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Original Article

Evaluation of Intraoperative Cytological Smears for Diagnosis of Brain Tumors with Special Reference to Immunohistochemistry

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Abstract

Context: Brain tumors are heterogeneous group of benign and malignant tumors of glial, meningeal, neuronal, embryonal, and lymphoid origin. Rapid intraoperative diagnosis of the nature of the tumor helps the surgeon to plan the extent of surgery and modify it accordingly. **Aims:** (1) To establish the validity and reliability of squash cytology in the intraoperative diagnosis of brain tumors. (2) To correlate with histopathological report and immunohistochemistry (IHC) profile. **Settings and Design:** Prospective, observational study. **Subjects and Methods:** A prospective, observational study was conducted in the Department of Pathology in collaboration with the Department of Neurosurgery of a tertiary care hospital. One hundred and seven patients with symptomatic or radiologically detected brain tumors were included in the study. Intraoperative squash smears were stained with hematoxylin and eosin and rapid papanicolaou stain. Cytological diagnosis was recorded and communicated to the surgeon. Cytological findings were corroborated with histological findings subsequently. GFAP, Ki-67, and ER-PR IHC were used as additional markers. **Statistical Analysis Used:** Software used in statistical analysis of our study was MedCalc version 11.6 (Mariakerke, Belgium: MedCalc Software 2011). **Results:** A total of 107 cases were included in the study. Meningioma was the most common lesion. Overall sensitivity in our study to diagnose benign and malignant tumors was 94.7% and the specificity is 97.6% with positive and negative predictive value of 94.7% and 97.6%, respectively. Diagnostic accuracy was highest in pituitary adenoma. **Conclusions:** The combination of meticulous evaluation of clinical, radiological, and cytological findings helps in accurate and rapid diagnosis of brain tumors.

Keywords: *Brain tumor, GFAP, Ki-67, squash cytology*

Introduction

The annual incidence of tumors of the central nervous system (CNS) ranges 10 to 17 per 100,000 persons for the intracranial tumors and 1 to 2 per 100,000 persons for intraspinal tumors.[1] Cytological techniques have been used for diagnosis of brain lesions since 1930 when Eisenhardt and Cushing advocated the use of touch preparation for rapid diagnosis of tumors.[2] In the vast majority of cases, the neurosurgeon's main reason for intra-operative neuropathologic consultation is to ensure that a diagnostic specimen was obtained in the least invasive manner and with few complications. Second, if the smear diagnosis is an infection or lymphoma, to make sure that additional biopsies are submitted in adequate quantity for microbiologic or flow cytometric studies, respectively; an infectious disease specialist or hematologist-oncologist on duty are immediately contacted to ensure institution of or modification to appropriate medical treatment. Similarly, it serves a guide to determine the extent of resection in embryonal tumors in children. For these two purposes, an intraoperative cytological examination has indeed been reported to be an excellent method compared to stereotactic biopsy.[3-10]

Subjects and Methods

A prospective, observational study was conducted in the Department of Pathology in collaboration with the Department of Neurosurgery of a tertiary care hospital including 107 patients presented with clinically symptomatic or radiologically detected brain tumors. Inclusions criteria were (1) A space-occupying lesions of the brain (primary) diagnosed by magnetic resonance imaging (MRI) FLAIR, computed tomography scan study. (2) Only tumorous lesions of the brain were included in the study. All spinal tumors (intra- and extra-axial), infectious diseases affecting

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the brain and miscellaneous conditions such as metabolic diseases were excluded from the study.

Proper approval from the Institutional Ethical Committee and informed consent from the parents in case of minor for the procedure were taken. History along with radiological data was documented. Intraoperative squash smears were prepared. Areas of interest are selected from fresh tissue (usually 2–5 bits were taken depending on the amount of tissue received). 1–2 mm cube of tissue with a scalpel was taken and placed on a glass slide. A second slide was pressed (perpendicular to the second slide) over the first slide with optimal pressure. All the slides were dipped into 95% ethanol (fixative), and brought to the laboratory, for staining with, hematoxylin and eosin (H and E) and rapid papanicolaou. Special cytochemical staining like periodic acid–Schiff, Ziehl–Neelsen, and Masson-Trichrome were performed whenever necessary. Depending on the cohesiveness of the cells, background, cytoplasmic-nuclear details and relation with the vessels, the final cytological diagnosis was made. Depending on the cytological findings, smears were broadly categorized according to the WHO classification. Tissue for histopathological examination was obtained as post-operative materials. Cytodiagnosis were compared taking histopathology reports as gold standard. WHO grade I and II were considered as benign and grade III and IV were considered as malignant. GFAP, Ki-67, and ER-PR immunohistochemistry (IHC) were used as additional markers.

Statistical analysis

Histopathological diagnosis of the tumors were taken as the "gold standard" and the reports of fine-needle aspiration cytology were statistically analyzed using Chi-square test, Mathews correlation test, sensitivity, specificity, positive and negative predictive value, diagnostic accuracy, etc., Software used in statistical analysis of our study was MedCalc version 11.6 (MedCalc Software Broekstraat 52 B-9030 Mariakerke Belgium).

Results

Clinical parameters

A total of 107 cases were included in the study. Meningioma was the most common lesion. Based on MRI finding, all cases were broadly categorized into supratentorial and infratentorial lesions. Supratentorial space occupying lesions of brain constituted 63% of cases were the predominant lesion in our study, followed by lesions at other sites (e.g., extra-axial), 19% and infratentorial (18%) location. Age of presentation of different brain tumors was between 2 and 70 years. Slight male preponderance was noted with male: female ratio 1.22:1 [Table 1].

Most of the space occupying lesions of brain presented with the symptoms and signs of cortical dysfunction (52%). Other important symptoms and signs comprised cerebellar and ophthalmic manifestations.

Cytological smear

The cytological smear patterns were grossly subclassified into gradient clumps, lumpy mounds, and diffuse patterns. Schwannoma, meningioma which were hard and leathery to smear comprised the lumpy mounds. Whereas the low-grade brain tumors like pilocytic astrocytoma and cerebellar hemangioblastoma produced diffuse patterns [Figure 1].

The provisional diagnosis of brain tumors was given by assessing the pattern of blood vessels and their relation

Table 1: Distribution of brain tumor cases according to age and site of lesion

MRI location	Diagnosis	<3 years	3-15 years	15-65 years	>65 years	Total (%)
Intra-axial						
Supratentorial	Glioma	1	1	26	1	27
	Craniopharyngioma		3	4		6.5
	Pituitary adenoma			6		6
	Central neurocytoma		1	1		2
	Ependymoma	1	1	3		5
	Metastasis			2		2
	Germinoma		1			1
	Others	1	3	4		7.5
Infratentorial	Medulloblastoma		2			2
	Pilocytic astrocytoma		2			2
	Myxopapillary ependymoma		1			1
	Metastasis			1		1
	Others		1	2	1	4
Extraaxial	Meningioma			24	1	24
	Schwannoma			8		7.5
	Melanocytoma			1		1
	Giant cell tumor			1		1
	Others			4		3.7

MRI – Magnetic resonance imaging

with the tumors cells. Major vessel pattern were thin-walled blood vessels, endothelial proliferation and that of Caterpillar arrangement. Low-grade gliomas and pituitary adenoma displayed thin walled vessels. Glioblastoma and metastatic deposits revealed thick vessels. In ependymoma, vessels resembled Caterpillar arrangement.

Cytological findings

Depending on cytological findings and proper clinicoradiological correlation, lesions were broadly categorized into four major groups.

Neuroepithelial tumors

Totally 31 cases presented with neuroepithelial lesions. Two were of pilocytic astrocytoma and seven were of glioblastoma. Majority were astrocytoma which mostly presented between 15 and 65 years, while most of the pilocytic astrocytomas were in children [Figure 4]. Astrocytoma which reveals moderate numbers neoplastic cells having fibrillary process and present around thin-walled blood vessels. Glioblastoma shows pleomorphism, mitotic activity, and endothelial proliferation. The presence of Rosenthal fiber is characteristic of pilocytic astrocytoma.

Tumors of meninges

Total number of meningeal tumors that were diagnosed were 25 cases, i.e., 23.36%. Syncytial cluster of meningothelial cells along with whorl formation was detected in majority cases. True psammoma bodies were identified in 7 cases.

Tumors originating in posterior fossa of brain

Brain tumors originating from the posterior fossa of brain comprised those space occupying the cerebellum, in and around the fourth ventricle of brain. Ependymoma comprises the bulk of tumors (23%) found in that region. Key features of ependymoma were feature is the presence of papillaroid fragments and caterpillar vessels. Furthermore, cerebellum being one of the most common site of secondary metastasis constitutes about 14% of all lesions found in the posterior fossa of the brain. Medulloblastoma comprised 9% of lesion showing densely packed cells having hyperchromatic nuclei focal molding.

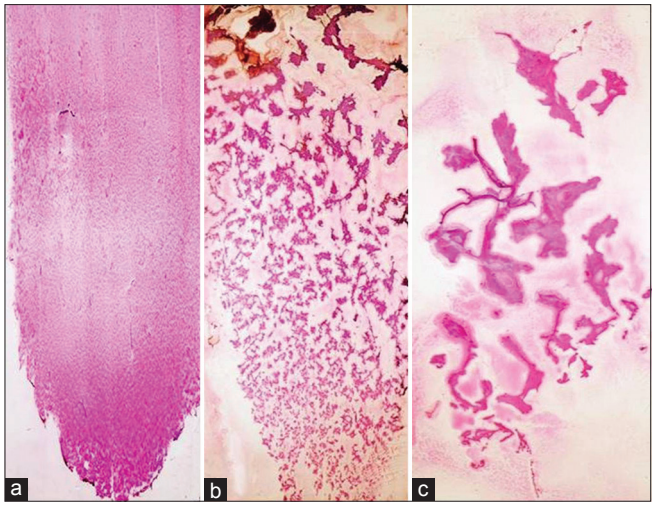

Figure 1: Smear pattern (a) Noncohesive pattern, (b) intermediate, (c) cohesive pattern

Tumors originating in suprasellar region of brain

Pituitary adenoma was the most common in the age group of 15–65 years including about 7% of total cases revealing monolayered cells without molding and cohesion. Craniopharyngioma being the next most common tumor found in the suprasellar region was equally distributed among the children, adolescent and the adults (4.6%). These tumor were recognized by the presence of squamous epithelium along with gliosis and Rosenthal fibers.

Ependymoma [Figure 2], medulloblastoma [Figure 3], craniopharyngioma, pituitary adenoma, germinoma, showed 100% diagnostic accuracy cytohistological correlation. Whereas, the most difficulty was found in the case of oligodendroglioma [Figure 4], due to the resemblance of this glioma with other low-grade glioma, lack of calcification, and no cytoplasmic clearing [Table 2 and Figure 5].

Totally 102 cases of 107 cases were able to be cytological correlated with histology. It was found that out of 20 diagnosed cytologically benign cases, 2 cases were found to be histologically malignant. Out of 82 cytologically malignant cases, only 1 case was found to be histologically benign. Five cases were inconclusive cytologically. Overall sensitivity in our study to diagnose benign and malignant tumors is 94.7% and the specificity is 97.6% with positive and negative predictive value of 90% and 98.7%, respectively. Two-sided $P < 0.0001$, which is considered extremely significant [Figure 6].

Immunohistochemical findings

Ki-67 expression in gliomas was assessed and mean value in Grade I-IV were 1%, 2.73%, 8%, and 18.84% [Table 3]. Tukey–Kramer multiple comparison test evident that *P* value of comparison between grade I and grade II is not significant, but all others are significant [Figure 7].

Female patients (14 cases) with meningioma expressed maximum PR receptor positivity [Table 4]. In male patients (9 cases), expression of PR receptors positive was nearly equal to PR receptor negative cases [Figure 8].

Discussion

Tumor of CNS represents 1.4% of newly diagnosed cancer cases. Although, the brain tumors are not only frequent but also high morbidity and poor survival rate make it diagnostic priority. A conclusive histopathological diagnosis of intracranial space occupying lesion is a primary requisite before planning further patient management. Conventionally, in staged procedures, squash cytology are

Table 2: Discordant and inconclusive cases

Discordant cases				Inconclusive cases
Grading error		Cell type error		
Low grade gliomas (II)	Anaplastic astrocytoma (III)	Meningioma (I)	Schwannoma (I)	Melanocytoma
Meningioma (I)	Atypical meningioma (II)	Small blue round cell tumor (IV)	Cerebellum	Cerebellar hemangioblastoma
High grade glioma (III)	Gliosarcoma (IV)	Glioblastoma (IV)	Metastasis	Hemangiopericytoma
Oligodendroglioma (II)	Anaplastic oligodendroglioma (III)			GCT of base of brain
				Inadequate tissue pieces

GCT – Giant cell tumor

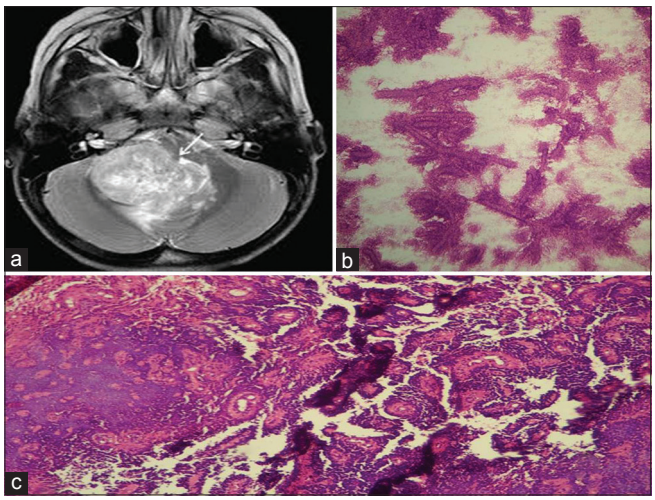

Figure 2: Ependymoma(a) Magnetic resonance imaging lesion in the postfossa near fourth ventricle. (b) Thick, rigid, serpentine vessels producing Chinese characters. Tumor cells polarized in their attachment to the vessel (H and E, ×40). (c) Uniform round nuclei and the conspicuous cellular polarity with a fibrillary matrix of processes extending to the vessel walls (HPE, ×40)

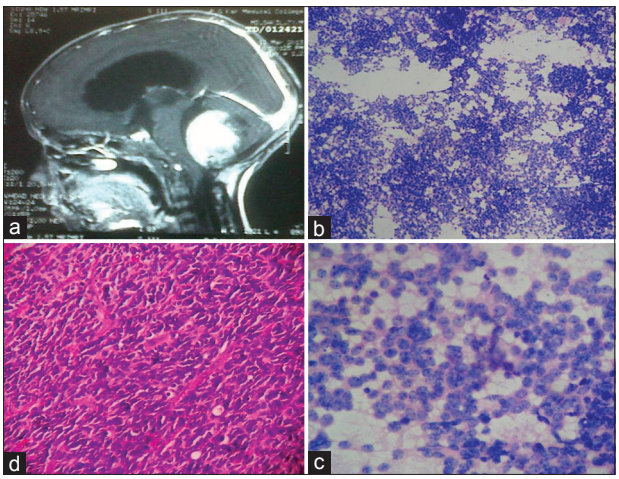

Figure 3: Medulloblastoma (a) Magnetic resonance imaging The T1 weighted scan shows a relatively well circumscribed but large mass expanding the central cerebellum (arrow). (b) Low microscopic powers reveal like blue marbles spread out in a box (H and E, ×40). (c) Cells with scant to no cytoplasm having nuclei displaying salt and pepper chromatin but no nucleolus. Nuclear molding seen (H and E, ×100). (d) Medulloblastomas having carrot cells with spongioblastic arrangement (H and E, ×40)

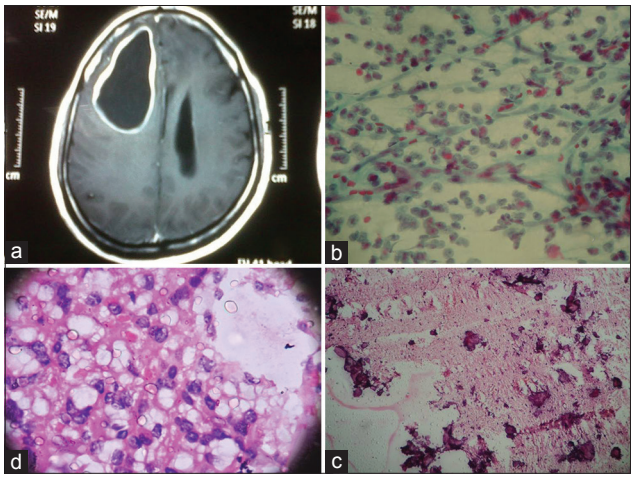

Figure 4: Oligodendroglioma (a) Magnetic resonance imaging Solid cystic sol in right frontoparietal region. (b) Cells form vague clusters. The smear disperses many of the nuclei away from the clusters. A weak or sparse glial matrix loosely ties the cells into these collections (H and E, ×100). (c) Calcification (H and E, ×100). (d) Halos in oligodendrogliomas (H and E, ×400)

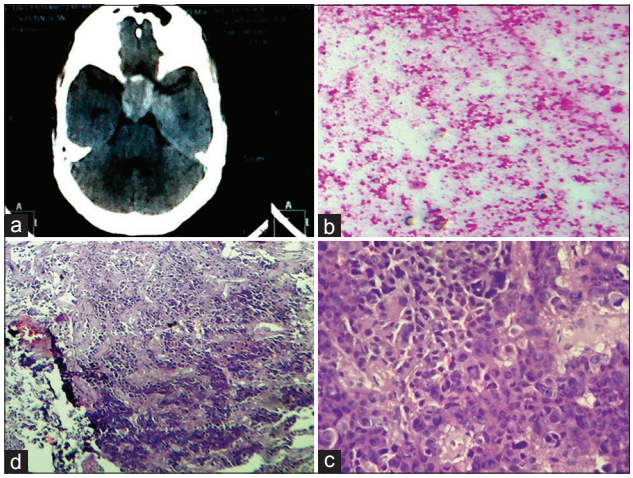

Figure 5: (a) Magnetic resonance imaging - A lobulated well defined mixed signal intensity sellar/suprasellar lesion. (b) Round cells arranged in short chains and circles against a granular background (H and E, ×100). (c and d) Round to oval cells with granular cytoplasm and arranged in diffuse pattern (H and E, ×400 and ×100)

preferred over frozen section where tissue is available for a detailed study, but reporting on the squash cytology smear is challenging in differentiating primary and metastatic tumor of the brain. H and E stained smear must be supplemented with ancillary IHC stain in difficult cases. Despite minor disagreement among experts, a sizable number of studies

have showed squash smears examination to have high sensitivity, specificity, and diagnostic accuracy.[11-15] Overall sensitivity in this study to diagnose benign and malignant tumors was 94.7% and the specificity is 97.6% with positive and negative predictive value of 94.7% and 97.6%, respectively.

Increased number of intraoperative consultation contributed by modern neuroimaging and neurosurgical modalities. Higher accuracy rates in squash cytology reporting can be achieved by following a simplified categorical approach and proper radiological correlation. Reporting of squash cytology smears starts with the gross evaluation of specimen followed by microscopic examination of cohesiveness of the cells, background, cytoplasm-nuclear details, and relation with the vessels. Technical expertise and proper training in neuropathology reporting are the prime need to ascertain good diagnostic accuracy. In this study, ependymoma, medulloblastoma, craniopharyngioma, pituitary adenoma, germinoma, showed 100% diagnostic accuracy cytohistological correlation. Whereas, the most difficulty was found in the case of oligodendroglioma, due to the resemblance of this glioma with other low-grade glioma.

We highlight certain advantages of squash cytology as intraoperative consultation

1. Rapid: Following methodical approach, early and accurate diagnosis is possible without consuming more time
2. Requirement of tiny tissue: Preparation of squash cytology requires only 1 mm of tissue which leads to less invasiveness
3. Sampling of multiple areas: Sampling from 2 to 5 areas

Table 3: Ki-67 in neuroepithelial tumors

	WHO I	WHO II	WHO III	WHO IV
Mean	1	2.7325	8	18.84285
SD	0.7071	1.349	1.414	3.687
Sample size (*n*)	2	20	2	7
SEM	0.5000	0.3016	1.000	1.393
Lower 95% CL	−5.383	2.101	−4.706	15.433
Upper 95% CL	7.353	3.364	20.706	22.253
Minimum	0.5000	0.8000	7.000	15.000
Median (50th percentile)	1.000	2.625	8.000	18.000
Maximum	1.500	4.800	9.000	25.000
Normality test KS		0.1263		0.1918
Normality test *P* value	Too few values	>0.10	Too few values	>0.10
Passed normality test		Yes		Yes

SD – Standard deviation; SEM – Standard error of mean; CL – Confidence limit; KS – Kolmogorov-Smirnov

Table 4: Tukey-Kramer multiple comparison test of Ki-67 expression

Comparison	Mean difference	*q*	*P*
Grade I versus Grade II	−1.733	1.576	>0.05 (NS)
Grade I versus Grade III	−7.000	4.723	<0.05*
Grade I versus Grade IV	−17.843	15.015	<0.001***
Grade II versus Grade III	−5.268	4.792	<0.05*
Grade II versus Grade IV	−16.110	24.751	<0.001***
Grade III versus Grade IV	−10.843	9.124	<0.001***

*,***Extremely significant, *P* value of comparison between Grade I and Grade II is NS, but all others are significant. NS – Not significant

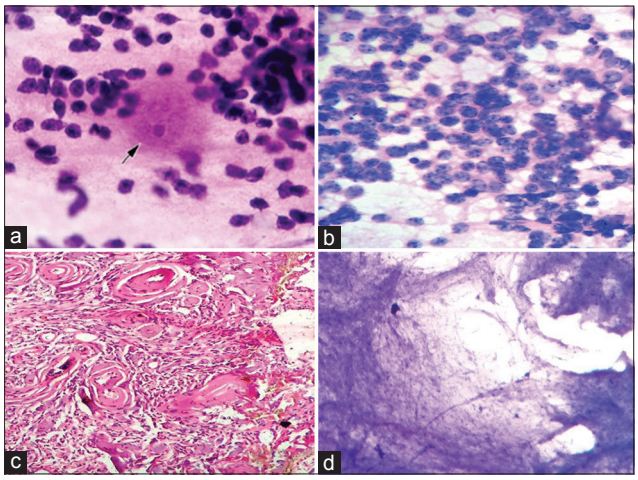

Figure 6: (a) Granular layer of cerebellum discordant with medulloblastoma (b), (c) Schwannoma discordant with meningioma (meningothelial) (d)

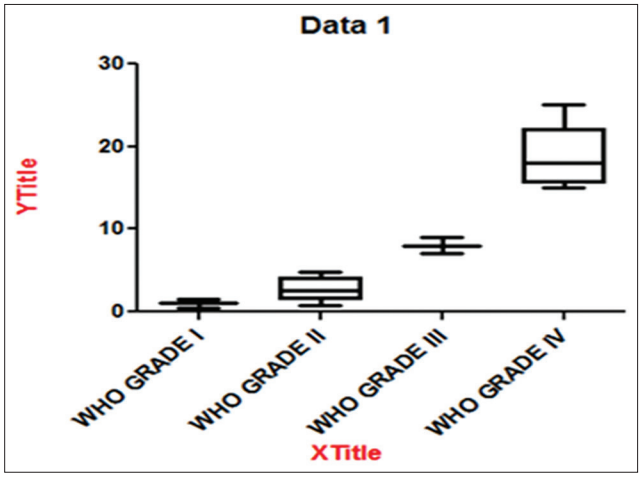

Figure 7: Box and whiskers graph, vertical of Ki 67 expression

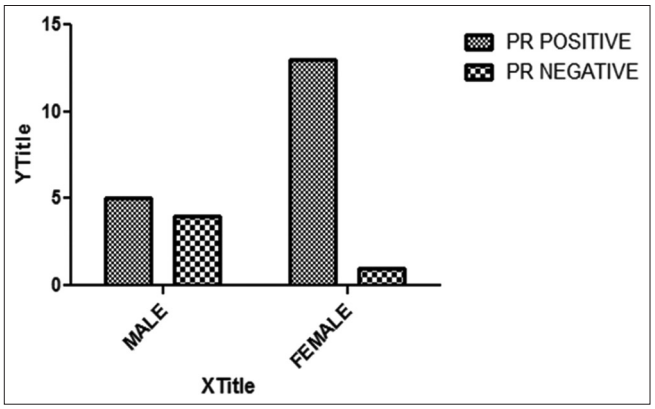

Figure 8: Expression of PR receptors in male and female cases of meningioma

is possible depending on neurosurgeon observation. Softest and darkest areas are usually sampled
4. No need of fresh frozen tissue: Unavailability of cryostat in all centers leads to a logistic problem in frozen section reporting
5. Smear pattern evaluation: Depending on the biophysical properties, smears reveal the cohesive or discohesive pattern. These cytological patterns help in triaging the lesions
6. Better nuclear-cytoplasmic details: Depending on technical expertise, accuracy of smears reporting varies from 86% to 95%
7. Assessment of resected margin: Sampling from the apparent gross tumor margin contributes in additional information like margin status
8. Additional testing: IHC assessment is possible.

However, there are certain disadvantages
1. Accurate localization is necessary
2. Mitosis and necrosis cannot be assessed
3. Architectural details are lost.

In summary, the squash cytology method performed satisfactorily throughout the evaluation, and the results compared well with our existing histopathological method for all benign and malignant lesions.

Conclusions

Squash smear evaluation is a sensitive, efficient, and time-saving alternative to frozen section as intraoperative consultation.

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Conflicts of interest

There are no conflicts of interest.

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