Research Article

Accuracy and utility of intraoperative squash smear cytology in neurosurgical practice

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Received: November 04, 2018; **Accepted:** November 21, 2018

ABSTRACT

Background: Intraoperative squash smear cytology (ISSC) is a rapid and reliable diagnostic tool that provides guidance to the neurosurgeon for precise targeting of the lesion and its surgical resection. It also helps the surgeon to modify the approach at surgery at times based on the preliminary impression of the lesion on cytology. **Objectives:** The present study was undertaken to assess the utility of ISSC for cytomorphological diagnosis in a resource-limited setting. The accuracy of the method was assessed by correlating cytological diagnosis with histopathological diagnosis. **Materials and Methods:** A total of 106 cases of central nervous system (CNS) tumors were examined by squash smear technique for cytological diagnosis which were then compared with histopathological diagnosis provided on paraffin-embedded sections. **Results:** Cytohistological correlation was available in all the 106 cases included in the study. Of these 106 cases, 96 were concordant with the final histopathological diagnosis, while 10 were discordant. Thus, complete correlation with final histopathological diagnosis was observed in 90.56% of cases. **Conclusion:** Intraoperative squash smear cytology proved to be a simple, rapid, and inexpensive technique for intraoperative consultation of CNS tumors in the absence of frozen section facility.

KEY WORDS: Central Nervous System Tumors; Squash Smear Cytology; Intraoperative Diagnosis; Neurosurgical Practice

INTRODUCTION

The lesions of central nervous system (CNS) are a result of various infections, inflammation or a wide spectrum of life threatening malignancies.[1] The clinical presentations of these lesions vary depending on the location of the lesion and may be similar for many diseases irrespective of the underlying etiology. In 10–30% of the cases, the radiological features are not conclusive of the etiology of the lesions, thus making it difficult for the surgeon to provide the patient with adequate therapy based on radiology alone.[2] A cytological or histopathological examination of the tumor tissue is essential for a definitive diagnosis of the lesion in question. Due to the closed architecture of the intracranial and intraspinal space-occupying lesions, fine-needle aspiration cytology is not possible, and hence, a rapid and reliable method of intraoperative diagnosis is needed for critical decisions regarding the targeting of the lesion and the extent of surgical resection.[3]

There are two principal techniques for establishing a rapid tissue diagnosis - frozen section and intraoperative squash smear cytology (ISSC). Frozen section interpretation of brain tissue may prove difficult at times as the brain tissue has higher water content and is predisposed to show ice crystal artifacts. In addition, in developing countries, frozen section

Access this article online	
Website: http://www.ijmsph.com	Quick Response code
DOI: 10.5455/ijmsph.2019.1132721112018	

has limited use in routine diagnostic practice because of limited laboratory resources due to financial constraints.[4]

The CNS squash smear cytology was first introduced in the 1930s[5] and has now been established as a method of intraoperative diagnosis of CNS tumors. The soft consistency of CNS tissue is best suited for ISSC, which, in fact, is a hindrance for frozen section. The smear preparation is a very simple method that provides a rapid tissue diagnosis within a few minutes of biopsy reaching the laboratory.[6-8]

The present study was undertaken to find the accuracy and utility of ISSC technique as a standalone method for the rapid diagnosis of CNS lesions.

MATERIALS AND METHODS

The study was a prospective study carried out at the Department of Pathology, Government Medical College, Srinagar, and associated Shri Maharaja Hari Singh Hospital, a referral hospital with tertiary care facility. After obtaining clearance from the Institutional Ethical Committee, all consecutive patients that presented at the Department of Neurosurgery, SMHS Hospital, during the study period of 3 years (from June 2015 to June 2018) with clinical features suggestive of space-occupying lesions in CNS and the same confirmed with radioimaging, either with computed tomography scan or magnetic resonance imaging (MRI) scan, were enrolled in the study. The age, sex, and clinical presentation of each case was noted. The patients were operated according to the clinical need for the confirmation of radiological diagnosis, symptom alleviation, and further management in the form of chemotherapy or radiotherapy.

All the cases were open biopsies. The tissue sample obtained by open biopsy was collected on a gel pad or wet cotton pad soaked with normal saline and provided to the pathologist, ensuring that tissue did not dry. Small 2–3 mm-sized tissue bits were taken on one end of the glass slides, and with the help of another glass slide, pressure was applied over tissue to crush it and it was smeared onto the slides. The amount of force required to smear the tissue varied depending on the consistency of tissue sample. About 4–6 smears were prepared for each case. The smears were immediately fixed in methanol and stained by quick H and E method. Briefly, the smears were dipped in Harris's hematoxylin for 5 min, quick rinsed in water, then dipped in acid alcohol (1% hydrochloric acid in 70% alcohol), followed by Scott's tap water, and counterstained with 0.5% eosin for 20 s. The slides were then mounted in Distyrene Plasticiser Xylene (DPX) [Table 1].

The smears were studied keeping in view the clinical and radiological information provided to the pathologist and an opinion regarding the presence of the lesional tissue, and the nature and type of lesion were informed to the surgeon. The time for the entire procedure was noted. The intraoperative cytological diagnosis was compared with final diagnosis on the formalin-fixed paraffin-embedded histopathological sections to assess the diagnostic accuracy of squash smears in CNS tumors [Table 2].

RESULTS

The present study aimed to evaluate the role of the squash smear technique in the diagnosis of central nervous tumors and its correlation with histopathology. A total of 106 cases of CNS tumors were included in the study. Of these cases, 83 (78.30%) were intracranial space-occupying lesions and 23 (21.69%) were spinal lesions. The age of the patients ranged from 4 years to 72 years.

Male-to-female ratio of 1.5:1 was observed. Patients presented mainly with headache, nausea, and vomiting. Seizures were the presenting complaint in many patients, while others presented with local symptoms according to the site of the brain involvement such as difficulty in speech, disorientation, paralysis of one side of the body, vertigo, tinnitus, or difficulty in hearing. Patients with spinal lesions presented with weakness of limbs depending on the level of spinal involvement.

Astrocytic tumors, pituitary adenoma, ependymoma, medulloblastoma, lymphoma, and metastatic and inflammatory lesions were easy to squash, whereas those difficult to squash included some meningiomas and schwannomas.

The average time taken from the removal of tissue from the brain to the communication of cytological diagnosis to the surgeon was 12–15 min.

Among the 83 intracranial lesions, 29 were diagnosed as meningioma, 23 as astrocytoma, 2 as oligodendroglioma, while we had 6 cases of pituitary adenoma and 4 cases of metastatic deposits to the brain, 3 cases of schwannoma, and 2 cases each of medulloblastoma, lymphoma and inflammatory pathology, an abscess, and a tuberculoma. There was also one case of retro-orbital dermoid cyst on cytology.

Among the 23 intraspinal lesions, we diagnosed 9 cases of schwannomas, 3 lymphomas, 4 ependymomas, and two cases of meningioma and an intraspinal dermoid cyst with granulomatous inflammatory reaction on cytology. There were two cases of non-specific spinal abscess on cytology.

In our study, we misinterpreted 10 cases on cytology. One case of meningioma (papillary subtype) was diagnosed as metastatic deposit due to papillae formation and highly atypical features [Figure 3a-d]. Two cases of astrocytomas were diagnosed as oligodendrogliomas [Figure 4a and b]. This could be due to sampling from the periphery of an astrocytoma where neoplastic astrocytes are admixed with neurons and oligodendrocytes with felt-like background. Two cases of ependymomas (one cerebral and another

Table 1: Cytological features of various lesions on squash smear

Cytological diagnosis	Features observed on squash smear
Meningioma	Cohesive syncytial clusters of plump spindle nuclei with whorl formation, abundant granular cytoplasm, and nuclear pseudoinclusions [Figure 1a and b]
Astrocytoma (Grade 2)	Moderately increased cellularity, irregular clusters, abundant glial fibrillary processes, no mitoses
Astrocytoma (Grade 3)	Markedly increased cellularity, nuclear pleomorphism, mitotic figures seen [Figure 2a and b]
Glioblastoma	Marked nuclear pleomorphism, mitotic figures, vascular proliferation, and tumor necrosis
Pituitary adenoma	Sheets of monotonous round cells with centrally placed vesicular nuclei with no distinct nucleolus, eosinophilic cytoplasm, and multinucleation may be seen
Ependymoma	Palisaded tumor cells around blood vessels with fibrillary matrix adjacent to blood vessels or at the edges of tumor cells, round-to-oval cells with pale-stippled chromatin and one or more nucleoli
Medulloblastoma	Densely packed cells with round- or carrot-shaped hyperchromatic nuclei and scant cytoplasm, nuclear molding and mitotic figures, rare rosettes
Schwannoma	Cohesive uniform spindle cells in twisted rope pattern, palisading nuclei, no nucleoli
Lymphoma	Neoplastic cell aggregates around blood vessels with dispersion. Nuclear pleomorphism with large immunoblasts and centroblasts having distinct cell borders, scant cytoplasm, granular chromatin, and prominent nucleoli
Metastasis	Cellular cohesiveness with molding of adjacent cells in the clusters, absence of glial fibrillary background, features depicting the site of the primary lesion
Abscess	Necrosis, abundant neutrophils
Tuberculoma	Lymphocytes, histiocytes, epithelioid cells, necrosis

Table 2: Comparative analysis of histopathological diagnosis with the cytological diagnosis offered on squash smears along with the accuracy of cytological diagnosis for each lesion

Diagnosis	Histopathology	ISSC	Accuracy (%)
Intracranial lesions (*n*=83)			
Meningioma	30	29	96.66
Astrocytoma	27	23	85.18
Oligodendroglioma	2	2	100
Ependymoma	2	1	50
Pituitary adenoma	6	6	100
Schwannoma	4	3	75
Metastasis	4	4	100
Lymphoma	2	2	100
Inflammatory	2	2	100
Hemangioblastoma	1	-	-
Dermoid cyst	1	1	100
Intraspinal lesions (*n*=23)			
Schwannoma	9	9	100
Lymphoma	3	3	100
Ependymoma	5	4	80
Meningioma	2	2	100
Astrocytoma	1	-	-

ISSC: Intraoperative squash smear cytology

spinal) were misinterpreted as astrocytomas on squash cytology [Figure 5a-c]. One case of low-grade astrocytoma was diagnosed as reactive astrocytosis of the brain, possibly due to erroneous sampling from the periphery of the main lesion. One case of high-grade astrocytoma was misdiagnosed as inflammatory lesion, possibly tuberculoma.

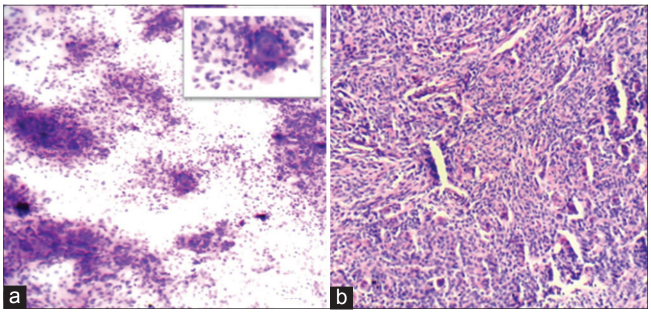

Figure 1: Meningothelial meningioma. Panel a: Squash smears (×10) revealed whorled pattern of meningothelial cells and oval nuclei in background with fine chromatin. A meningothelial whorl shown in the inset (×40). Panel b: H and E stained sections revealed the presence of meningothelial meningioma

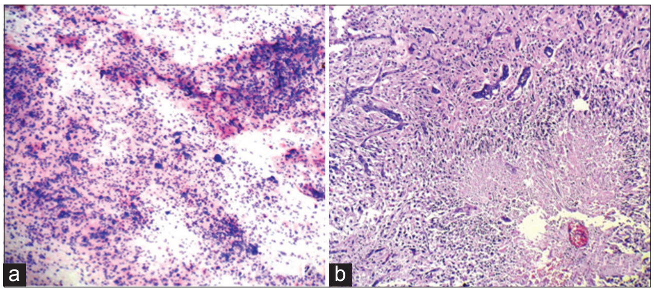

Figure 2: Glioblastoma multiforme (GBM). Panel a: Squash smears (×10) revealed scattered bizarre cells with marked nuclear and cytoplasmic pleomorphism. Lack of necrosis and absent endothelial cell proliferation in the material received resulted in a diagnosis of anaplastic astrocytoma. Panel b: H and E stained sections of the tumor revealed features of GBM, with marked pleomorphic tumor cells, necrosis, and presence of endothelial cell proliferation

This could be due to the misinterpretation of coagulative necrosis of glioblastoma multiforme as caseous necrosis of a tuberculoma. One case of spinal astrocytoma was diagnosed as ependymoma on crush cytology, while one case each of cerebellopontine angle schwannoma and cerebellar hemangioblastoma were misinterpreted as meningioma and benign mesenchymal tumor, respectively [Table 3].

Correct diagnosis percentage on squash cytology was 90.56% in our study when compared with histopathology as the gold standard.

DISCUSSION

The diagnosis and management of CNS tumors have been relatively difficult as compared to other visceral tumors due to the inaccessibility of these lesions for simple tissue diagnostic procedures such as fine-needle aspiration cytology or incision biopsy. With the advances in neuroimaging, the management of such tumors has been revolutionized and the advent of stereotactic biopsy or endoscopic approach has made it possible to approach these inaccessible lesions to obtain tissue for diagnosis. At the same time, it has also increased the responsibility of a pathologist toward the proper interpretation of the material provided to him for diagnosis.[9,10]

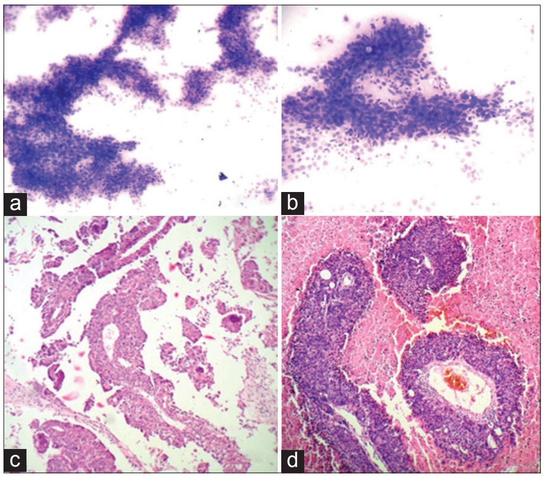

Figure 3: Papillary meningioma. Panel a (×10) and b (×40): Squash smears showing acellular tumor with multiple papillary clusters and individual scattered tumor cells. The cells are elongated and are arranged around blood vessels, forming papillae. This smear was diagnosed erroneously as metastatic deposits of an epithelial malignancy. Panel c and d: H and E stained sections of the same tumor revealed features of a papillary meningioma, with multiple papillary clusters of tumor cells centered on blood vessels

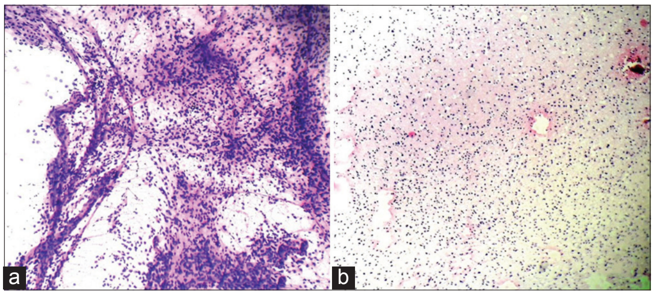

Figure 4: Low-grade astrocytoma. Panel a: Squash smears (×10) revealed features of a neoplasm with round monomorphic nuclei in a fibrillary background. The presence of fibrillary material, fine capillary network, and clinging of cells around vessels led to the erroneous diagnosis of oligodendroglioma. Panel b: H and E stained section of the same tumor revealed features of a low-grade astrocytoma

The advantages of squash cytology are that it is a simple and rapid procedure that needs minimum equipment and technical skill. Squash smears can be prepared from very tiny specimens allowing tissue to be preserved for paraffin embedding.[11] Several studies conducted to compare the results of squash cytology with frozen section have concluded that the accuracy of cytology was as good as frozen section[12-14] and cytology alone can guide the neurosurgeon in the intraoperative period in a resource-limited setting where frozen section is not performed routinely for logistic and technical reasons.[15]

In the present study, the intraoperative diagnosis was sought on open craniotomy specimens. We had to ask for more tissue in five cases due to the inconclusive nature of smears prepared in the first instance, which was provided by the surgeon without any difficulty. However, with the advent of stereotactic biopsy, a very limited tissue is available for evaluation and CNS cytology is the best method for checking the adequacy of the procedure.[7] Therefore, practicing CNS squash cytology in open biopsies will provide excellent teaching material for the cytopathologist.[16,17]

In several studies conducted to correlate squash cytology with histopathology, the diagnostic accuracy of squash cytology was ranging from 87% to 97%.[18-20] In our study, the accuracy of squash cytology was 90.56% when compared with histopathology as the gold standard which was comparable to other studies. Our study, however, confirms the reliability of CNS squash cytology, and further, multicenter study with larger number of patients will make us confident of its value as a standalone intraoperative diagnostic procedure in resource-limited setting where frozen section facility is not available.

Table 3: Discordant cases

Number of cases	Diagnosis on histopathology	Diagnosis on ISSC
1	Papillary meningioma	Metastasis of papillary tumor
2	Astrocytoma	Oligodendroglioma
1	Astrocytoma	Reactive gliosis
2	Ependymoma	Astrocytoma
1	Astrocytoma Grade IV	Inflammatory (tuberculoma)
1	Astrocytoma	Ependymoma
1	Schwannoma	Meningioma
1	Hemangioblastoma	Benign mesenchymal tumor

ISSC: Intraoperative squash smear cytology

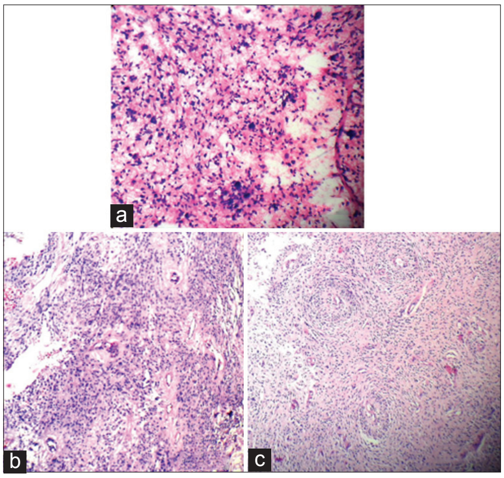

Figure 5: Ependymoma. Panel a: Squash smears (×10) revealed tumor with small round monomorphic cells. There was no evidence of rosettes in the material examined. The tumor was misdiagnosed as low-grade astrocytoma. Panel b and c: H and E stained section of the tumor revealed features of an ependymoma

Another significant aspect of our study was to assess the degree to which our intraoperative CNS squash cytological diagnosis helped the neurosurgeon in the management of patients. On discussing each case with the operating surgeon, our consultation was of help in five cases where the pre-operative MRI diagnosis was not offered or was proved to be wrong. A case of tentorial lesion reported as tentorial meningioma on MRI proved to be a case of medulloblastoma on crush smear. A case of the left parietal lesion with satellite lesions reported as high-grade glioma on MRI proved to be a case of metastatic deposit of an epithelial malignancy on crush smears. A case of cerebellar vermis mass in a 4-year-old girl reported as primitive neuroectodermal tumor on radiology proved to be a pilocytic astrocytoma. A case of frontal lobe lesion reported as glioblastoma on MRI turned out to be a tuberculoma on crush smears. A case of filum terminale mass in an 8-year-old boy where MRI diagnosis was not offered proved to be a granulomatous inflammatory lesion on crush smears. All these cytological diagnoses were confirmed on histopathology and helped the neurosurgeon in modifying the surgical approach as was seen in some other studies as well.[15,21]

CONCLUSION

In the end we can conclude that intraoperative squash smear cytology is easy to perform, inexpensive, permits high diagnostic accuracy and is a useful alternative in centers lacking frozen section infrastructure. It can provide a preliminary diagnosis in CNS lesions enabling the surgeon to plan further management on operating table.

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How to cite this article: Khuroo MS, Hamdani SM, Alam SS, Sahaf BR, Dar NQ, Bhat RA. Accuracy and utility of intraoperative squash smear cytology in neurosurgical practice. Int J Med Sci Public Health 2019;8(2):130-135.

Source of Support: Nil,**Conflict of Interest:** None declared.