

Diagnostic accuracy of squash cytology in central nervous system tumors

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Abstract

Introduction: CNS tumor requires intraoperative decision making regarding the extent of tumor removal. Clinical examination and imaging studies are not sufficient enough to predict the biological behavior of the tumors. Squash cytology is a quick method of evaluation of cytomorphologic features prepared from smear technique and provide the preliminary diagnosis and aid in intraoperative decision making by differentiating neoplastic from non neoplastic and benign from malignant lesions. The aim of this study is compare the diagnostic accuracy of squash cytology to that of histopathological examination.

Methods: This study consists of 36 specimens from both brain and spine subjected to both squash cytology and histopathological evaluation. The squash preparation and histopathological finding were later compared and diagnostic accuracy calculated.

Results: Gliomas are the most common tumor encountered and the accuracy of Squash cytology obtained was 71%. In meningioma, 100% diagnostic accuracy was obtained however, there was limitation in accurately predict the subgroup of tumor by squash cytology alone. Other neoplastic lesions included in this study were Schwannoma, Oligodendroglioma, Ependymoma, mixed tumors and others. Overall, the accuracy predicted by squash cytology is found to be 77.8 % in this study.

Conclusion: Squash cytology is rapid and reliable method of tissue diagnosis that aid in intraoperative decision making regarding the extent of Central Nervous System tumor excision

Introduction

Central nervous system (CNS) tumors comprise of brain and spinal cord tumors with varying clinical signs and symptomatology, as well as, imaging characteristics. The tumors can range from truly benign to overt malignant; however, the pathological differentiations from clinical and radiological parameters is often confusing.^{1,2} At times, intraoperative decision-making can be difficult due to the tumor location, pre-operative and anticipated neurological deficits. Incomplete resection of benign pathology can deny patient with the chance for cure. However, overzealous attempt for complete resection in a patient with malignant pathology can increase neurological deficits and surgical morbidity and decrease quality of life without increasing survival benefit.³

Squash cytology (SC) is a method of quick evaluation of cytomorphologic features prepared from smear technique that could provide the preliminary diagnosis, separating the low grade tumors from the high grade ones as well as differentiating non-neoplastic from neoplastic lesions.^{4,5,6} Unnecessary surgeries can be avoided in non-neoplastic lesions. As such, it can distinguish tumors amenable to gross total resection (i.e. Ependymoma) from tumors that are not (i.e. Germinoma); and it also enables tissue allocation for tumor banking, molecular testing, culture or other ancillary techniques.^{7,8,9} Previous studies have correlated the diagnostic efficacy of SC with formal histopathological (HP) evaluation. It has been found to be as high as 85%.

Few studies have been done in India but to the best of our knowledge, no such study has been yet carried out in our country. Hence, this study was undertaken to correlate cytomorphological diagnosis by squash smear technique to that of histopathological examination of CNS neoplastic lesions and to determine the diagnostic accuracy of SC.

Methods

This study was conducted over a period of five months from November 2015 to March 2016 at the Departments of Neurosurgery and Pathology in Tribhuvan University Teaching Hospital. There were a total of 36 cases of CNS neoplastic lesions, who had both SC and HP evaluation. The tumor tissue obtained during the time of surgery was separated into two specimens. Specimen one was promptly transported in isotonic normal saline and sent for SC while the specimen two was fixed and transported in 10% formalin at the end of surgery for HP evaluation. The specimen for SC was squash smeared and 4 Giemsa stain and 2 pap stain were prepared and evaluated. Relevant clinical and radiological data were recorded. Cytological and HP findings were recorded separately. Final comparison between HP observations and SC impressions was made. Correlation between these two techniques was graded as complete agreement, partial agreement or no agreement (i.e. disagreement). Complete agreement was defined as complete concordance with tumor classification and grading. If the SC could pin point the tumor but failed to grade it, was regarded as a partial agreement. Disagreement or no agreement meant complete misinterpretation in the SC reports compared to HP evaluation.

Results

A total of 53 operations were performed for CNS neoplasms over the period of five months. Thirty-seven patient specimen were subjected for SC and HP evaluation, however, only 36 specimens were available for analysis as one patient biopsy specimen was misplaced.

The mean age of the patients was 40.2 (\pm 20.9) years, the age ranging from 4 to 72 years. In regard to gender, there were 19 females and 17 males. Thirty-one specimens were from brain and 5 from spine.

The SC and HP evaluation of all 36 specimens are represented in detail in Table 1.

Table1: Comparative reports of histopathological examination and squash cytology

SN	HPE (n= 36)	SC (n = 36)
1. Glioma (n=11)		
	GBM (7)	GBM (4) HGG (1) Metastasis (1) Oligodendroglioma (1) Pilocytic Astrocytoma (2) Craniopharyngioma (1) HGG (1)
	Pilocytic Astrocytoma (4)	
2. Meningioma (n = 8)		
	Meningiothelial (4)	Meningiothelial (2) Transitional (2)
	Transitional (3)	Transitional (3)
	Psammomatous (1)	Transitional (1)
3. Schwannoma (n = 6)		
	Schwannoma (6)	Schwannoma (4) Neurofibroma (1) Ependymoma (1)
4. Oligodendroglioma (n = 2)		
	Oligodendroglioma II (1)	Oligodendroglioma II (1)
	Anaplastic Oligodendroglioma III (1)	Glial Tumor III/IV
5. Ependymoma (n = 2)		
	Ependymoma (1)	Ependymoma (1)
	Myxopapillary Ependymoma (1)	Myxopapillary Ependymoma (1)
6. Mixed Tumors (n = 1)		
	Oligoastrocytoma (1)	Oligodendroglioma (1)
7. Others (n = 6)		
	Adamantinomatous Craniopharyngioma (2)	Craniopharyngioma (2)
	Cavernous Hemangioma (1)	Inconclusive (1)
	Diffuse Large B-Cell Lymphoma (1)	NHL, High Grade (1)
	Hemangioblastoma (1)	Acellular Smear (1)
	Metastasis (1)	Metastasis (1)

The CNS neoplasms are heterogeneous.² The tumors both benign and malignant were included (Table 1). Overall, glioma was the most commonly-encountered tumor in the studied population, constituting of 30.5% of the final diagnosis. High grade glioma (HGG) included World Health Organization (WHO) grading III and IV of Astrocytoma. WHO grade I and II were considered as low grade glioma (LGG). In this study, there were a total of 7 cases of Glioblastoma Multiforme (GBM) leveled as WHO grade IV.

In cases with HGG, complete agreement between SC and HPE was obtained in four specimens and partial agreement in one specimen. Hence, overall accuracy of SC for HGG was found to be 71.4% (5/7). Similarly, there were four cases with LGG; but SC complete agreement with HPE was obtained in only 50 % (n = 2) and partial agreement in 25% (n = 1); and, hence, the overall agreement was 75% (3/4). Figure 1A and 1B shows the cytological features and histopathological features of Glioma.

Among benign brain tumors, meningioma is very common. There were altogether eight cases of meningioma as reported in the final HPE, accounting for 22.2% (8/38). Meningothelial (n = 4), Transitional (n= 3) and Psammomatous (n= 1) subtypes of meningioma; all belong to the WHO grade I tumors. The complete agreement was observed in 62.5 %; and partial agreement was observed in 37.5 %. Fig 2A and Fig 2B demonstrates the characteristic cytomorphologic and histopathological features of meningioma.

Other tumors in this study included Schwannoma, Oligodendroglioma, Ependymoma and so forth. Schwannomas included in this study comprises vestibular schwannomas, as well as, spinal schwannomas. For all types of tumors, the complete and partial agreement and disagreement are represented in the following Table 2.

When the agreement was dichotomized only as agreement, which includes complete and partial, or no agreement, there were a total of 28 concordances out of 36 specimens, of comparing SC with final HPE. Thus, on calculating the accuracy of 77.8 % was achieved using SC method.

Discussion

Intraoperative decision-making may change depending upon the intraoperative impression of CNS tumor texture, consistency, vascularity and so on. The heterogeneity of tumors types, the differing aggressiveness of tumors even in similar classification and new advancement in molecular diagnosis and immunohistochemical printing, renders need for confirmation of the pathology to decide upon the extent of resection. At the same time, acquiring sufficient tissue for such procedures is of paramount importance.^{8,10} Fluid cytology may be used for cystic lesions, but for solid lesions SC and frozen section are two methods available to the surgeons. SC has several advantages over frozen section, especially in CNS lesions. It provides a rapid and reliable diagnosis, requires small sample size, allows tissue to be preserved for paraffin embedding, needs minimal technical equipment, is less expensive, and gives better cellular detail. Moreover, the high water and fat content and innate fragility of brain tissue leading to freezing artifacts and poor quality frozen sections makes this technique superior to frozen sections.^{9,10,11} Furthermore, impression of non-neoplastic lesions especially infective or inflammatory may allow surgeon to avoid resection near or at eloquent area and prevent neurologic deficit.^{7,12,13}

The accuracy of SC in this study was found to be 77.8 %, which is in concordance with other studies (80 – 97 %).^{4,5,6,13,14,15} The differing accuracy in various literatures could be due to the extremely heterogeneous nature of the lesions included for analysis. Some have included

Table 2: Correlation of squash cytology with histopathological examination among various tumor groups.

Tumor Group	Agreement			Total	Accuracy (%)
	Complete	Partial	No		
Craniopharyngioma	0	2	0	2	100
Ependymoma	2	0	0	2	100
Glioma	6	2	3	11	72.7
Hemangioblastoma	0	0	1	1	0
Hemangioma	0	0	1	1	0
Lymphoma	0	1	0	1	100
Meningioma	5	3	0	8	100
Metastasis	1	0	0	1	100
Mixed tumors	0	0	1	1	0
Oligodendroglioma	1	1	0	2	100
Schwannoma	4	0	2	6	66.7
	19	9	8	36	77.8

inflammatory and infectious, while others have taken spinal lesions as well. In addition, the inability to squash the fibrous lesions and inclusions of such lesions may have influenced the accuracy in some studies. The relatively low accuracy of SC in our study could possibly be due to the inclusion of cavernous hemangioma and hemangioblastoma which inherently have low SC accuracy.^{3,10}

We were able to get 100% diagnostic accuracy of SC in meningioma. Others have recorded around 83% agreement. The difference may be due to absence of fibrous meningiomas in our study. Other than fibrous type, the meningioma smear is relatively easy to prepare. It showed variable cellularity, high in syncytial type, low fibrous type. The cells were fairly uniform, arranged in syncytial clusters or in whorls, with eosinophilic cytoplasm and poorly-defined boundaries. The nuclei were oval with delicate chromatin and presence of intranuclear inclusions in some. Psammoma bodies were noted in some cases.^{1,14,16,17} (Fig 2A and 2B)

Figures

Figure 1A and 1B: Squash Cytology and Histopathological Examination of Glioblastoma Multiforme

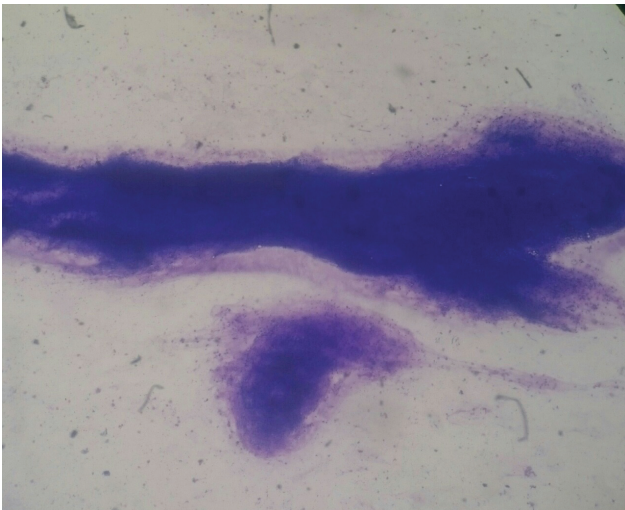


Figure 1A: Squash smear: GBM showing pleomorphic tumor cells in glial background. Occasional bizarre looking cells present and many proliferating capillaries also seen.

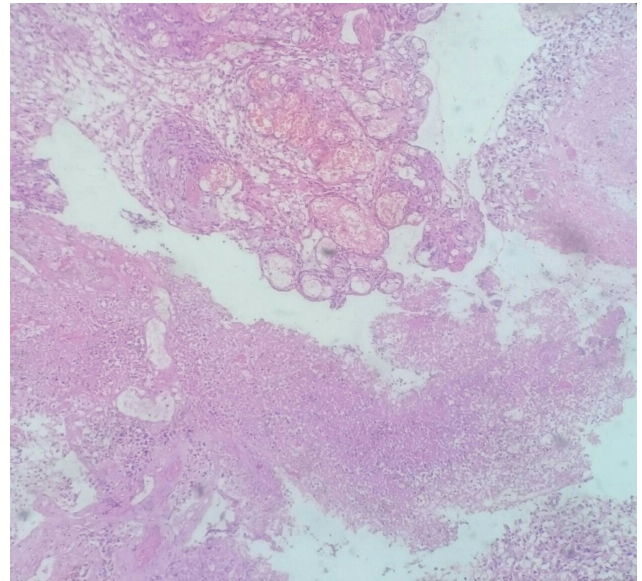


Figure 1 B: Tissue Section GBM: Diffuse proliferation of polygonal to elongated tumor cells with marked pleomorphism. Multiple foci of necrosis and endocapillary proliferation seen.

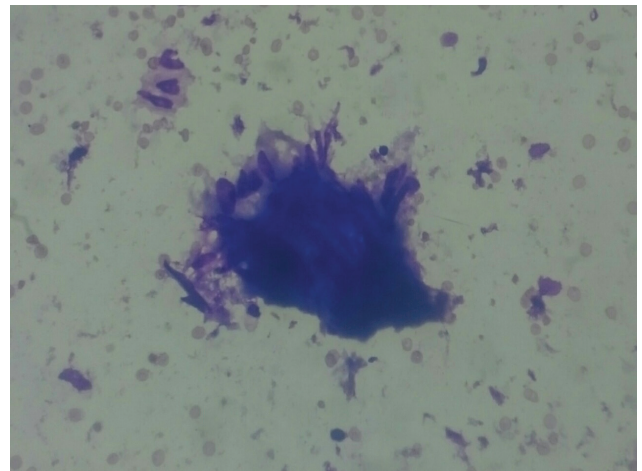


Figure 2A and 2B: Squash Cytology and Histopathological Examination of Transitional Meningioma

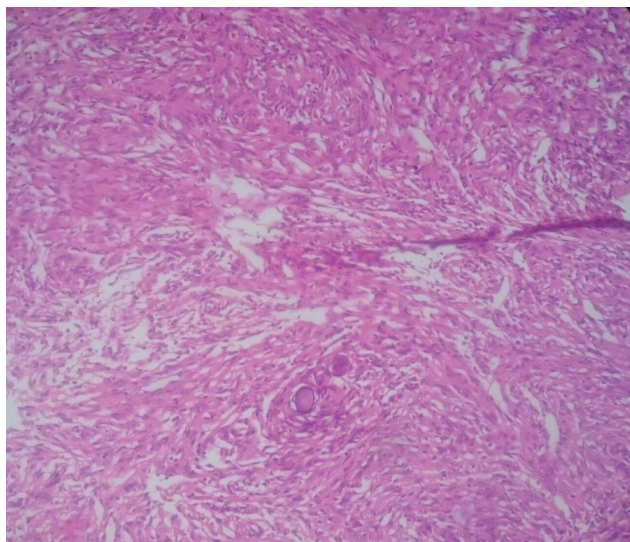


Figure 2A Squash Smear and 2B Tissue section of Transitional Meningioma showing spindle shaped cells having scant cytoplasm with elongated nuclei and inconspicuous nucleoli along with meningotheelial cells having round to oval nuclei. Psammoma bodies also seen.

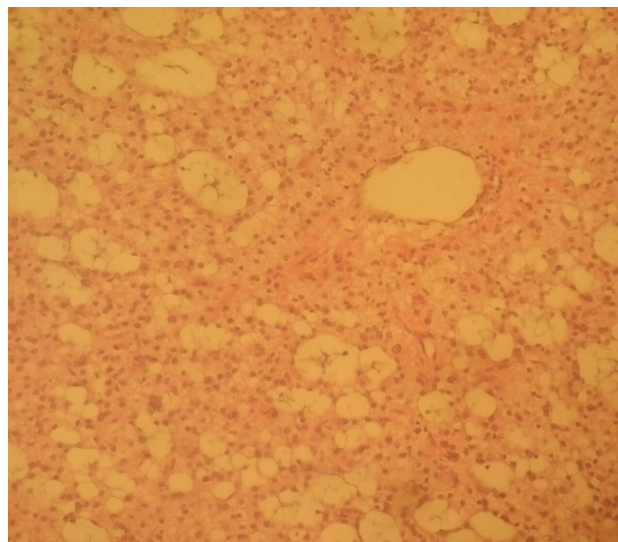


Figure 3B: Tissue Section: Oligoastrocytoma showing diffuse proliferation of tumor cells in fibrillary background showing moderate pleomorphism. Tumor cells are round to oval with irregular nuclei and irregular nuclear rim. Few fragments showing oligodendroglial component composed of monotonous round nuclei and perinuclear halo. Some areas shows microcytic pattern.

Figure 3A and 3B: Squash Cytology of Oligodendroglioma and Histopathological Examination of Oligoastrocytoma

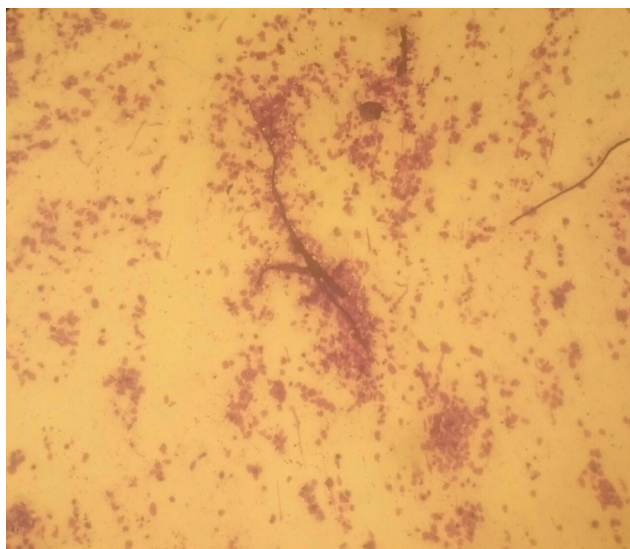


Figure 3A: Squash Smear: Oligodendroglioma showing monomorphic to mildly pleomorphic tumor cells in a glial background with thin walled capillaries.

In cases of high grade glioma, under grading of lesion is a problem in SC. The fibrillary background common to astrocytoma will be visualized. However, the necrotic tissue (one of the key components of HGG diagnosis) is deliberately left behind during smear preparation. In this study, the accuracy rate of only 63% for glioma is less than the reported percentage in other studies.^{5,10,11,13} This may be due to the polymorphic nature of HGG itself. The stereotactic biopsy may yield better accuracy in terms of tissue selection for the smear preparation.^{1,6,14} (Fig 1A and 1B)

On the other hand, the tumors, such as ependymoma, oligodendroglioma and mixed tumors had good accuracy as well in concordance with reported elsewhere. The unique features of granular background and microcalcification visible on the smear makes identification of oligodendroglioma easier; but the area from where squash smear is made, might not adequately represent the entire tumor. So, it is not uncommon to see fibrillary background in the histological examination, thus miss the mixed tumors.^{1,6,15,18,19,20} (Fig 3A and Fig 3B) The use of stereotaxy and/or intraoperative Computed Tomography or Magnetic Resonance Imaging may increase the chance of getting into representative area and might have better SC yield.⁶

Conclusion

SC is a less expensive, reliable and rapid diagnostic method with high accuracy on comparison with final HP evaluation of CNS neoplasm. Intraoperative decision-making regarding the extent of surgical resection and adequacy of biopsy can be assessed with incorporation of this tool in regular surgical practice of CNS tumors, particularly in developing countries. Further studies with a larger sample size is recommended to explore this technology further.

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