

Squash Smear Cytology, CNS Lesions – Strengths and Limitations

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ABSTRACT

Introduction: Cytodiagnosis of CNS Space Occupying Lesions (SOLs) by squash smear preparation is a rapid, inexpensive and fairly accurate diagnostic tool. Most of the studies till date have proved its utility in intraoperative consultation.

Aim: This study was undertaken to evaluate accuracy of squash smear cytology and to highlight as well as analyze the diagnostic pitfalls.

Materials and Methods: This study was conducted in department of pathology, Shri Guru Ram Rai Institute of Medical and Health Sciences. Intraoperative squash smears of two hundred and twenty two cases of CNS SOLs were studied. Cytological diagnoses were correlated

with histopathological evaluation which was taken as gold standard. Discordant smears were reviewed and causes were analyzed.

Results: An accuracy of 83.78% was achieved by squash smear cytology. The discrepancies due to interpretational errors were seen in 61.1% cases and sampling errors accounted for 38.9% cases. Cyto-histological discordance was noted more in oligodendrogliomas and grading of gliomas.

Conclusion: Squash smear cytology is a fairly reliable diagnostic tool in intraoperative neuropathological consultation. Thorough sampling of tissue submitted, clinicoradiological correlation while reporting and awareness about the diagnostic pitfalls help in achieving reasonable accuracy.

Keywords: CNS SOLs, Discordance, Intraoperative cytology

INTRODUCTION

The incidence of Central Nervous System (CNS) tumors varies from 10-17 per lakh persons per year for intracranial and 1-2 per lakh persons per year for intraspinal tumors [1]. The knowledge of location and size of CNS tumors along with their histological type and grade are important for patient management. Recent advances in radiological techniques can correctly assess the spatial delineation of tumors but defining the exact nature of these lesions remains in the province of pathologists.

The volume of tissue available for the pathologist varies with the procedure adopted - open craniotomy or stereotactic biopsy.

Squash smear preparation is a simple and rapid methodology for fairly accurate intraoperative diagnosis of CNS space occupying lesions [2-4].

The main goal of intraoperative cytodagnosis in stereotactic biopsies is to confirm the adequacy of the tissue. This goal has been achieved by many centres as revealed by various

studies [5]. In case of open biopsy an intraoperative diagnosis warrants taking of additional tissue for ancillary techniques (for infections and lymphomas), helps the surgeon to decide on extent of resection and makes it possible to institute adjuvant therapy in immediate postoperative period. Last, but not the least, an intraoperative pathological diagnosis is of great value to the anxious family members of the patients.

A reasonably high accuracy of squash cytodagnosis can be achieved if cytological smears are evaluated taking the clinical and radiological picture into consideration.

This study has been undertaken to share our experience with crush cytology and analysis of the diagnostic pitfalls.

MATERIALS AND METHODS

This prospective longitudinal study was conducted in the Department of Pathology of Shri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun and was approved by the research and ethical committee of the institution. A total of two hundred and twenty two cases were included in

the study. All cases of CNS space occupying lesions, which were subjected to intraoperative smear cytology from January 2009 to August 2015 at our hospital, were included.

The samples were transported from the operation theatre in normal saline with requisition forms bearing the clinico-radiological information of the patients without any delay.

The tissue bits were grossly inspected and squash smears were made from all areas which appeared grossly different. Half of the number of prepared smears were fixed in 100% methanol and stained with H&E stain while the other half were stained with rapid Leishman's stain. A turn around time of 30 minutes was targeted to render intraoperative diagnosis.

Cytological diagnosis were correlated with histopathological diagnosis which is accepted as gold standard and observations were recorded. A detailed re-evaluation of thirty six discordant smears was done and causes analyzed.

RESULTS

We evaluated 220 cases and Sensitivity and specificity of 83.78% was achieved by squash smear cytology. Correct cytological diagnosis was rendered in nearly 84% cases (186/222) and 16% (36/222) were misinterpreted.

The age wise distribution is tabulated in [Table/Fig-1]. Maximum number of cases was seen between 41-60 yrs (50%). There were only a few cases below 10 yrs of age. The benign and malignant neoplasms were in almost equal proportion {Benign 113/222 (50.9%) and malignant were 109/222 (49.09%)}

A reasonably high accuracy of cytological diagnosis was noted in cases of pituitary adenomas, meningiomas, epidermoid cysts and schwannomas ($p < 0.05$) as compared to high grade gliomas ($p > 0.05$) [Table/Fig-2].

The cyto-histological discrepancy was seen more in the grading of tumors as is evident from [Table/Fig-3]. Amongst the 36 discordant cases, 61.12% cases were wrongly interpreted while sampling error was encountered in 38.88% cases.

The overall accuracy and accuracy of squash smears specifically for cases of meningioma, pituitary adenoma and metastasis were comparable with other similar studies as is evident from the table [Table/Fig-4].

DISCUSSION

CNS space occupying lesions are diverse entities. For optimizing therapy, grading of the tumor is as important as the knowledge of exact histogenesis of the lesion.

Intraoperative cytopathological consultation should first comment on the adequacy of the tissue followed by diagnosis

and grading of the tumor if possible. The report should preferably indicate the level of certainty of the diagnosis based on the specific features of that particular lesion.

Serial Number	Age Group(Years)	Number of Cases
1	0-10	06
2	11-20	12
3	21-30	30
4	31-40	33
5	41-50	50
6	51-60	61
7	61-70	15
8	71-80	12
9	81-90	03

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

Histopathological Diagnosis	Total Cases N=222	Correct Cytological Diagnosis	Accuracy (%)
Meningioma	62	59	95.16
Pituitary Adenoma	21	21	100
Epidermoid Cyst/ Craniopharyngioma	06	06	100
Schwannoma	09	09	100
Primitive Neuroectodermal Tumor (PNET) /Medulloblastoma	11	09	81.81
Non Hodgkin's lymphoma	03	02	66.66
Hemangioblastoma	05	04	80
Atypical Teratoid / Rhabdoid Tumor (ATRT)	02	01	50
Choroid Plexus Papilloma	03	02	66.66
Pineal Parenchymal Tumor of intermediate differentiation	01	00	00
Low grade Astrocytoma	09	08	88.88
High grade Astrocytoma	48	38	79.16
Low grade Oligodendroglioma	05	03	60
High grade Oligodendroglioma	08	04	50
Mixed Oligoastrocytoma	08	04	50
Ependymoma	03	03	100
Subependymal Giant Cell Astrocytoma (SEGA)	01	01	100
Metastasis	11	09	81.81
Reactive/Inflammatory	06	03	50

[Table/Fig-2]: Correlation of histopathological and cytological diagnosis.

Final Histo Diagnosis	Cytological Diagnosis	Cases Misdiagnosed On Cytology
Low grade Astrocytoma	High grade Astrocytoma	01
High grade Astrocytoma	Low grade Astrocytoma PXA with Anaplasia Malignant Spindle Cell Tumor	08 01 01
Low grade Oligodendroglioma	Low grade Astrocytoma High grade mixed	01 01
High grade Oligodendroglioma	Metastasis Reactive/Inflammatory High grade Astrocytoma	02 01 01
Mixed Oligoastrocytoma	Astrocytoma	04
Meningioma Angiomatous Meningioma Clear Cell Meningioma	Glioma Meningioma Meningioma	01 01 01
Medulloblastoma Medulloblastoma(Anaplastic variant)	Small cell variant GBM Medulloblastoma	01 01
NHL	Lymphoid cell infiltrate	01
Reactive/Inflammatory	Low grade Gliomas	03
Hemangioblastoma	No opinion offered	01
Metastasis Metastasis Papillary CA	GBM Metastasis - Amelanotic Melanoma	01 01
ATRT	PNET	01
Pineal Parenchymal Tumor of Intermediate Differentiation	Pineoblastoma	01
Choroid plexus papilloma	Ependymoma	01

[Table/Fig-3]: Details of discordant cases.

Various Studies	Squash smear Accuracy In				
	Total cases	Cases of Meningiomas	Cases of Pituitary Adenoma	Cases of Metastasis	Cases of Glioma
Present study	83.78%	95.6% (59/62)	100% (21/21)	90.9% (10/11)	74.3% (61/82)
Tilgner et al., [3]	81.3%	81.6% (40/49)	83.3% (5/6)	92.7% (357/385)	92.4% (3078/3330)
Patty et al., [4]	87%	93.5% (29/31)	-	-	79.2% (42/53)
Shah et al., [2]	89.7%	60% (12/20)	100% (15/15)	100% (7/7)	87% (81/93)

[Table/Fig-4]: Comparative analyses of total and few specific tumor cases with other studies.

Squash smear preparation was first introduced for rapid intraoperative diagnosis by Eisenhardt and Cushing in 1920 [6]. It is now an acceptable method of intraoperative consultation in CNS lesions. It is a fairly reliable technique with an accuracy ranging from 85%-93% [7, 8].

The present study revealed an accuracy of 83.78% that was comparable with other studies. The diagnostic errors were encountered more with oligodendrogliomas, mixed gliomas and reactive gliosis.

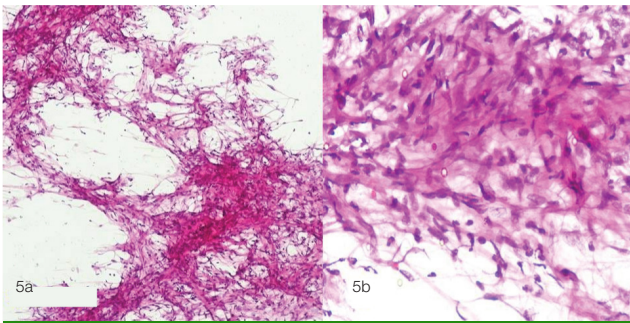
Sampling error was the cause of discordance in 38.8% of cases. This is unavoidable considering the small amount of tissue received for squash cytology.

Absence of focal features in tissue submitted for squash preparation resulted in wrong subtyping of meningiomas, primitive tumors and mixed gliomas. Erroneous down grading

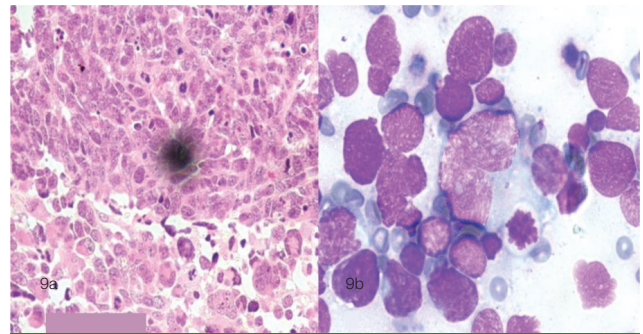
of gliomas was also due to non-representative tissue. Hence, in a radiologically high grade tumor, if the squash smears show otherwise, it is better to communicate to the operating surgeon that a more representative tissue is required.

One case of GBM showed only pleomorphic spindle cells on squash smears and this was called a malignant spindle cell tumor. Histopathology showed a considerable sarcomatous change which was inadvertently selectively sampled for cytology. A more extensive sampling of the tissue received for cytology and a radiological correlation would at least have placed GBM in the differential diagnosis.

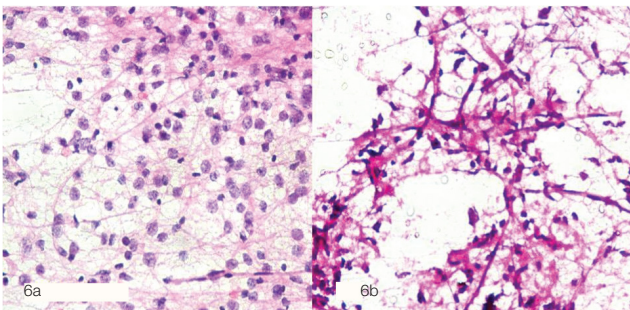
A fairly reasonable accuracy of 95% was noted with intraoperative cytodagnosis of meningiomas, when equipped with radiological finding of dural tail along with characteristic findings of lobules and whorls of monotonous cells on squash smear preparation. In absence of above,



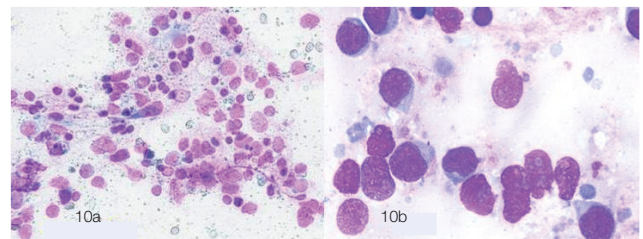
[Table/Fig-5a-b]: a– smear shows cellular fragments with apparent fibrillary background , meningioma. (b)– smear shows broad intercellular bridges, meningioma.



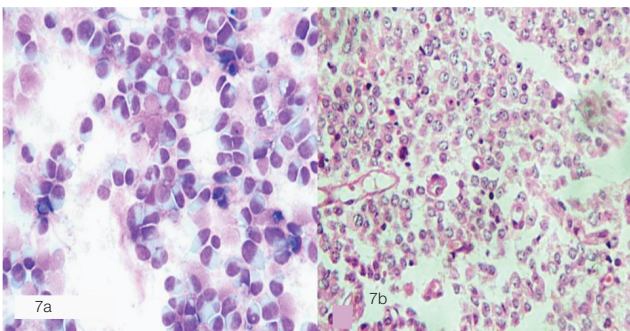
[Table/Fig-9a-b]: (a) – section shows anaplastic large cells in anaplastic medulloblastoma (H&E HPE) (b) – smear shows typical small cells of medulloblastoma.



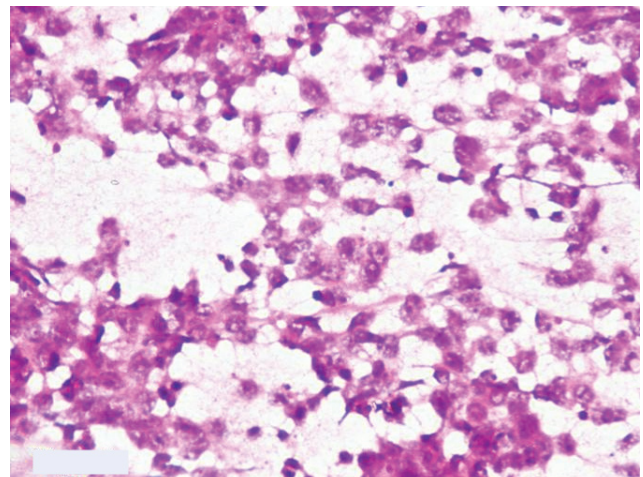
[Table/Fig-6a-b]: (a) – smear shows Round monomorphic nuclei in a fibrillary background, oligodendroglioma. (b) - smear shows spindling of nuclei of cauterized oligodendroglioma.



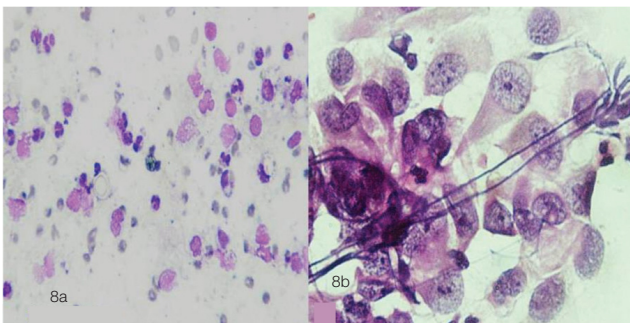
[Table/Fig-10a-b]: (a)– smear shows Polymorphous lymphoid cells, in PCNSL.(b) – Another area with a few monomorphic atypical lymphoid cells, PCNSL.



[Table/Fig-7a-b]: a &b - smear shows Round cell tumor with no fibrillary background, anaplastic oligodendroglioma.



[Table/Fig-11]: Shows atypical malignant cells in a fibrillary background.



[Table/Fig-8a-b]: (a) - smear shows scant degenerating nuclei with neutrophils and (b) shows Occasional cluster of mini gemistocytes, oligodendroglioma, same case.

a more nuanced observation aids in arriving at the correct diagnosis.

One of the cases of meningiomas studied, had an unusual growth pattern such that the tumor's anatomic relationship with dura and brain was obscured on imaging. This being cellular with an apparent fibrillary background was called glioma on cytology [Table/Fig-5a]. Review of the smears revealed high cellularity without pleomorphism and mitosis. The interconnecting bridges between cells and cell clusters

erroneously thought to be glial matrix were thicker [Table/Fig-5b]. A glioma of such cellularity would definitely show features of anaplasia which were lacking in this case.

Since, Oligodendroglial neoplasms are more chemosensitive, efforts at precise diagnosis have resulted in an increase in their detection from 5% to 25-33% of all gliomas [9,10].

On squash smears oligodendrogliomas are characterized by evenly spread smears of moderate cellularity and scant fibrillary matrix. Tumor cells have round nuclei with scant cytoplasm. Four of nine cases were wrongly typed in our study, with an accuracy of only little more than 50%. A poor correlation was also experienced by N Krishnani et al., [11].

It was observed that the presence of round nuclei of dispersed tumor cells was suggestive of oligodendroglioma irrespective of the amount of background fibrillary matrix. Lower grade oligodendrogliomas had considerable fibrillary matrix with monomorphic cells having round nuclei and scant cytoplasm [Table/Fig-6a]. Presence of fibrillary matrix should not exclude the diagnosis of oligodendroglioma. Surgical cautery may cause spindling and wrinkling of round nuclei of oligodendrogliomas making it difficult to give a correct diagnosis as had happened in three of the cases included in the study [Table/Fig-6b]. As the grade of oligodendrogliomas increases it was observed that, the fibrillary matrix decreased and the number of cells with eccentric cytoplasm (minigemistocytes) increased. This predominance of cells with moderate well defined cytoplasm in a background free of fibrillary matrix prevented us from giving the correct diagnosis of the tumor in two cases [Table/Fig-7a&7b]. A case of anaplastic oligodendroglioma showed predominantly necrosis with inflammatory cells [Table/Fig-8a] and the few atypical round cells present in the smear were missed. A careful search on review of smears showed a few interspersed minigemistocytes with similar nuclei [Table/Fig-8b].

Definite grading of a CNS tumor cannot be reliably done by imaging [12]. The presence or absence of enhancement helps but is not always very specific. Accurate grading of gliomas is of great importance to decide the management and predict prognosis. A study by Mitra et al., showed an accuracy of 90.6% to establish the lineage of gliomas and 92.6% to grade the gliomas by squash cytology [13]. 80.7% of astrocytomas could be accurately graded in this study. Debris due to tissue cauterization was misinterpreted as necrosis and resulted in upgrading of a low grade astrocytoma. It is better to correlate smear assessment with radiological findings and any discrepancy should then be objectively analyzed. Presence of necrosis without required pleomorphism and cellularity should be questioned.

The cases of GBM were easily diagnosed, however, grade

3 astrocytomas were not due to the loss of hallmark mitotic figures during squash smear preparation. In such cases, we relied on a subjective analysis of degree of cellularity and pleomorphism; hence there was a discrepancy in 8.33% of high grade gliomas.

Medulloblastomas show variable morphology both on cytological and histopathological examination. Presence of sheets of larger polygonal cells with moderate amount of cytoplasm is seen in anaplastic large cell medulloblastoma, but may confuse the cytopathologist during intraoperative consultation. One such case was observed during the present study wherein the histopathology showed sheets of large pleomorphic cells admixed with classical small cells [Table/Fig-9a]. The smears revealed predominantly small cells and hence were correctly called primitive [Table/Fig-9b]. It is reiterated that medulloblastoma encompasses several tumors with different genetic and biological behaviors. Hence, a tumor with variant morphology can be a medulloblastoma in the correct clinicoradiological setting.

Differential diagnosis of small cell primitive lesions includes small cell variant of GBM. These invariably have a glial component. One case was reported as small cell variant of GBM instead of PNET due to misinterpretation of necrotic granular background as glial matrix.

Primary CNS lymphomas are seen both in immunocompromised and immunocompetent patients. When these solid masses of lymphoma are sampled for squash cytology, they yield cellular smears, showing predominantly discohesive cells with high grade nuclei and one or several nucleoli making the diagnosis quite evident. Tumor cells at the periphery of the solid growth infiltrate the surrounding brain parenchyma and evoke a strong gliotic reaction. The gliotic tissue binds the normally discohesive lymphoma cells along with reactive T-cells into the matrix. Such smears may not be as representative of a lymphoma and a definite diagnosis may not be possible as experienced in one of the three cases of lymphoma [Table/Fig-10a]. A close examination of the enmeshed cells and cells lying in singles will definitely help in arriving at the correct diagnosis [Table/Fig-10b].

Reactive gliosis is associated with all kinds of brain injury, both neoplastic and non neoplastic [14]. It is very important to recognize gliosis, intraoperatively especially when a biopsy is taken at or near the grossly indistinct transformation between normal and diseased brain. If gliosis is present the surgeon may be guided to take a deeper more representative biopsy. Cytological features of reactive gliosis include moderate heterogeneous cellularity, fibrillary background, increased blood vessels without endothelial proliferation, perivascular lymphohistiocytic infiltrate along with haemosiderin laden macrophages. Failure to accurately appreciate these features

in gliotic smears led us to misdiagnose reactive gliosis as a low grade glioma in three cases.

Densely vascular hemangioblastomas are known to smear poorly because of abundant reticulin thus yielding pauci cellular smears. Contrary to this, four out of the five cases of hemangioblastomas revealed cellular smears with numerous polygonal stromal cells and vacuolated cytoplasm in the present study. In one case a diagnosis was not offered due to presence of thick vascular fragments and occasional stripped nucleus.

Metastatic brain tumors are the commonest brain malignancies and their incidence is expected to rise due to increased survival of patients with extracranial neoplasms [15].

Further it is estimated that 30% brain metastasis are diagnosed synchronously or at times precede the detection of a primary tumor elsewhere in the body [16]. Hence, it is not very infrequent that tissue from such cases is received for intraoperative assessment.

Eleven cases in this study were diagnosed as metastatic malignancies on histopathological examination. Nine of these were correctly diagnosed and typed on squash cytology achieving an accuracy of 81.8%. In both the discordant cases, there was no history of a known primary malignancy. One was erroneously diagnosed as GBM, while the other was wrongly typed.

The background matrix of metastatic malignancy is devoid of fibrillary matrix, thus easily differentiated from a high grade primary tumor like GBM. This distinction is blurred if gliotic tissue near the metastatic site is also included in the tissue submitted for squash cytology as seen in one of the cases [Table/Fig-11]. In such cases it is helpful to observe the edges of clusters of tumor cells, if any, which are sharp and well defined in metastasis. Moreover most of the metastasis show brisk mitosis some of which can be still seen after squash preparation. Mitosis in metastasis is spotted more easily than in GBM.

LIMITATIONS

A major limitation of squash cytology preparation is rendering of intraoperative diagnosis in a limited short time frame. This is further compounded by lack of clinico-radiological information at times. Hence, it is recommended that the report should include degree of certainty of specific diagnosis offered, to assist neurosurgeon to take correct intraoperative decisions. Non-representative tissue submitted for squash cytology also results in discrepancies. In stereotactic biopsies, though, this helps the surgeon to take a deeper more representative tissue for histopathological evaluation.

CONCLUSION

Squash smear preparation is a simple and reliable tool for intraoperative diagnosis. A cytopathologist should be aware of artifactual findings of cytology smears and offer a diagnosis keeping the clinico-radiological picture in mind. Thorough sampling, complete and astute observation, wise interpretation along with maintaining a reasonable turnaround time is mandatory. Simultaneous re evaluation of discordant cases also helps in improving subsequent squash cytology reporting.

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