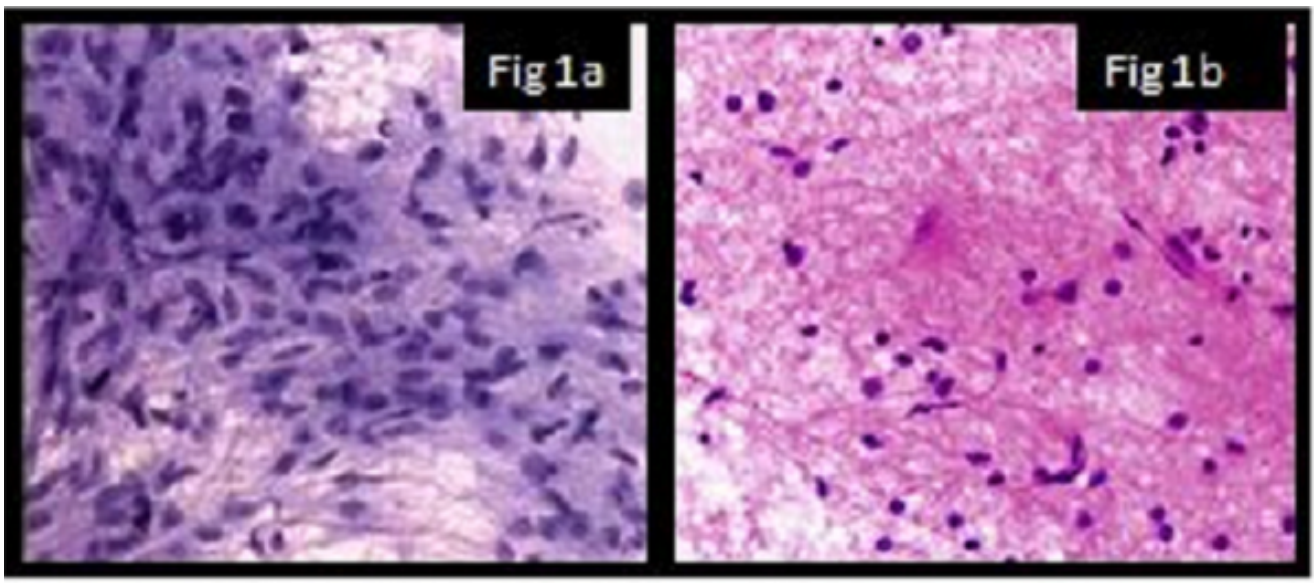

Fig. 1: Glioma; **a:** Glial cells with rounded nuclei with relatively open chromatin in a find fibrillary squash (Toluidine blue; 10X); **b:** Histopathology (H&E 10X)

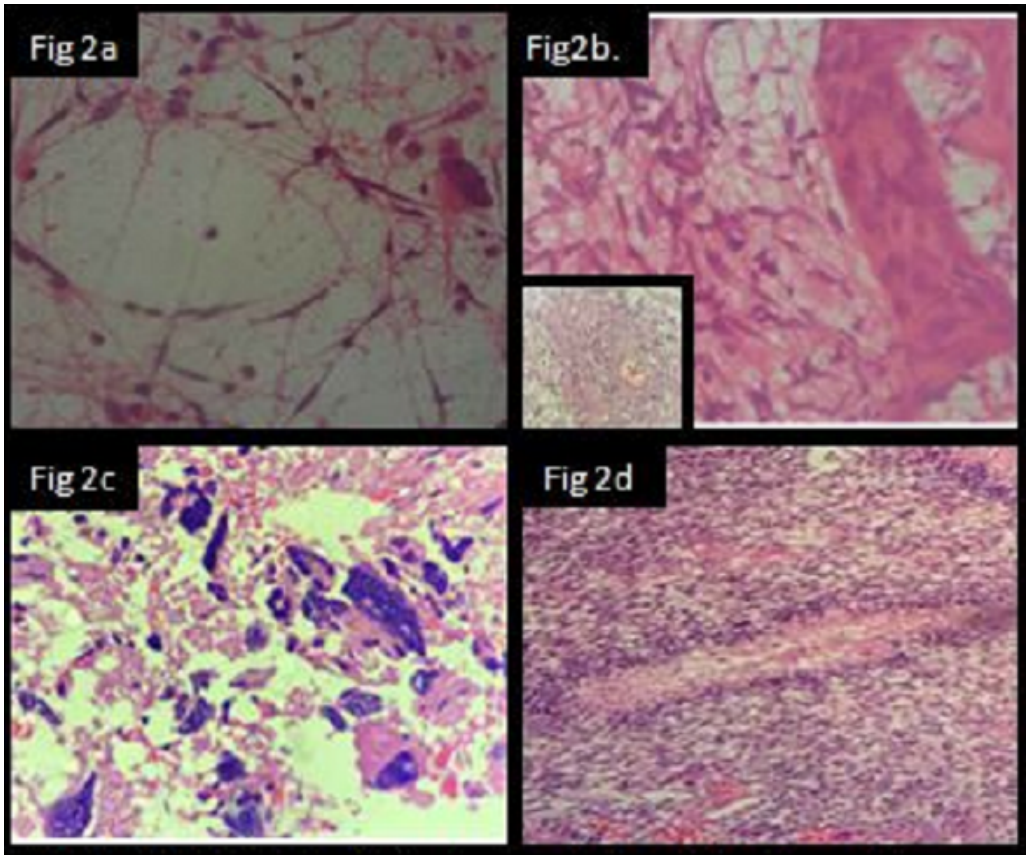

Fig. 2: Glioblastoma; **a:** Markedly cellular pleomorphic malignant astrocytic cells in well-formed fibrillarymesh work with hyperplastic blood vessel, bizarre astrocytic cells against afibrillary background, tumor giant cells; **b:** Palisading necrosis inset shows glomeruloid proliferation of blood vessel

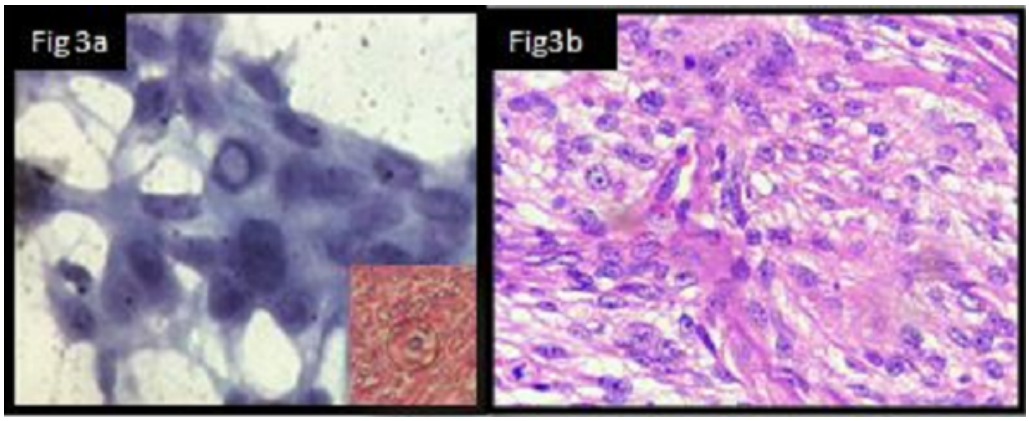

Fig. 3: a: Pictomicrograph shows meningothelial cell with oval nucleus andsome showing intranuclear vacuolations, squash (toluidine blue; 40X) **b;** Histopathology (H&E; 40X)

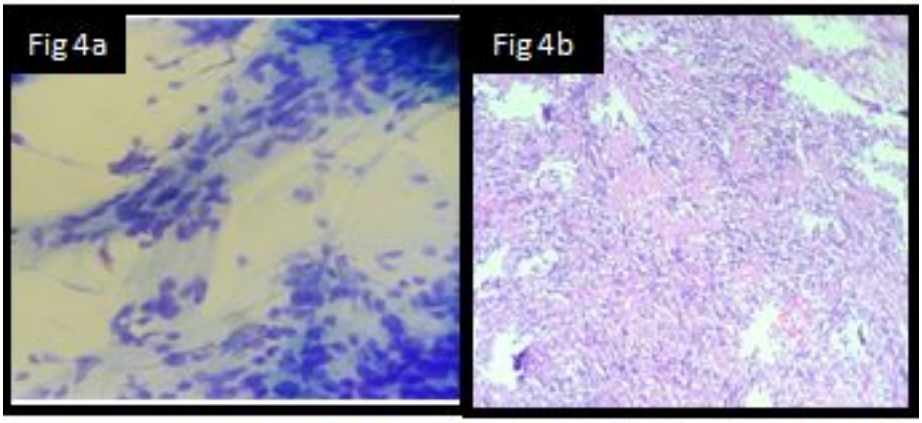

Fig. 4: a: Smear showed spindle shaped cells with wavy nuclei on a fibrillary background, squash (toluidineblue; 10X); **b:** Verocay bodies(H&E 10X)

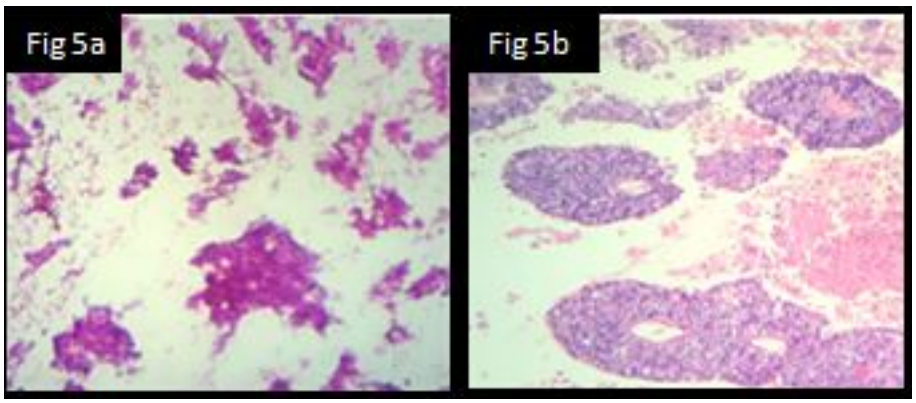

Fig. 5: a: Metastatic adenocarcinoma: cohesive cluster of malignantepithelial cells; **b:** Histopathology (H&E 10X)

it is very important to identify the reactive gliosis with glioma and then biopsy taken. Typically, in gliosis, reactive astrocytes tend to be evenly distributed, with slightly enlarged and eccentric nuclei, abundant, eosinophilic cytoplasm with stellate longer, tapering processes and low nucleus/cytoplasmic (N/C) ratio. In contrast, low-grade fibrillary astrocytomas have uneven distribution of neoplastic cells with increased N/C ratios, irregular hyperchromatic nuclei with coarse chromatin, shorter, thinner and more variably intersecting processes, mitotic figures especially atypical ones, and at times microcystic change

The next most common tumors were meningioma includes 8 cases Radiologically, meningiomas are isointense to cerebral cortex in MR images and show homogenous contrast enhancing tissue in MR and CT with “dura tail” smears. On squash they revealed firm rubbery soft tissue showing psammoma bodies, fibrillary matrix, nuclear inclusion, and a characteristic whorling pattern was seen along with a few spindle cells in some areas.our study showed 100% concordance.

The next group of tumors encountered was schwannomas which constituted 6 (11.5%) showed 100% concordance in accuracy. They were difficult to smear and cytosmears showed cohesive fragments of spindle cells and subsequent histopathology revealed a distinctive biphasic pattern composed of Antoni A and Antoni B areas with interlacing bundles of uniform cells in parallel array of palisades (verocay bodies).

Next most common tumors in present study are metastasis which constituted about 4/52 cases. Metastatic carcinomas displayed cohesive nests of atypical cells with high-grade with nuclear anaplasia, prominent nucleoli, frequent mitoses and a necrotic background.

Our study also included two cases of medulloblastoma and two cases of lymphoma which showed similar findings on histopathological diagnosis.

One case of clinical diagnosis of dysembryoblastic neuroepithelial tumor was made which on squash smear showed small uniform cells with delicate capillary vasculature and microcystic pattern admixed were large ganglion cells and on histopathology typically seen as

floating neurons.

One case of small blue round cell tumor was diagnosed on squash which correlated with histopathology and the final diagnosis was lymphoma on immunohistochemistry.

This study showed diagnostic accuracy of 95.9% which corelated with other studies.

5. Conclusion

Our study shows a high degree of cyto-histological correlation (95.92%). With better and precise radio imaging the percentage of cyto-histological correlation can improve and increase further. Some cases will always require histopathological study and/or immunohistochemical markers for definitive diagnosis, but for most of the lesions cytology of the CNS tumors performed intraoperatively fulfills all the determinants of an excellent diagnostic modality. Squash also replaces frozen section in diagnosis for CNS tumors. Frozen section needs cryostat, technical experience and ice crystal artifacts makes squash a better diagnostic modality for CNS tumor intraoperatively.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Eisenhard L, Cushing H. Diagnosis of intracranial tumor by supravital technique. *Amj Pathol*. 1930;6:541–52.
2. Sumit Mitra et al squash preparation: A reliable diagnostic tool in Intraoperative diagnosis of central nervous system tumors. *J Cytol*. 2010;27(3):81–5.
3. Nanarang. intraoperative diagnosis of CNS lesions. comparision squash smear, touch, imprint and frozen section. *J Cytol*. 2015;32(3):153–8.
4. Asha T, Shanker SK, Rao TV, Das S. Role of squash smear technique for rapid diagnosis of neurosurgical biopsies- A cytomorphological evaluation . *Indian J Pathol Microbiol*. 1989;32:152–60.
5. Agrawal M, kumar Chandrakar S, Lokwani D, Purohit MR. Manju Raj Purohit; 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.
6. Shukla K, Parikh B. Accuracy of cytopathological diagnosis of central nervous system tumors in crush preparations. *Indian J Pathol Microbiol*. 2006;49:483–6.
7. Powel SZ. Introperative consultation cytologic preparations and frozen section in central nervous system. *Arch Pathol Lab Med*. 2005;129:1635–52.
8. Boopathy T, Karthika V. An analysis of CNS tumors in squash preparation with histological correlation. *J Dent Med Sci*. 2014;16:81–6.
9. Shreshta S, Thapa BK. smear technique for intraoperative diagnosis of central nervous system neoplasms. *J Pathol Nepal*;2014(7):544–8.
10. 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*;2017:221–4.
11. Jindal A, Kaur K, Mathur K, Kumari V, Diwan H. Intraoperative squash smear cytology in CNS lesions: A study of 150 pediatric cases. *J Cytol*. 2017;34(4):217–20.
12. Cancer Stat Facts: Brain and Other Nervous System Cancer. National Cancer institute;Available from: https://seer.cancer.gov/statfacts/html/brain.html.
13. Patil SS, Kudrimoti JK, Agarwal RD, Jadhav MV, Chuge A. Chuge Utility of squash smear cytology in intraoperative diagnosis of central nervous system tumors. *J Cytol*. 2016;33(4):205–9.
14. Acharya S, Azad S. Squash smear cytology, CNS lesions- Strengths and limitations. *Natl J Lab Med*. 2016;5:1–005.
15. Sanjeev K, Aparna B, Anuradha K, Brijesh T, Sanjay K, Neetika S. Intraoperative squash cytology of CNS and spinal cord lesions with histologic correlation. *Ann Pathol Lab Med*. 2020;3(2).
16. Nori SNS, Lakshmi PVBR. A study to determine the accuracy of CNS lesions by squash smears and correlation with histopathology. *Int J Pathol*. 2017;4:21–8.
17. Chand P, Amit S, Gupta R, Agarwal A. Errors, limitations, and pitfalls in the diagnosis of central and peripheral nervous system lesions in intraoperative cytology and frozen sections. *J Cytol*. 2016;33(2):93–7.
18. Deb P, Sharma S. Intraoperative neurocytology of primary central nervous system neoplasia: A simplified and practical diagnostic approach. *J Cytol*. 2011;28(4):147–58.

Author biography

P Tejaswi Postgraduate

K Shirisha Assistant Professor

Ashok Kumar D Professor

Cite this article: Tejaswi P, Shirisha K, Kumar D A. A study of squash smear cytology in intraoperative diagnosis of CNS tumors and its histopathological correlation. *Indian J Pathol Oncol* 2020;7(3):441-446.