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Original Research Article

Study of Squash Smear Cytology in Intraoperative Diagnosis of CNS Tumors- A Tertiary Care Centre Experience in Western Odisha

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Conflict of interest: Nil

Abstract:

Background: Intraoperative squash smear cytology is an effective method for the prompt diagnosis of neurosurgical specimens. This method is easy, rapid, inexpensive and designed to evaluate the precision of intraoperative squash smear cytology in identifying space-occupying lesions within the Central Nervous System (CNS).

Aims and Objectives: To evaluate the value of squash smear cytology for rapid intraoperative diagnosis of CNS tumors. To know the pattern of CNS tumors cytologically. To determine the limitations of squash smears.

Methods: Intraoperatively, tumor tissues obtained from the neurosurgeon were subjected to a squash smear examination at the Department of Pathology, VIMSAR, Burla. The tissue samples were gently squashed between glass slides, stained with rapid Hematoxylin and Eosin (H & E) and Diff-Quik stain, and examined under a microscope. The cytological features were documented and correlated with subsequent histopathological diagnoses obtained from formalin-fixed, paraffin-embedded sections.

Results: Eighty cases were received from the Department of Neurosurgery, of which 9 were non-neoplastic cases and 71 had neoplastic lesions. Meningioma accounted for the largest group of tumors among the neoplastic lesions, with 35 cases (49.29%). A total of 65 cases (91.54%) showed complete correlation with histopathology, one case (1.40%) showed partial correlation, and 5 (7.04 %) were discordant.

Conclusion: Squash smear cytology is a reliable, rapid, and cost-effective method for intraoperative diagnosis of CNS tumors.

Keywords: CNS Tumors, Squash Smear Cytology, Intraoperative Diagnosis.

Introduction

The Central Nervous System (CNS) tumors, including brain tumors, are relatively uncommon neoplasms. It contributes to approximately 3.1% of all cancer incidences as well as mortality in India. Globally, approximately 3,22,000 new cases were estimated in 2022.[1]

The neoplasms of the brain present significant challenges for neurosurgeons, clinicians, and pathologists because of CNS's complex architecture and vital functions. Therefore, they require correct diagnosis, surgical removal, and effective treatment while minimizing damage to surrounding healthy tissue. The pathologist has two basic methods for quickly determining a tissue diagnosis: frozen section and CNS squash smear cytology (CSC). Frozen section is superior to squash smear cytology for the assessment of architectural features, especially when the tissue is firm to hard in consistency. However, because brain tissue is prone to displaying ice crystal artifacts, analyzing a frozen portion can be difficult. CNS tumors have a gel-like consistency and are very soft; squash smear cytology allows better nuclear details and provides rapid diagnosis and does not need any sophisticated instrumentation. [2-4]

First introduced by Eisenhardt and Cushing in early 1930, CSC has now been established as a method of intraoperative diagnosis of CNS tumors.[2] Pathologists have accepted squash smear cytology as a simple, rapid, inexpensive, fairly accurate, and dependable tool for intraoperative diagnosis.[5] This study aims to evaluate the utility of intraoperative squash cytology from a neurosurgeon's perspective.

Materials and Methods

This is a prospective study conducted in the Department of Pathology at VIMSAR, Burla. The study was carried out after receiving approval from the institutional ethical review committee. Eighty neuropathological specimens received between August 2022, and July 2024 were evaluated. The patients included in the study were those who were admitted to the Department of Neurosurgery at VIMSAR, Burla, due to space-occupying lesions of the brain and spinal cord. The specimens were received intraoperatively. Both the specimen container and the requisition forms were inspected to ensure that all the information was correct. First the specimen was thoroughly examined. Areas that were necrotized or hemorrhagic were not smeared. Only the viable tissue was selected. Next, 0.5–1 mm^2 of tissue that had been cut with a scalpel was placed on a clean, labeled glass slide. The tissue was then squeezed using the second slide and rapidly pulled across the first slide to create the smear, which produced a rather homogeneous tissue layer. The right amount of pressure was carefully applied for the tissue to spread properly. Both fixed wet and dry smears were made. For wet smears, two to three smears were quickly fixed in isopropyl alcohol for five minutes and stained with rapid Hematoxylin and Eosin stain. Another two to three smears were dried immediately and stained with Diff-Quik stain. The cytological findings were correlated with histopathological diagnosis. Immunohistochemistry was also advised in some cases for confirmation and subtyping.

Results

During the two-year study period from August 2022 to July 2024, the total number of general pathology specimens received at the Department of Pathology, VIMSAR, Burla, was 7645. The prevalence of CNS lesions reported in the Department of Pathology, VIMSAR, Burla, was 1.04%. A total of 80 CNS lesions were received, of which 71 cases were found to be CNS tumors. The age range in this study was 13 to 80 years, with a mean of 45.08 years. The highest incidence of CNS tumors was observed to occur in the fifth and sixth decades, accounting for 25.36% each. (Table 1) Overall, the male-to-female ratio for CNS tumors was 1:1.36. CNS tumors were most commonly found in the supratentorial area, accounting for 63.38% of all cases (45 out of 71 cases).

Table 1: Age Distribution of CNS Tumours

Age Group	Frequency	Percentage
<10 yr	00	00%
11-20 yr	06	08.46%
21-30 yr	07	09.85%
31-40 yr	13	18.30%
41-50 yr	18	25.36%
51-60 yr	18	25.36%
61-70 yr	07	09.85%
>71 yr	02	02.82%
Total	71	100%

Cytological examination revealed 35 cases (49.29%) of meningioma, followed by astrocytoma (19 cases, 26.76%), Schwannoma (10 cases, 14.08%), metastasis (4 cases, 5.63%), medulloblastoma (2 cases, 2.81%), and Ependymoma (1 case, 1.40%) (Table 2).

On subsequent histopathological and immunohistochemical examination, 34 out of 35 cases of meningioma were correctly diagnosed in cytology with a diagnostic accuracy of 97.14%. The diagnostic accuracy of schwannomas was 90%, glial tumors 89.47%, and metastatic lesions 75%.

Out of 71 cases, a correct correlation was obtained in 65 cases, with an overall diagnostic accuracy of 91.54%. In the present study, five cases were discrepant, and one case of metastasis showed partial correlation in typing the tumor deposits.

Table 2: Diagnostic Accuracy of Squash Cytology

CNS Tumours	No. of cases in squash cytology	Correct cytological diagnosis	Misdiagnosed cases	Diagnosis offered	Diagnostic Accuracy (%)
Meningioma	35	34	1	Astrocytoma	97.14%
Astrocytoma	19	17	2	Ependymoma	89.47%
Schwannoma	10	09	1	Meningioma	90.00%
Metastasis	04	03	1*	Metastatic adenocarcinomatous deposits	75.00%
Medulloblastoma	02	01	1	Ependymoma	50.00%
Ependymoma	01	01	-	-	100.00%
Total	71	65	6		91.54%
(1* = 1 case partially correlated)					

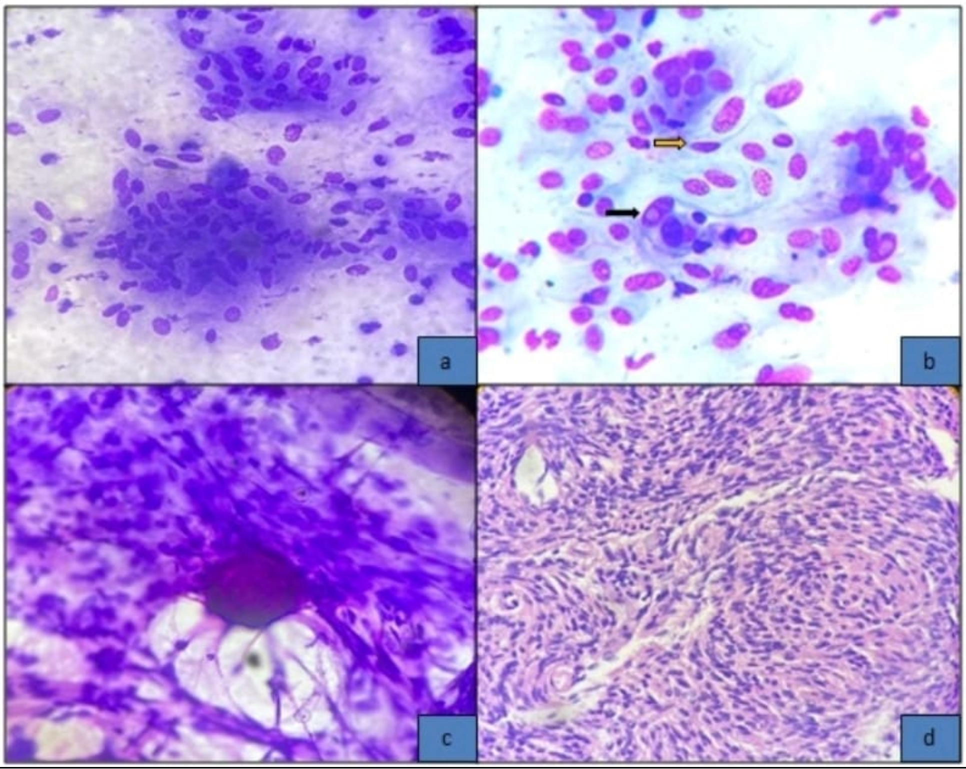

Figure 1: (a) Squash smear cytology of meningioma showing syncytial clusters of plump spindle cell nuclei with indistinct cytoplasm and whorl formation (Diff-Quik x100). (b) Squash smear cytology showing intranuclear inclusions (black arrow) and nuclear grooving (yellow arrow) (Diff-Quik x400). (c) Squash smear showing concentric, lamellated, basophilic psammoma body (H&E x400). (d) Histopathology of Meningothelial Meningioma, showing meningothelial cells in whorls (H&E x400)

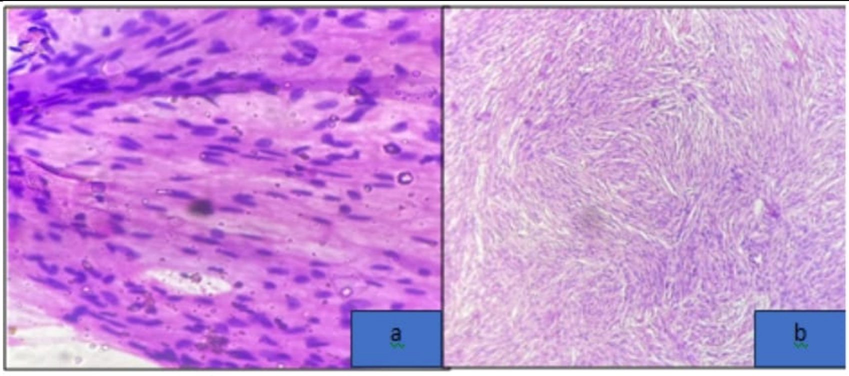

Figure 2: (a) Discrepancy case – Schwannoma on squash cytology (Diff-Quik x400). (b) Fibroblastic Meningioma on histopathology- Monomorphic elongated cells with spindle nuclei arranged in fascicles (H&E x100)

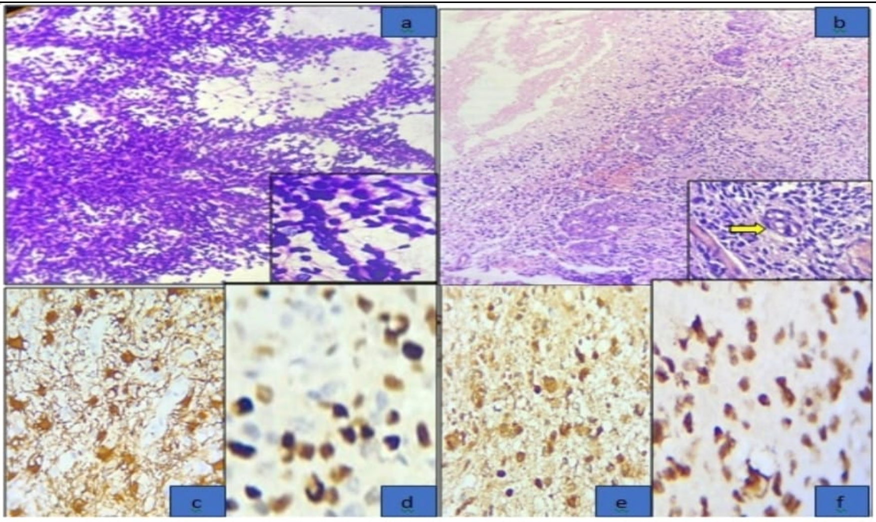

Figure 3 (a) High-grade glioma: Squash smear showing a marked increase in cellularity, nuclear pleomorphism, and glial fibrillary processes in the background (H&E x100), inset showing pleomorphic nuclei H&E x400). (b) Histopathology of High-grade glioma showing areas of necrosis (H&E x100), inset showing microvascular proliferation with glomeruloid vessels (H&E x400). (c-f) Astrocytoma grade 4 - Neoplastic cells are positive for GFAP, Ki- 67, IDH, and ATRX respectively (IHC, x400).

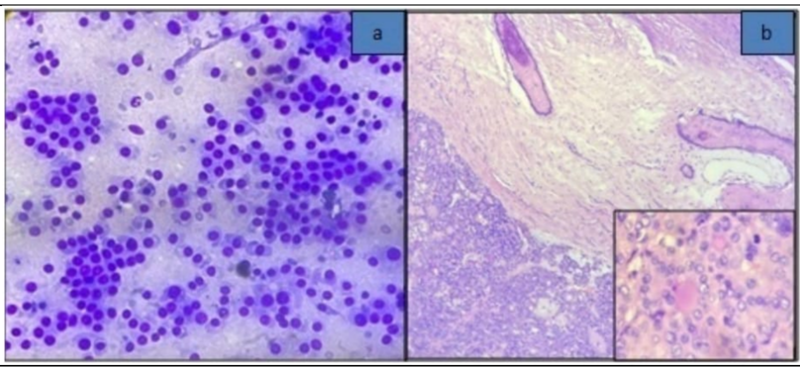

Figure 4:(a) Metastatic follicular carcinoma: Squash cytology smear showing monomorphic round cells in a dispersed population and follicular pattern in a thin colloid background. (Diff-Quik, x400). (b) Histopathology showing trabeculae of skull bone and metastatic deposits of thyroid follicular carcinoma (H&E x100), inset showing microfollicular pattern of thyroid follicular carcinoma (H&E x400)

Discussion

Squash smear cytology is a simple and rapid procedure. Since CNS tumors are soft and friable, they are easier to stain and provide more detailed cellular information. The pathologist will be able to provide an accurate cytological diagnosis with the aid of the patient's specific location of the tumor, clinical presentation, and radiological findings. In this study, a total of 80 cases of CNS lesions were received at the Department of Pathology, VIMSAR, Burla, in the two-year study period.

Out of 80 CNS lesions, 71 cases (88.75%) were neoplastic, and 9 cases (11.25%) were non-neoplastic. The non-neoplastic lesions included arachnoid cysts, epidermal inclusion cysts, and inflammatory lesions. Of 71 CNS tumors, the youngest patient was a 13-year-old child, and the oldest was 80 years old. The maximum incidence of CNS tumor was found to occur in the 5th and 6th decades, which was comparable with Acharya et al., where the maximum numbers of cases were seen in 41-60 years.[6] Our study showed female predominance with M:F = 1:1.36, similar to the study conducted by Omon et al.[7] But studies done by Khuroo et al., Tejaswi et al., and Jindal et al. showed a slight male preponderance.[8-10]

Out of 71 cases, 35 cases (49.29%) were diagnosed as Meningioma in histopathology, followed by Astrocytoma 18 cases (25.35%), and Schwannoma 9 cases (12.67%). Meningiomas were more common than Astrocytomas in our study; this was also observed in the study by Khuroo MS et al. [8] However, according to studies by Mitra S et al. and Kalpana et al., the incidence of Astrocytoma was higher than that of Meningioma. [5,11] Table-3

Meningiomas constituted largest category accounting for 49.29%. The most common age group was 51-60 years (40%) followed by 41-50 years 37.14%), which is in parallel with the study done by Patel P. J. et al, where the most common age group was 5th & 6th decades.[12]

Out of the 35 cases of Meningioma, 25 (71.42%) were female patients and 10 (28.58%) were male, accounting for the female preponderance with M: F = 1:2.5. Female preponderance in Meningioma is found all over the world, which is also found in the study by Ostrom et al. [13] This is due to the effect of sex steroids. Meningiomas rarely develop in prepubertal children when circulating sex hormones are low. Meningiomas are known to express progesterone and estrogen receptors. It has been demonstrated that hormone replacement therapy raises the risk of Meningioma in women. [14-16] Meningiomas are diagnosed easily with squash cytology. Meningothelial cells in syncytial pattern, whorl formation, psammoma bodies, intranuclear inclusion, and nuclear grooving were seen in variable proportions in our study. (Figure 1a-d) These diagnoses were confirmed and subtyped in histopathology.

Out of the 35 cases of meningioma, 34 were diagnosed correctly. Only one was misdiagnosed and reported Schwannoma as the fibrous areas mimicked Antoni A areas of Schwannoma. Spreading was easy, and on microscopy, the cells appeared as intact whorls with oval to elongated bland-appearing nuclei. On histopathology, it was reported as Fibroblastic Meningioma (Figure 2a-b).

The next most common tumors were Astrocytoma. 19 cases of astrocytoma were diagnosed on squash cytology with 2 discrepent cases which on histopathology diagnosed as Ependymoma. Hence the diagnostic accuracy was 89.47%. It is inappropriate to grade Astrocytoma based on squash cytology, as Astrocytoma varies significantly in grade from one area to another within a single tumor. [11,17] The Low-grade glioma showed low to moderate cellularity, with the tumor cells having round, oval to slightly elongated nuclei rimmed by pale-staining cytoplasmic processes. Squash smears of high-grade gliomas were highly cellular and showed tumor cells against a fibrillary background with necrotic debris and marked endothelial proliferation. Subsequent histopathology sections showed high cellularity with bizarre tumor cells, large areas of necrosis, mitosis, pseudo-palisading around tumor necrosis, and endothelial proliferation (Figure 3 a-b). Similar findings were reported by Tejaswi et al. [9] Subsequent immunohistochemical studies helped in the confirmation and subtyping of CNS tumors (Figure 3 c-f). In this study, one case of Astrocytoma was misdiagnosed as Meningioma in squash cytology due to the presence of bland-appearing elongated spindle-shaped cells, and some vague whorl-like structures. However, in histopathology, it was revealed as grade 3 Astrocytoma.

Out of 10 cases, 9 cases (12.67%) of Schwannoma were correctly correlated which is comparable to research by Sumit et al and Kalpana et al who found the tumor accounted for 12% to 13%. [5,11] Schwannomas were firm and difficult to spread. Squash cytology showed spindle cells in fascicles with vague Verocay bodies and frayed rope appearance. Histopathology showed cellular areas with nuclear palisading (Antoni A) and hypocellular (Antoni B) areas. Nine cases out of ten were accurately diagnosed. In one case, Meningioma was misdiagnosed as Schwannoma because of the presence of elongated spindle-shaped cells and vague Verocay body formation (Figure 2 a-b).

Our study documented four cases of brain metastases from tumors elsewhere in the body, which was in accordance with the study by Khuroo MS et al. [8] Two cases with adenocarcinomatous deposits from lung carcinomas and one from follicular carcinoma of the thyroid gland in squash cytology were reported (Figure 4 a-b). In one case, metastatic carcinomatous deposit possibly squamous cell carcinoma was suggested because of the presence of polygonal to elongated cells and tadpole-like cells present discretely and in clusters along with areas of necrosis. The case was diagnosed as papillary adenocarcinomatous deposit in the cerebellum in histopathology. The immunohistochemistry showed CK 7, CK19, TTF 1, and Napsin A positive along with CK 20, PAX 8, and GFAP negative with metastasis from lung cancer. A study by Mark W. Becher et al. also revealed that metastatic carcinoma cells were negative for GFAP but positive for cytokeratin.[18]

Two cases of Medulloblastoma were diagnosed in squash smear cytology, one of which was correctly diagnosed, and another one was diagnosed as Clear Cell Ependymoma on histopathology. The discrepancy was due to the presence of round cells with scant cytoplasm and hyperchromatic nuclei, which also lacked the rosette formation typical of Ependymoma.

In our study, four cases of Ependymoma were diagnosed histopathologically. One case of Ependymoma was correctly identified in squash smear cytology, but two cases were incorrectly diagnosed as Astrocytoma and one as Medulloblastoma on cytology.

The statistical analysis of the study showed that the sensitivity of Meningioma, Astrocytoma, Schwannoma, metastasis, and Ependymoma was found to be 97.14%, 100%, 100%, 75%, and 25%, respectively. The specificity of Meningioma, Astrocytoma, Schwannoma, metastasis, and Ependymoma was found to be 97.22%, 96.30%, 98.39%, 98.52%, and 100%, respectively. Ependymoma and Medulloblastoma were only reported once in the present study, so the statistical analysis did not add much value. Different studies (Table-4) show that the accuracy of squash cytology varies from 85% to 96%. [9,10,19-21] In the present study, out of 71 tumor cases analyzed, a complete correlation with histopathology was achieved in 65 cases. One case of metastasis to brain showed partial correlation. The overall diagnostic accuracy was found to be 91.54%. This finding is in parallel with the study done by P. Kadiyala et al. [19]

Table 3: Comparative Incidence of CNS Tumours in India

CNS Tumours	Khuroo MS et al study [8]	Kalpana et al study [11]	Tejaswi et al Study [9]	Incidence in the Present Study
Meningioma	36.14%	19.13%	15.38%	49.29%
Astrocytoma	32.53%	39.23%	40.38%	25.35%
Schwannoma	04.81%	11.96%	13.64%	12.67%
Metastasis	04.81%	2.87%	3.6%	05.63%
Ependymoma	06.02%	1.91%	1.82%	05.63%

Table 4: Diagnostic Accuracy of Squash Cytology in Various Studies - an Overview

Authors	Number of cases	Correlate with HPE	Accuracy
P. Tejaswi et al [9]	52	47	95.92%
Jindal A et al [10]	150	141	94%
P Kadiyala et al [19]	64	58	91%
Shukla et al [20]	140	135	96.42%
D. Goel et al [21]	3057	2598	85%
Present study	71	65	91.54%

Hence Squash cytology is a useful adjuvant in centres without intraoperative frozen sections and can be utilized as a quick way to diagnose CNS lesions. However, it should be borne in mind that squash cytology is only a preliminary investigation and should not be used solely for diagnostic or therapeutic purposes. It has to be confirmed by histopathology. Immunohistochemistry should be included whenever necessary.

Limitations observed in Squash Cytology were low cellular yield, difficult to spread and obtain representative sample. Lesions such as Meningiomas and Schwannomas were difficult to smear due to the increased fibrous component or calcification, often rendering smears of poor quality.

Conclusion

Squash cytology is a sensitive and specific modality for diagnosing space-occupying lesions of the brain and spinal cord. The method is easy, rapid, and inexpensive. Details of cellular morphology are well seen in squash cytology. Hence, squash cytology can be used as a reliable diagnostic tool in developing countries like India. Despite its benefits, squash cytology should only serve as a preliminary study. The gold standard for confirmation is always histopathology. It should never be used for diagnosis or treatment. Moreover, squash cytology is a fast and accurate method for cytologically diagnosing CNS lesions when used by a pathologist with experience, and combined with clinical and radiological data, it significantly aids neurosurgeons in surgical decision-making, potentially improving patient management outcomes.

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