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Accuracy and diagnostic utility of squash smear in rapid intraoperative diagnosis of CNS tumor in tertiary care center - A 100 case study

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Abstract
Introduction: Intraoperative squash smear cytology is a simple and reliable technique for rapid intraoperative diagnosis of neurosurgical specimen. Squash smear technique has recently gained importance because of image-guided stereotactic biopsies. It helps the neurosurgeons to decide the extent of the surgery. The soft consistency of CNS tissue is best suited for squash cytology which is in fact hindrance for frozen section.
Aims and objectives: To study the accuracy and utility of intraoperative squash smear in the diagnosis of central nervous system (CNS) tumors and to find out the incidence of various CNS tumors.
Materials and methods: Total of 100 retrospective cases with clinical diagnosis were studied from September 2018 to December 2019 (15 months) at department of histopathology, B.J.M.C. and Civil Hospital, Ahmedabad.
Results: Out of 100 cases, in 11 cases smears were inadequate or showed only necrotic material. Therefore diagnosis by squash smear was possible in only 89 cases. Out of 89 cases, 76 cases showed concordance, 6 cases showed partial concordance and 7 cases were discrepant on comparison of squash smear cytology with histopathology. 85.4% diagnosis of squash smear cytology matched with its histological reporting. With highest incidence of Astrocytoma followed by Meningioma. One case of Central Neurocytoma, Round cell tumor and Melanoma each were also recorded.
Conclusion: Intraoperative Squash Smear cytology technique can provide rapid, simple, reasonably accurate and reliable diagnosis to the operating surgeons. It helps the neurosurgeons in determining the modality of treatment. Astrocytoma was the most common tumors followed by Meningioma.

Keywords: Squash smear, Astrocytoma, Meningioma

Introduction
Intraoperative squash smear cytology is a simple and reliable technique for rapid intraoperative diagnosis of neurosurgical specimens. Squash smear has gained importance due to image guided stereotactic techniques [1]. It helps the neurosurgeons to decide the extent of the surgery [2]. Intraoperative diagnosis of space occupying lesions of the Central Nervous System helps neurosurgeons to modify the approach at surgery. The ideal intraoperative method used should be accurate, rapid and should allow the preservation of tissue for paraffin section study [5]. Frozen section provides good cytomorphological details and for histological typing if there is no limitation of tissue availability. However, it requires costly equipments and may cause ice crystal formation particularly in astrocytoma and the freezing artifacts cause distortion of architecture. The advantages of squash smears are that it is easy to smear CNS tumor with good cellularity, can be done even when the sample is limited and intraopertaive diagnosis can be rendered within 10 minutes [1]. This study is aimed to reveal the accuracy and diagnostic utility of squash smear in rapid intraoperative diagnosis of CNS tumors in a tertiary care hospital of Ahmedabad, Gujarat where 100 cases were studied.

Materials and methods
In this study, a total of 100 cases were collected from September 2018 to December 2019 (15 months) at department of histopathology, B.J.M.C. and Civil Hospital, Ahmedabad. All the patents were subjected to magnetic resonance imaging (MRI). Clinical diagnosis of CNS tumor was made and was correlated with radiological findings.

Small bits of brain tissue obtained intra-operatively were sent to histopathology department in normal saline; then squash smears were prepared and were stained with Hematoxylin and Eosin (H and E) stain. Then they were observed under the microscope and diagnosis was made. The reports were informed to the neurosurgeons. Remaining tissue was processed for histopathology. Comparison between diagnosis of squash smear cytology and histopathology was made and results were noted. The tumors were classified according to the World Health organisation (WHO) classification of CNS neoplasms 2016. The observations were then subjected to appropriate statistical analysis methods.

Results

Out of 100 cases, in 11 cases smears were inadequate or showed only necrotic material. Therefore diagnosis by squash smear was possible in only 89 cases.

Out of 89 cases, 76 cases showed concordance, 6 cases showed partial concordance and 7 cases were discrepant on comparison of squash smear cytology with histopathology. The diagnostic accuracy of squash smear cytology was found to be 85.4% with highest incidence of Astrocytoma followed by Meningioma. One case of Central Neurocytoma, Round cell tumor and Melanoma each were reported.

Table 1: Incidence of CNS tumors diagnosed in the present study using Squash smear (89cases).

Cns tumors	No. Of cases	% of incidence
Astrocytoma	30	33.7
Meningioma	19	21.3
Ependymoma	10	11.2
Medulloblastoma	7	7.8
Metastasis	7	7.8
Oligodendroglioma	5	5.6
Schwannoma	5	5.6
Pituitary adenoma	3	3.37
Central neurocytoma	1	1.1
Round cell tumor	1	1.1
Melanoma	1	1.1

In table 1 as mentioned, Out of 100 cases of CNS tumors diagnosed by histology, comparision with squash smear was available only for 89 cases. The most common CNS tumor was Astrocytoma, followed by Meningioma, Ependymoma, Medulloblastoma and metastasis, oligodendroglioma, Schwannoma, followed by Pituitary adenoma, Central neurocytoma, Round cell tumor and Melanoma.

Table 2: Age and Sex wise distribution of CNS tumors as diagnosed by Squash smear.

Age	Male	Female	Total
0-15	9	4	13
16-30	10	7	17
31-45	11	19	30
46-60	8	7	15
61-75	8	3	11
76-90	3		3
Total	49	40	89

In table 2, Out of 89 cases that were compared using squash smear and histopathology techniques, overall cases show slight Male predominance. But higher incidences of tumors is seen among Females in age group 31-45 and 46-60 years. Maximum number of cases are seen in the age group of 31-45 years. The youngest case was of 4 months. The eldest case was of 78 years.

Table 3: Incidence of Astrocytomas according to their WHO grading and variants using squash smear.

Astrocytomas	Male	Female	Total	Incidence %
WHO GRADE I	3	4	7	23
Pilocytic Astrocytoma	2	2	4	
WHO GRADE II	8	3	11	36
Fibrillary Astrocytoma	3	1	4	
Pleomorphic Xanthoastrocytoma	3	1	4	
WHO GRADE III	4	3	7	23
Anaplastic astrocytoma	4	3	7	
WHO Grade IV	3	1	4	13
GlioblastomaMultiforme	3	1	4	
Astrocytoma reported without grading or variant	1		1	3
Total	19	11	30	

In Table 3, Astrocytomas were divided on the basis of their WHO grading and their variants. Out of total 7, astrocytomas graded WHO Grade I, Only 4 were Pilocytic Astrocytoma. Out of total 11, astrocytomas graded WHO Grade II, Only 4 were Fibrillary Astrocytoma and 4 were Pleomorphic Xanthoastrocytoma. All WHO grade III astrocytomas& Grade IV astrocytomas were reported as anaplastic astrocytoma and Glioblastoma Multiforme. Only one astrocytoma was reported without any grade or describing its variant.

Table 4: Incidence of Meningioma according to their WHO grading and variants using squash smear.

Meningioma	Male	Female	Total	Incidence %
Grade I	2	9	11	57
Psammomatous Meningioma	1	3	4	
Fibroblastic Meningioma	0	3	3	
Meningoepithelial Meningioma	1	3	4	
Grade II	0	2	2	10
Atypical Meningioma	0	2	2	
Grade III	1	3	4	21
Anaplastic Meningioma	1	3	4	
Meningioma reported without grading	1	1	2	10
Total	4	15	19	

In table 4, Out of 11 cases of Grade I meningioma that were reported, 4 cases belonged to Psammomatous Meningioma, 3 cases belonged to Fibroblastic Meningioma and 4 cases belonged to Meningoepithelial Meningioma. Out of 2 cases of Grade II meningioma that were reported, both the cases belonged to Atypical Meningioma. Out of 4 cases of Grade III meningioma that were reported, both the cases belonged to Anaplastic Meningioma.

Table 5: Cases with complete concordance based on gender.

CNS tumors	Male	Female	Total
Astrocytoma	16	10	26
Meningioma	3	14	17
Ependymoma	5	3	8
Medulloblastoma	4	2	6
Metastasis	6	0	6
Oligodendroglioma	3	1	4
Schwannoma	2	2	4
Pituitary Adenoma	1	1	2
Central Neurocytoma	1	0	1
Round cell tumor	1	0	1
Melanoma	1	0	1
Total	43	33	76

In table 5, out of 30 cases of astrocytoma complete concordance was seen in 26 cases. Out of 19 cases of meningioma complete concordance was seen in 17 cases. 8 cases of ependymoma out of 10, 6 cases of medulloblastoma out of 7, 6 cases of metastasis out of 7, 4 cases of oligodendroglioma out of 5, 4 cases of Schwannoma out of 5, 2 cases of pituitary adenoma out of 3 and all cases of central neurocytoma, round cell tumor and melanoma show complete concordance i.e is 76 of the total 89 cases showed complete concordance.

Table 6: Causes of Partial concordance

Sr No.	Squash Smear Findings	Histopathological Findings
1	Astrocytoma who grade ii	Astrocytoma grade i
2	Astrocytoma grade ii	Anaplastic astrocytoma
3	Astrocytoma	Fibrillary astrocytoma
4	Meningioma/ Ependymoma	Ependymoma who grade i
5	Meningioma	Transitional meningioma
6	Glioma grade ii	Anaplastic astrocytoma gradeii

In table 6, 6 cases showed partial concordance out of 89 cases on squash smear.

Table 7: Causes of Discordance

Cause	No. of Cases
Mixed gliomas causing diagnostic difficulties due to dominance of one histological pattern	6
Reactive Gliosis diagnosed as Astrocytoma	1
Total	7

In table 7, Out of 7 cases showing discordance, 6 cases were of mixed gliomas which caused difficulties in diagnosis and 1 case of reactive gliosis which was reported as astrocytoma.

Discussion

Out of all the 100 cases collected during the 15 month period, 11 of the cases only showed inadequate or necrotic material, so they were excluded. The cases where the intraoperative diagnosis was same as the Final diagnosis with an accurate assessment of cell line and benign versus malignant were assigned to be in complete concordance**.** 76 cases showed complete concordance, i.e. 85% of the cases showed complete concordance. Partial concordance was considered in cases in which +/- 1 grade of deviation in tumor grading was registered or there were two differential diagnosis or specific variant of lesion was not mentioned. 6 cases showed partial concordance i.e only 7% of cases showed partial concordance.

No concordance or discordance was considered to cases where intraoperative diagnosis was not confirmed by final histological diagnosis. 7 cases showed disconcordance i.e only 8 % of cases showed discordance.

Causes of Discrepancies occurred due to:

1) Sampling errors
2) Squash smear preparation errors
3) Staining errors
4) Diagnostic errors

The accuracy of squash smear technique found in this study is 85.4%, which is comparable to Bharti jha et.al whose accuracy was 82.35% [3], kumarverma et.al whose accuracy was 88.9% [4] and mitra *et al.* whose accuracy was 88.5% [6]. It was found that for meningioma, there is preponderance of meningioma in females while in CNS tumors are more common in males which correlates with study of Zorludemir S *et al.* [7],

Conclusion

Intraoperative Squash Smear cytology technique can provide rapid, simple, reasonably accurate and relatively cheaper diagnosis as compared to frozen sections to the operating surgeons.

It helps the neurosurgeons in determining the modalities of treatment.

Astrocytomas are the most common tumors followed by Meningiomas.

Age group wise study shows predominance of tumor in the a ge group of 31-45 year.

Overall accuracy of squash smear technique when compared to paraffin section is 85.4%.

Squash smear technique can be used as complementary to frozen sections for rapid diagnosis of intraoperative cases.

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