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# Intraoperative Squash Cytology in CNS Lesions – How Fine is the Resolution?

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#### Abstract

Introduction: With the advent of minimally invasive/ stereotactic neurosurgical techniques accurate localization of tumor and differentiation from surrounding normal brain parenchyma has become imperative to give maximal benefit of surgery and reducing collateral neurodeficit. Despite the advancement of non-invasive radiological tools, direct evidence from the microscope during the operation guides the surgeon and gives the patient optimal benefit of the surgery. The aim of this study is to correlate intraoperative squash cytology and final histopathological diagnosis and find out efficacy and reliability of squash as a diagnostic tool.

**Method/study design:** A cross sectional observational study conducted in the Department of Neuropathology from August 2018 to June 2019. All neurosurgical cases received for both intra-operative diagnosis and histopathology sample were included.

**Results:** Total ninety four cases of squash smear and corresponding histopathology with immunohistochemistry was studied and overall sensitivity of squash was calculated to be 91.3%. Individual sensitivity, specificity and positive predictive value for major lesions were also calculated.

**Conclusion:** The role of intraoperative diagnosis in CNS tumors need not be overemphasized. It can achieve targeting of lesion and provide guidance to the neurosurgeon in modifying and monitoring the surgical approach.

Keywords: Intraoperative cytology · Squash cytology · Neuropathology · CNS tumors · Concordance

# Introduction

Present concept is that high resolution and specialized neuroimaging precludes the need for intraoperative pathology consultation [1,2]. Then what is the stance of intraoperative diagnostic techniques like squash and imprint cytology in such a scenario? Neurosurgical operations were once considered grave with high morbidity and mortality however present situation is not so. Refinement of procedures and minimally invasive techniques have come up however here comes the need for pre-operative and intraoperative diagnostic accuracy [1-4]. Sometimes, when surgeon and radiologist both are at a cross road the pathologist's opinion becomes imperative. Neurosurgery differs from other surgery in the basic technique that a neurosurgeon goes through the lesion and not around it so margin clearance is not the aim of intraoperative CNS cytology. The aim of neurosurgeons is to minimize residual neurodeficit along with removal of the lesion. Intraoperative cytology helps to confirm that proper diagnostic tissue is biopsied in minimally invasive procedures or very small lesions or lesions present in dangerous areas that control vital body functions [3-7]. It helps surgeon manage unexpected lesions like if a suspected tumor turns out to be inflammatory then surgical management changes and also additional sample for microbiology needs to send in sterile container in addition to histopathology sample [8]. One has to be very careful in handling brain biopsy tissue since amount of tissue is usually very scarce and rebiopsy is not an option if additional test is required.

The aim of this study is to find out concordance between intraoperative cytology and final histopathology in central nervous system tumors and evaluate how much diagnostic accuracy can be achieved for individual tumors.

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# **Materials and Methods**

This study was performed in the Department of Neuropathology in collaboration with the Department of Neurosurgery and Neuroradiology.

Time period of the study was from August 2018 to June 2019.

This study was a cross sectional observational study.

Our study population comprised all neurosurgical patients of any age (2- 80yrs) whose tissue was submitted for both intraoperative cytology and later for histopathology. Both tumor and non-tumor pathology of brain and spine was included.

Intraoperative squash and imprint cytology was done and stained with rapid hematoxylin and eosin stain (time taken is 5 minutes). Reporting was done by two pathologists and after discussion provisional diagnosis was informed to the operating neurosurgeon. Whole procedure from receipt of sample to communication of report took average 10 minutes.

For permanent sections the specimen was grossed and sectioned as per standard protocol and after processing H&E stain was done along with additional stains like PAS, Z-N stain, reticulin stain when required. For final confirmation immunohistochemistry was done when necessary.

Ethical clearance and consent was taken from the institution's ethical clearance committee.

# **Result and Analysis**

Total number of cases in this study is ninety four out of which eighty eight (88) cases were from brain and six (6) cases were from spinal surgeries. Eighty seven (87) cases were of neoplastic etiology and seven (7) cases were inflammatory/infective. Most common lesion is Glioblastoma (WHO grade IV) followed by Diffuse astrocytoma, Oligodendroglioma, Metastatic tumors, Lymphoma and Meningioma. Overall sensitivity of squash smear in intraoperative diagnosis as per our study is 91.3% as shown in Table 1. Sensitivity and specificity of squash for individual cases were calculated when sample size was more than five for that lesion. Squash cytology has high sensitivity(Sn), specificity(Sp) and positive predictive value(PPV) in diagnosis of both low grade gliomas (Sn-88.2%, Sp-98.7%, PPV-93.75%) and high grade gliomas (Sn-92.86%, Sp-95.45%, PPV-89.65%).Specificity of 100% was calculated for metastatic tumors and lymphomas. Overall in lesions where squash diagnosis was relied on for planning further surgical modification like inflammatory vs. metastatic tumors, low grade vs. high grade lesion etc the efficacy of squash was high as shown in Table 2.

Previous studies on accuracy of intraoperative cytology show results comparable to this study [9-18].

## Discussion

The aim of CNS intraoperative cytology is to provide a reliable intraoperative diagnosis and guide the surgeon in targeting and resecting of lesion [3,4,5,6,19]. For closed stereotactic surgery it is sufficient to state whether sample is adequate and representative or not [3,4,7,8,18,20]. For open surgical resection a more detailed information is required by the surgeon to plan further course of surgery and management.

In our study evaluation of smear was done based on the below [21]:

- Smearing pattern (cohesive/ dyscohesive/ mixed)
- Background (fibrillary/ clear/ necrotic/ myxoid or mucoid/granular/ vacuolated)
- Type of cells (glial/ganglion like/round/ epithelioid/ poorly differentiated/ mixed or polymorphic)
- Pattern of cellular arrangement (papillae/ whorls/ rosettes/ arborizing blood vessels)
- Cellular elements (Rosenthal fibres/ eosinophilic granular bodies/ lymphoglandular bodies/ mucin/ keratin).

In this study total sample size was ninety four (94). The largest number of cases were high grade glioma (29.8%) followed by low grade glioma (18.1%), metastatic tumors (10.6%), lymphoma (9.6%) and so forth.

The advantage of this study is that sample size is large and each case was reported by 2-3 pathologists so biased reporting was minimalised. The limitation of this study is that the distribution of cases is not a true representation of incidence of these lesions. These statistics reveal which lesions are more frequently operated and require intraoperative cytology diagnosis. Another limitation of squash cytology is fibrous/firm/focally calcified tissue for which procedure could not be done. Some inflammatory and pilocytic astrocytoma were hence missed.

Individual categories of CNS lesions are being discussed below with their classical description and some challenging cases encountered.

#### Low grade glioma

Clinically they present with progressively developing symptoms over months in the form of speech, vision, behavioral or seizures [21-23]. Imaging show ill-defined low intensity lesions without contrast enhancement [21,22]. Grossly low grade gliomas are gray to pink or translucent soft masses and can be identified from normal tissue before smearing. Smear shows low to moderate cellularity with abundant background fibrillary processes. Numerous thin walled blood vessels are seen more so in oligodendroglioma along with calcification occasionally [1,21,23]. Glial cells show minimal pleomorphism as shown in Figure 1.

The main concern in diagnosis of low grade glioma is differentiating it from normal tissue and gliosis. The surgeon wants to know whether sampled tissue is diagnostic. Gross total resection is aimed when dealing with low grade gliomas specially pilocytic astrocytoma. However this may not be possible in many cases due to location of the lesion. Therefore when small sample can be biopsied it is absolutely essential to guide the surgeon in taking representative sample.

Table 1. Distribution of CNS lesions diagnosed in histopathology with immunohistochemistry (considered as gold standard) and the concordance and discordance rates between squash and histopathology.

S. No	Type of lesion	No of cases in histopathology	No of cases in squash	Concordance	Discordance (squash +, HP - )
1	Pilocytics astrocytoma	3	2	1	1
2	Low grade glioma	17	16	15	1
3	High grade glioma	28	29	26	3
4	Meningioma	7	7	7	0
5	Nerve sheath tumour	3	3	3	0
6	Metastasis	10	9	9	0
7	Chordoma	2	2	2	0
8	Primary CNS lymphoma	9	9	9	0
9	Embryonal tumour	3	3	2	1
10	Neurocytoma	2	2	2	0
11	DNET	1	0	0	0
12	Chronic inflammatory lesion	3	4	3	1
13	Granulomatous lesion	4	4	4	0
14	Cyst	1	1	1	0
15	Granular cell tumor	1	0	0	0
	TOTAL	94	91	84	7

Table 2. Individual sensitivity, specificity and positive predictive value of commonly occurring CNS lesions.

S.No	Histopathological diagnosis	No. of cases	Sensitivity	Specificity	<b>Positive Predictive Value</b>
1	Low grade glioma	17	88.24%	98.7%	93.75%
2	High grade glioma	28	92.86%	95.45%	89.65%
3	Metastatic tumors	10	90%	100%	100%
4	Lymphoma	9	100%	100%	100%
5	Meningioma	7	100%	100%	100%
6	Inflammatory/ granulomatous lesions	7	100%	98.86%	87.5%

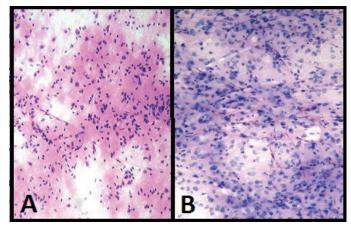


Figure 1. Photomicrograph of low grade glioma (WHO grade II). A – Diffuse astrocytoma. Dense fibrillary background with increased cellularity, mild nuclear atypia and thin capillaries. B – Oligodendroglioma. Uniform round cells without cytoplasmic processes in a granular vacuolated background and plenty of thin capillaries. (H&E, 100X).

As per our study out of 17 cases of low grade glioma (astrocytoma and oligodendroglioma, WHO grade II), 15 cases were diagnosed correctly. Two cases diagnosed as high grade in cytology due to presence of increased nuclear pleomorphism and microvascular proliferation turned out grade II oligodendroglioma in histology. Another case diagnosed as low grade glioma was diagnosed as DNET in histology.

Pilocytic astrocytoma - In ideal cases smear consists of bipolar cells with bland nuclei and Rosenthal fibres in a fibrillary background. However most of the cases in this study could not be diagnosed in squash due to technical limitations. In most cases tissue was firm so proper smear preparation was not possible and one case was pilocytic astrocytoma with regressive changes so morphology in squash could not be identified.

#### High grade glioma

This category includes grade III and IV lesions. Radiologically high grade gliomas can be confused with metastasis and sometimes inflammatory lesions. Therefore it is required to differentiate them in intraoperative cytology since management is different for each. Cytology of high grade glioma can show variable cell population ranging from pleomorphic glial cells to high grade undifferentiated cells. Vascular proliferation and necrosis is usually present as shown in Figure 2.

We received 28 cases of high grade glioma out of which 26 were identified correctly in squash, 2 cases were missed. One case was diagnosed as inflammatory and another case as metastatic in squash. Out of three cases diagnosed as high grade glioma in squash two cases were eventually found as low grade after immunohistochemistry, another case was diagnosed as embryonal tumor in histopathology.

#### Lymphoma

Most primary CNS lymphomas (PCNSLs) arise in immunocompromised individuals but they can be seen sporadically in healthy individuals also. 95% of PCNSLs are non-Hodgkin lymphoma of diffuse large B- cell type. Radiologically they are seen as homogenous contrast enhancing uni- or multifocal lesions.<sup>[5,6]</sup> Smears are highly cellular with monolayer sheets of dyscohesive cells with background of necrosis and lymphoglandular bodies. Individual cells are intermediate to large size with finely dispersed or coarse chromatin with one or more nucleoli. Mitosis, apoptotic bodies and tingible body macrophages are frequently found as shown in Figure 3. In our study we found imprint smear better than crush preparation to appreciate the morphology of the cells. All of the nine cases were diagnosed correctly in imprint/squash. Most cases of lymphoma were confused with tumefactive demyelination in radiology therefore squash report was critical in diagnosis.

#### **Metastatic tumors**

Metastatic tumors are most common neoplasms in adults. Most common tumors metastasizing to brain are from lung (50%), breast (14%), skin/ melanoma (10%), urinary tract (6%) and colon (5%) [16,21,23]. In children

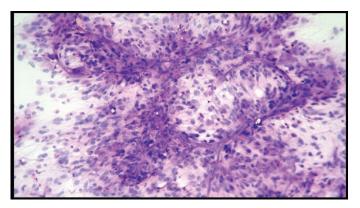


Figure 2. Glioblastoma. Smear showing pleomorphic astrocytic cells and vascular proliferation. (H&E, 100X).

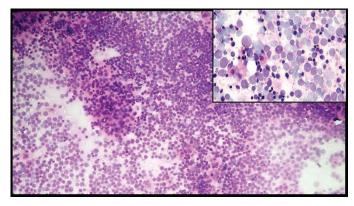


Figure 3. Diffuse large B cell lymphoma. Highly cellular smear with round cells and tingible body macrophages. (H&E, 40X) Inset showing lymphoid blasts and background lymphoglandular bodies. (H&E, 400X).

small blue round cell sarcoma like embryonal rhabdomyosarcoma, Ewing's sarcoma/PNET are most common malignancy metastasizing to brain [21,24,25]. Radiologically metastatic tumors are sometimes confused with inflammatory lesions, high grade gliomas, cysts etc. A significant portion of metastatic tumors show no primary tumors therefore stereotactic biopsies are done for diagnosis. Squash diagnosis is imperative when pre-operative diagnosis is not established. Smears shows abundant dyscohesive or cohesive cells with increased N:C ratio. Smears reveal features as those of primary neoplasm including specialized differentiation (papillae, mucin, keratinization, neuroendocrine features or melanin) as shown in Figure 4. In our study period we received ten metastatic tumors out of which nine were diagnosed correctly in squash and one case of pineal region tumor was provisionally diagnosed as germ cell tumor but turned out to be metastatic poorly differentiated squamous cell carcinoma.

#### Meningioma

Meningioma usually does not require squash diagnosis unless radiological picture or intraoperative finding does not corroborate with the diagnosis. Smear in meningioma is composed of cells in syncytial clusters with oval nuclei with occasional cytoplasmic pseudoinclusions and micronucleoli [16,21,26,27]. Occasionally whorls may be present as shown in Figure 5. We encountered seven meningioma cases all of which were identified in squash smear including two atypical meningioma

#### Inflammatory lesions

Inflammatory CNS lesions often mimic neoplasia both clinically and radiologically. In such compromising situation intraoperative microscopic study of cytology can answer the question- neoplastic or non-neoplastic? Unequivocal identification of large number of inflammatory cells or abundant macrophages in a smear rules out the diagnosis of neoplasia. Smear from inflammatory tissue may show acute inflammatory cells in pyogenic cerebritis or chronic inflammatory cells with granulation tissue formation in organizing abscess or hematoma. Features of gliosis are sometimes evident in inflammatory lesions which may be mistaken as glioma. Wellformed granulomas though rare are not unheard of in granulomatous lesions like tuberculosis as shown in Figure 6. We received seven cases of inflammatory lesions including granulomatous. All seven were identified in squash as non-neoplastic lesions. However one case diagnosed as inflammatory due to presence of necrosis and inflammatory cells later turned out as glioblastoma in histopathology. We had four cases of tuberculosis in this series, in two of them granulomas were identified in squash and in two samples coagulative necrosis and inflammation were present.

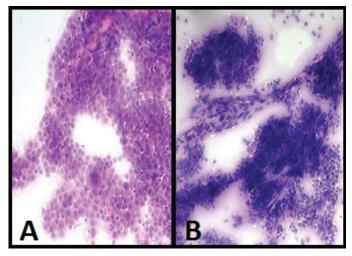


Figure 4. Metastatic tumor. A – Metastatic Squamous cell carcinoma. Sheets of polygonal cells with eosinophilic cytoplasm and frequent mitoses. B – Metastatic adenocarcinoma. Pleomorphic metastatic cells forming three dimensional crowded groups and vague papillae. (H&E, 100X).

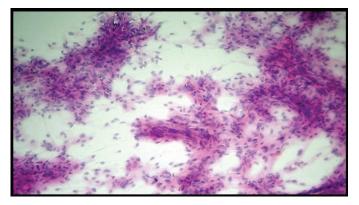


Figure 5. Meningioma. Small groups of uniform cells with oval nuclei and ill defined cytoplasm in syncitial clusters and single cells in a clean background. (H&E, 100X).

Usually these samples do not require squash diagnosis. Squash preparation is difficult from these samples as mostly tissue is firm and fibrous. Smears show cohesive fragments of spindle cells with tapered nuclei [21,27]. We received three nerve sheath tumors which were identified in squash also.

#### Embryonal tumor

These cover a group consisting of undifferentiated or poorly differentiated neuroepithelial tumor usually presenting in children [21,22,25]. Smear shows high cellularity composed to dyscohesive small hyperchromatic primitive looking cells as shown in Figure 7. We received three samples out of which two were diagnosed correctly in smear and one was mistaken as high grade glioma.

#### Chordoma

Chordoma is an uncommon brain tumor arising from notochordal remnants. In smear they show characteristic physaliferous cells with bubbly cytoplasm and bland nuclei in a myxoid background as shown in Figure 8. We received two chordoma samples one presenting as pituitary adenoma and another also in clival region.

#### **Glioneuronal tumors**

Glioneuronal tumors comprise a heterogenous group that has a common feature of consisting at least partly of cells with neuronal differentiation. We received two samples of neurocytoma diagnosed correctly in squash. Smears displayed high cellularity showing sheets of uniform round cells

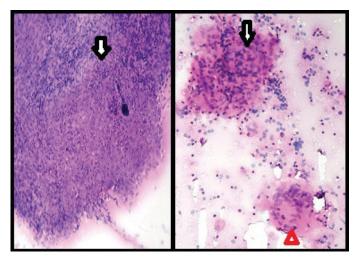


Figure 6. Granulomatous inflammation. A – Dense cluster of inflammatory granulation tissue with vague granuloma and necrosis (arrow). B – Inflammatory smear with epithelioid cell granuloma (arrow) and multinucleate giant cells (arrowhead). (H&E, 100X).

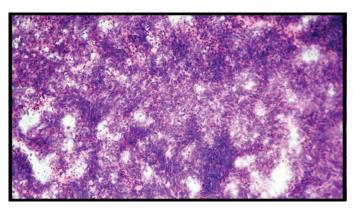


Figure 7. Embryonal tumor. Tightly packed sheets of dark blue 'primitive looking' cells. (H&E, 40X).

with scant cytoplasm occasionally forming rosettes as shown in Figure 9. Thin capillaries are present in the background.

#### Dysembryoplastic neuroepithelialtumor (DNET)

Dysembryoplastic neuroepithelialtumor have characteristic radiological findings. Smear shows small round oligodendrocyte like cells in a mixed fibrillary background. We had one case of DNET which was diagnosed as low grade glioma in squash as shown in Figure 10.

#### Granular cell tumor

Granular cell tumor is a rare tumor of posterior pituitary. We had one such case which was diagnosed a pilocytic astrocytoma in squash based on location and low grade features. The granularity of the cytoplasm was not well appreciated in squash.

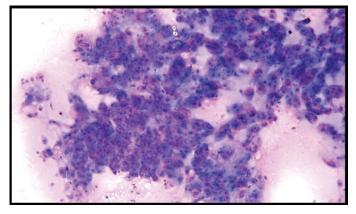


Figure 8. Chordoma. Varying proportion of physaliferous cells with bubbly cytoplasm and small cells with fusiform or round morphology in a chondromyxoid background. (H&E, 100X).

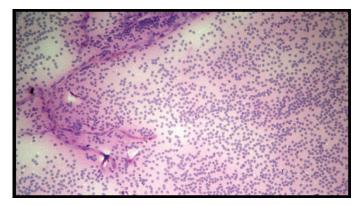
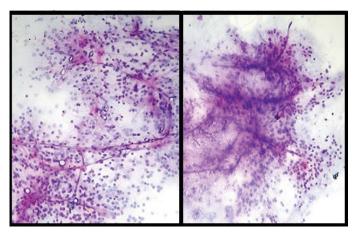


Figure 9. Central Neurocytoma. Highly cellular smear with diffuse pattern of small round cells and branching capillaries. (H&E, 40X).



**Figure 10**. Dysembryoplastic neuroepithelial tumor. Small round oligodendrocyte like cells (OLCs) in a fibrillary myxoid background. (H&E, 40X).

#### **Cerebral cysts**

Cerebral Cysts are usually benign and not sent for squash however one case of epidermoid cyst was sent and could be diagnosed correctly based on presence of lamellated keratin and radiological features.

# Conclusion

Intraoperative cytology has been accepted by pathologists as technically simple, rapid, inexpensive, fairly accurate, and dependable intraoperative diagnostic tool. The correlation between intraoperative cytology and histopathology is high. In adjunct with adequate clinical history and radiological finding, frozen sections and intraoperative cytology when combined has almost 100% sensitivity and specificity. Our study emphasizes that even with new molecular classification of CNS lesion in practice morphological assessment of cytology can provide a fairly accurate diagnosis.

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#### Disclosure statement

The authors have no conflicts of interest to declare.

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