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UTILITY OF INTRAOPERATIVE SQUASH SMEAR CYTOLOGY IN CNS LESIONS

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ABSTRACT

Objective

Intraoperative squash smear cytology (ISSC) is a simple, rapid, inexpensive and fairly accurate tool to diagnose CNS neoplasms intraoperatively. It can help the surgeon to modify the approach intraoperatively based on preliminary impression of lesion on cytology. Aim of this study is to find out the accuracy and utility of ISSC technique as a standalone method for rapid diagnosis of CNS lesions.

Materials And Methods

A total of 94 cases of CNS lesions were examined by squash smear technique for cytological diagnosis which were then compared with histopathological diagnosis provided on paraffin embedded sections which was taken as gold standard.

Results

An accuracy of 80.4% was achieved by Squash smear cytology. Of the total 94 cases, 82 were concordant with the final histopathological diagnosis, while 12 were discordant. The discrepancies were due to interpretational errors in 8% of the cases and sampling errors in 4% of the cases.

Conclusion

Squash smear cytology proved to be a simple, rapid, inexpensive and fairly accurate tool for intraoperative diagnosis of CNS lesions. Thorough sampling, clinicoradiological correlation and awareness about possible diagnostic pitfalls while reporting helps in achieving better diagnostic accuracy.

Keywords: CNS lesions, Discordant, Intraoperative squash smear cytology, Meningioma

INTRODUCTION

Central nervous system lesions are the product of various infections, inflammations or a wide spectrum of life threatening malignancies. The clinical presentation of various CNS lesions may appear similar despite their varied locations so knowledge of site and size of tumor along with its histopathological grading is essential for patient management. In approximately 10-20% of the cases, radiological features are inconclusive of the etiology of the lesions. Thus it is difficult for the surgeon to aid the patient prognosis **[1]**. Hence, a cytological or histopathological examination of the tumour tissue is essential for a definite diagnosis of the lesion. Fine needle aspiration cytology is not possible due to closed architecture of the intracranial and intraspinal space occupying lesions. There are two principal methods 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 developing countries like India, due to the limited laboratory and financial resources, frozen section has limited use in routine diagnostic practice **[2]**. Therefore, a rapid and reliable method of intraoperative diagnosis like intraoperative squash smear cytology is needed for critical decisions regarding the targeting of the lesion and the extent of surgical resection.

This study was undertaken to find the accuracy and utility of Intraoperative Squash Smear Cytology (ISSC) technique as a standalone method for the rapid diagnosis of CNS lesions as well as to detect and highlight the diagnostic pitfalls.

MATERIALS AND METHODS

A prospective study was performed in the department of Pathology S.B.K.SMI & R.C, Waghodia, Gujarat during December 2021 to December 2022.

A total of 94 cases of different CNS lesions were examined by squash smear technique for cytological diagnosis which were then compared with histopathological diagnosis provided on paraffin-embedded sections.

The age, sex and clinical presentation of each case was noted. 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.

Small 2-3 mm sized tissue bits were taken on 1 end of 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 smears were immediately fixed in methanol and stained by quick H and E method. The smears were studied keeping in view the clinical and Radiological information provided to us, and an opinion regarding the presence of lesional tissue, and the nature & type of lesion were informed to the surgeon.

The intraoperative cytological diagnosis was compared with final diagnosis of the formalin fixed paraffin embedded gold standard histopathological sections to assess the diagnostic accuracy of squash smears in CNS lesions.

Data collected were statistically analyzed and comparison between the intraoperative diagnostic procedure squash cytology and final histopathological diagnosis was made. The results were classified into the following categories: True Positive (presence of tumor correctly diagnosed); True Negative (absence of tumor correctly diagnosed); False Negative (the squash cytology failed to diagnose as tumor) and False Positive (squash cytology incorrectly diagnosed as tumor).

RESULTS

The study was a prospective study of 94 CNS space-occupying lesions, with the youngest patient aged 2 years and oldest, aged 63 years. The age wise distribution is tabulated in **Table 1**. Maximum number of cases was seen between 41-50 years (30.85%) as seen in **Table 1**. Patients presented mostly with seizures, headache, nausea, vomiting, vertigo, tinnitus and difficulty in hearing. Some presented with local symptoms according to the site of involvement such as difficulty in speech, disorientation, paralysis of one side of body, or falling suddenly.

Among the 94 intracranial lesions, 25 were diagnosed as meningioma, 23 as astrocytoma, 10 as oligodendroglioma, while we had 4 cases of pituitary adenoma and 4 cases of metastatic deposits to the brain. We had 4 cases of schwannoma, and 5 cases of medulloblastoma as shown in **Table 2**. In our study, meningiomas (incidence = 26.6%) were the most common tumors, followed by astrocytomas (23.4%) and oligodendrogliomas (10.6%). Metastatic lesions were diagnosed in four patients on squash smear which included metastasis from the breast, lung, and kidney. The cases with the same diagnosis and grade on cytology and histopathology were

considered as complete correlation as mentioned in **Table 2**. Benign were slightly more predominant than malignant. {Benign 51/94 (54.25%) and malignant were 43/94 (45.75%)}.

The cases where intraoperative cytological diagnosis did not correlate with the histological examination were categorized as discrepant cases. Out of 94 cases, correct cytological diagnosis was obtained in 82 cases (80.4%) and 12 cases (19.6%) were misinterpreted, of which 8 cases were discrepant in diagnosis whereas 4 cases showed sampling error. The causes of diagnostic discrepancies are described in **Table 3**. Sampling error was seen in fibroblastic meningioma as it is a tough tissue which is difficult to spread, in Choroid plexus papilloma and craniopharyngioma due to lack of representative tissue and inadequate tissue obtained.

The statistical analysis of various CNS lesions in our study is described in **Table 4**. It is seen that overall sensitivity of squash smear cytology of CNS lesions is 80.3% whereas the overall specificity is 98.8%. The overall positive predictive value (PPV) is 77.2% while the overall negative predictive value (NPV) is 99.1% which is comparable with other studies.

Table 1: Age Wise Distribution

Serial Number	Age Group (Years)	Number of Cases
1	0-10	07
2	11-20	08
3	21-30	12
4	31-40	15
5	41-50	29
6	51-60	18
7	61-70	07

Table 2: Cytological and Histological Diagnosis of Various CNS Lesions Along With Diagnostic Accuracy

Sr. No.	DIAGNOSIS	CYTOLOGY	HISTOLOGY	DIAGNOSTIC ACCURACY
1.	ASTROCYTOMA LOWGRADE	6	7	85%
2.	ASTROCYTOMA HIGHGRADE	13	15	87%
3.	EPENDYMOMA	4	5	80%
4.	OLIGODENDROGLIOMA	9	10	90%
5.	MEDULLOBLASTOMA	5	5	100%
6.	SCHWANNOMA	4	4	100%
7.	NEUROFIBROMA	4	4	100%
8.	MENINGIOMA	23	25	92%
9.	HEMANGIOLASTOMA	1	2	50%
10.	CRANIOPHARYNGIOMA	2	3	67%
11.	PITUITARY ADENOMA	4	4	100%
12.	METASTATIC TUMORS	4	4	100%
13.	CHOROID PLEXUS PAPILLOMA	0	1	0%
14.	EPIDERMOID CYST	1	1	100%
15.	TUBERCULOSIS	1	1	100%
16.	GLIOSIS	1	3	33%

Table 3: Various Causes of Diagnostic Disparities

Tumor Group	Total no. of discrepant cases	Cause of the discrepancy
Low grade astrocytomas	01	Grading error (over grading)
High grade astrocytomas	02	Grading error (under grading)
Gliosis	02	Cellularity increased but distribution not recognised, failure to find macrophages
Ependymoma	01	Absence of rosette pattern with diffuse fibrillary background
Oligodendroglioma	01	Presence of excessive fibrillary matrix, spindling and wrinkling of round nuclei
Hemangioblastoma	01	Low cellularity
	Total 08	

Table 4: Statistical Analysis of Various CNS Lesions In Our Study

Sr. No.	TUMOUR	TP	TN	FP	FN	SENSITIVITY	SPECIFICITY	PPV	NPV
1	HIGH GRADE ASROCYTOMA	13	76	3	2	86.7%	96.2%	81.3%	97.5%
2	LOW GRADE ASTROCYTOMA	6	85	2	1	85.7%	97.7%	75%	98.8%
3	OLIGODENDROGLIOMA	9	83	1	1	90%	98.8%	90%	98.8%
4	EPENDYMOMA	4	87	2	1	80%	97.7%	66.7%	98.8%
5	MEDULLOBLASTOMA	5	88	1	0	100%	98.9%	83.4%	100%
6	SCHWANNOMA	4	88	2	0	100%	97.8%	66.7%	100%
7	NEUROFIBROMA	4	89	1	0	100%	98.9%	80%	100%
8	MENINGIOMA	23	67	2	2	92%	97.1%	92%	97.1%
9	HEMANGIOLASTOMA	1	91	1	1	50%	98.9%	50%	98.9%
10	GLIOSIS	1	91	0	2	33.3%	100%	100%	97.8%
11	METASTATIC TUMORS	4	90	0	0	100%	100%	100%	100%
12	PITUITARY ADENOMA	4	90	0	0	100%	100%	100%	100%
13	CHOROID PLEXUS PAPILLOMA	0	93	0	1	0%	100%	0%	98.9%
14	CRANIOPHARYNGIOMA	2	91	0	1	66.7%	100%	100%	98.8%
15	EPIDERMOID CYST	1	92	1	0	100%	98.9%	50%	100%
16	TUBERCULOSIS	1	93	0	0	100%	100%	100%	100%

A reasonably high accuracy of cytological diagnosis was noted in cases of pituitary adenomas, metastatic tumors, medulloblastoma, epidermoid cysts and schwannomas as compared to high grade gliomas, gliosis and choroid plexus papillomas.

DISCUSSION

CNS lesions are varied and diverse. For optimizing therapy, grading of the tumor is as essential as the knowledge of exact histogenesis of the lesion. Squash smear preparation was first introduced for rapid intraoperative diagnosis by Eisenhardt and Cushing in 1920 **[3]**. It is now an important method of intraoperative consultation in CNS lesions. Intraoperative cytopathological consultation should first comment on the adequacy of the tissue followed by diagnosis. Few studies also compared results of squash cytology with frozen section and concluded that the accuracy of cytology was as good as frozen section **[4-6]**. According to Nigam *et al*., a well-prepared squash smear may eliminate the need for frozen section or at least

influence its interpretation **[7]**. Also, elimination of freezing artifact **[8]** and reduced cryostat contamination from potentially infected tissue make squash cytology superior over frozen section. [9] Some studies suggest that squash preparation provides better morphology than frozen section with even minute biopsy material **[10, 4, 11]**. Thus, though frozen section was considered complementary to squash cytology **[12]**, the latter alone can guide neurosurgeon during intra-operative period in many of the institutes where frozen section is not available. The strength of squash cytology is that it is simple, rapid, cost effective, robust, provides good cellular details, allows tissue to be preserved for paraffin embedding, need minimum equipment and technical skill personnel. It can easily be availed at the operative site. One of the most important advantages is that even very tiny specimens are suitable for smear preparation, which is extremely important in surgical procedure from intracranial lesions.

Accuracy of cytological diagnosis depends on good cytological preparation and staining of the smear. Smearing of the tissue with ease depends on the consistency of the tumor. The soft and friable tissues in the CNS tumors are easy to smear and thus provide good cellular details. This is especially exhibited in gliomas, medulloblastomas, pituitary adenomas, most of the meningiomas, and metastatic tumors. The diagnostic errors were encountered more with oligodendrogliomas, mixed gliomas and reactive gliosis.

Reactive gliosis is associated with all kind of brain injury, both malignant as well as non malignant. Whenever the biopsy is taken from at or near the grossly indistinct transformation between normal and diseased brain, it is very important to rule out gliosis. The surgeon is advised to take a deeper or more representative biopsy if gliosis is present. Moderate heterogenous cellularity, fibrillary background, increased blood vessels without endothelial proliferation and perivascular lymphohistiocytic infilterate along with hemosiderin laden macrophages are some of the important cytological features of gliosis. Failure to accurately appreciated these features in gliotic smears lead us to misdiagnosed reactive gliosis as low grade glioma **[13]**.

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 **[14, 15]**. 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. Of 10 cases, 9 were correctly typed in our study, with an accuracy of 90%. 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. As the grade of oligodendrogliomas increases it was observed that the fibrillary matrix decreased and the number of cells with eccentric cytoplasm increased. 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.

Definite grading of a CNS tumor cannot be reliably done by imaging **[16]**. The presence or absence of enhancement helps but is not always very specific. The grade of astrocytomas varies within a single tumor, and hence, grading of astrocytoma on cytology occasionally poses problem. 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 **[17]**. 86% of astrocytomas could be accurately graded in this study. One case of glioblastoma was under graded as anaplastic astrocytoma due to the absence of necrosis and no thick-walled vessels could be appreciated. Another case of anaplastic astrocytoma was misgraded as low-grade astrocytoma due to only occasional mitoses in the smear sampled. 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.

In case of hemangioblastoma, because of abundant reticulin it yields pauci cellular smears. In our study there was 1 case where representative tissue was not submitted and gliotic tissue was misinterpreted as pilocytic astrocytoma. In cases when the tissue is not representative or the cytology findings are not consistent with the radiological findings, at times, we can ask for more tissue to be submitted intraoperatively which would reduce the discrepancy **[13]**.

In case of ependymoma it shows fairly monotonous cells with oval/ round nuclei with finely stippled chromatin with

prominent fibrillary background. Multiple layers of nuclei surrounding vascular structure forming rosettes and papillae also seen. The absence of any rosette pattern with diffuse fibrillary background led to misinterpretation of one case of ependymoma as astrocytoma in our study **[18]**.

Metastatic brain tumors are the commonest brain malignancies and their incidence is expected to rise due to increased survival of patients with extracranial neoplasms **[19]**. Further it is estimated that 30% brain metastasis are diagnosed synchronously or at times preceed the detection of a primary tumor elsewhere in the body **[20]**. Hence, it is not very rare that tissue from such cases is received for intraoperative assessment. 4 cases in this study were diagnosed as metastatic malignancies on histopathological examination. All of these were correctly diagnosed and typed on squash cytology achieving an accuracy of 100%. The background matrix of metastatic malignancy is devoid of fibrillary matrix, thus easily differentiated from a high grade primary tumor like GBM. Moreover most of the metastasis show brisk mitosis some of which can be still seen after squash preparation, which is spotted more easily than in GBM.

Although smears provide good cytological details, a few diagnostic difficulties were faced. There were 12 discrepant cases in our study. Tissue from unrepresentative areas led to erroneous diagnosis in discrepant cases. The present study revealed an accuracy of 80.4% when compared with histology as the gold standard. This was due to limitations such as short and critical time frames, lack of clinic-radiological information, non representative tissue submitted, etc.

Thus the exact location, radiological findings, and clinical presentation of the patient help the pathologist in making the cytological diagnosis with reasonable accuracy. The role of pathologists, however, in the intraoperative setting should be to provide sufficient preliminary information for optimal surgery rather than to provide a precise diagnosis and accurate grade for each case.

CONCLUSION

We can conclude that intraoperative squash smear cytology is easy to perform, inexpensive and permits high diagnostic accuracy. A cytopathologist should be aware of artifactual findings of cytology smears and offer a diagnosis keeping the clinico-radiological picture in mind. Thorough sampling, comprehensive observation, wise interpretation along with maintaining a

reasonable turnaround time is mandatory. Intraoperative squash can provide a preliminary diagnosis in CNS lesions thus enabling the surgeon to plan further management on the operating table itself. Simultaneous re evaluation of discordant cases also helps in improving subsequent squash cytology reporting. Thus, we can say that squash smear cytology is immensely important in CNS lesions to guide the intraoperative decisions and ultimately decreasing the morbidities of prolonged surgeries.

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Author Contribution

Dr. Jigna Prakashbhai Patel contributed to study designing, literature search and review, data acquisition and statistical analysis.

Dr. Aishwarya Mehul Patel contributed to study designing and data analysis.

Dr. Heli Umesh Shah contributed to manuscript preparation and editing.

Dr. Rushika Nimesh Parikh contributed to statistical analysis and editing.

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