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A Comprehensive Study on Squash Smear Cytology with Correlation to Histomorphological and Clinicoradiological Findings in Central Nervous System Lesions

Dr. Monisha Gupta¹, Late Dr. Pinki Pandey², Dr. Roopak Aggarwal³, Dr. M Faheem⁴

¹All India Institute of Medical Sciences (AIIMS), Gorakhpur Senior Resident
Department of Transfusion Medicine and Immunohematology

Email id- Moniyashtanu123@gmail.com, Orcid id- 0009-0007-4461-6842

²Uttar Pradesh University of Medical Sciences, Saifai (UPUMS, Saifai) Former Head of
Department of Pathology, UPUMS, Saifai

Email id- pnkdxt@yahoo.in, pnkdxt@gmail.com, Orcid id- 0000-0002-8521-7588

³Uttar Pradesh University of Medical Sciences, Saifai Professor in the department of
pathology, UPUMS

Email id- drroopakaggarwal@gmail.com, Orcid id- 0000-0002-4919-3443

⁴King George University of Medical Sciences (KGMU), Lucknow Assistant Professor
Email id- mohdfaheem@gmail.com, Orcid id- 0000-0002-6004-9709

ABSTRACT:

Objective: To highlight the cytomorphological variations observed in lesions of the CNS and the accuracy of diagnosis made in squash preparation as compared to histopathology as the standard method of diagnosis and correlate the histopathological diagnosis with the clinical & radiological diagnosis.

Methods: A total of 100 sequential cases that were suitable for both squash cytology (cytopathology) and paraffin section histopathology (histology) were examined. Biopsy smears were subjected to rapid hematoxylin and eosin staining along with May–Grünwald–Giemsa staining for cytological evaluation. Concordance was reported with 95% confidence intervals calculated using the Wilson score method. Chance-corrected agreement was assessed using the multi-class Cohen's kappa coefficient and interpreted according to the Landis and Koch benchmarks. Sensitivity, specificity, predictive values, and accuracy were calculated for each lesion category on a one-versus-rest basis.

Results: From those cases examined, a category of diagnosis could be made by the squash cytology in each. Cytohistological concordance was 95.0%, with almost perfect agreement (kappa 0.94). Intra-group concordance was seen in glioma cases (30/33, 90.9%), meningioma cases (18/19, 94.7%) and craniopharyngioma cases (4/5, 80.0%) and was 100% for the remaining other lesions. With regards to the overall accuracy of the radiology compared to histopathology, the result was 94.0% (kappa 0.93). Five discrepancies were highlighted in the squash cytology, of which two could only be clarified by immunohistochemistry.

Conclusion: The amalgamation of clinical and radiological observations, along with histological confirmation, establishes squash smear cytology as a remarkable preoperative diagnostic technique for central nervous system disorders. It is also quick, accurate, and

dependable. It is most reliable for common types and should be used with caution for rare tumours, for which the estimates in this series are not very precise.

1. INTRODUCTION

Tumors of the central nervous system (CNS) represent a minor portion of all human cancers, yet they can lead to considerable illness and mortality because of their positioning in vital areas and the enduring consequences of certain neurosurgical interventions. The pathologist is often called on to make an immediate diagnosis during surgery to assist the surgeon in determining the extent of surgery and what further tissue is needed. Developed by Eisenhardt and Cushing from their work with neurosurgical tissue, this method takes advantage of the soft, cellular consistency of most neoplastic CNS lesions to provide well-spread samples in minutes, and is unique in that it only requires the use of glass slides and simple stains and a light microscope [1].

This technique is also inexpensive, and doesn't require a cryostat or the uninterrupted power supply of such systems, making it attractive for resource-limited settings. There are reports of high concordance between squash cytology and final histopathology in several studies [2,3,4]. Despite its diagnostic utility, interpretation may be challenging due to several well-recognized morphological pitfalls. Reactive gliosis can closely mimic pilocytic astrocytoma, spindle-cell clustering may simulate schwannoma, and pseudo-rosette-like formations may resemble ependymoma. Hence, integration of the cytomorphological findings with the patient's clinical presentation and radiological features is essential to avoid diagnostic pitfalls and ensure accurate diagnosis [5,6].

The diagnostic framework for CNS tumours has evolved considerably with successive revisions of the WHO classification. The present study was conducted using the 2016 WHO Classification of Tumours of the Central Nervous System, which was based predominantly on histomorphological features, supplemented by immunohistochemistry. The subsequent 2021 WHO Classification of Tumours of the Central Nervous System (WHO CNS5) introduced a more integrated histomolecular approach, incorporating molecular alterations into tumour classification and diagnosis. In particular, diffuse gliomas are now defined by specific molecular alterations, including IDH mutation and 1p/19q codeletion, with additional molecular markers such as ATRX and TERT status contributing to tumour characterization and classification [7]. However, the implementation of WHO CNS5 in routine clinical practice may be challenging in resource-limited settings because several of its diagnostic categories require access to specialized molecular investigations, which may not be routinely available, affordable, or feasible in all centres. Although rapid molecular assays and deep-learning-based techniques are increasingly being investigated for intraoperative diagnosis and may facilitate timely tumour classification, their widespread clinical availability remains limited [8,9]. In view of these practical limitations and the limited availability of comprehensive molecular testing during the study period, the present study used the 2016 WHO CNS classification as the reference standard for diagnosis. This approach enabled consistent application of histomorphological and available immunohistochemical criteria across the study population while acknowledging the subsequent evolution towards an integrated molecular classification.

In this context, the following objectives were outlined: (1) To characterize the cytomorphological features of different lesions in the brain and spinal cord as observed in squash preparations. (2) To assess the diagnostic precision of squash cytology compared to paraffin-section histopathology, including immunohistochemistry if necessary. (3) To explore the relationship between clinical and radiological observations and histopathological diagnoses. Unlike previous studies by Kumar et al. [10], Kumarguru et al. [11] and Philip et al. [12], which evaluated individual diagnostic modalities separately, the present study adopts a correlative, single-cohort design that integrates multiple modalities within the same cohort. This approach enables a more comprehensive assessment of diagnostic performance and represents a methodological distinction from the existing literature, in which individual modalities were predominantly evaluated in isolation.

2. MATERIALS AND METHODS

2.1 Study design, setting and cases

The study was done in the Department of Pathology, Uttar Pradesh University of Medical Sciences, Saifai, Etawah from January 2021 till June 2022 with the approval from Ethics committee of the institute. A cross-sectional observational study was conducted with retrospective and prospective data. All consecutive patients with intracranial or spinal mass who presented for surgery with a biopsy or resection were screened for possible inclusion, regardless of age. Those cases met the following criteria in which there had sufficient tissue for both intraoperative squash cytology and paraffin-section histopathological examination. Those specimens in which tissue was inadequate were judged insufficient for either modality and were excluded from this study.

Squash cytology was not intended to evaluate the adequacy rate nor the diagnostic yield of the technique, as the tissue adequacy was used as an inclusion criterion. As a consequence, consideration was focused on the 100 cases in which sufficient and interpretable material for both modalities were available. Patients' clinical information was collected, and all relevant information was collected before surgery, such as patient's age, history, radiologic results like preoperative computed tomography (CT) and magnetic resonance imaging (MRI) characteristics of the lesion.

2.2 Squash preparation and staining

The small tissue specimen ($\sim 1 - 2 \text{ mm}^3$) was compressed between two glass slides, and spread between them evenly to form smears of pairs. While half of the smears were promptly fixed in 95% ethanol and stained with rapid hematoxylin and eosin stain, the other half were air dried and stained with May-Grunwald Giemsa. The specimen was received by the surgeon within 20 minutes of which an intraoperative cytological diagnosis was given.

2.3 Histopathological Analysis

The residual tissue was routinely fixed, processed and embedded in paraffin, followed by staining and preparation of 4-5 micrometer sections with haematoxylin and eosin. Tumor diagnosis was interpreted according to the 2016 World Health Organization (WHO) Classification of Tumors of Central Nervous System, which served as the reference standard for interpretation of the diagnosis of the lesions.

2.4 Immunohistochemical correlation

Immunohistochemistry (IHC) was used to assess the formalin-fixed, paraffin embedded (FFPE) tissue in cases where a diagnosis could not be reached on morphologic grounds. A panel of suitable markers was chosen based on the differential diagnosis, including: GFAP; S-100; neurofilament; and Ki-67 etc. Morphological features and the IHC stain were combined to make the final histopathological diagnosis.

2.5 Radiological Diagnosis

All patients received computed tomography (CT) or magnetic resonance imaging (MRI) or both before surgery. Pre-operative diagnosis in the radiology was assessed with final histopathological diagnosis. These analyses were done on the broad modality level (e.g. glioma, meningioma, nerve sheath tumour etc.) to provide a uniform comparison across modalities.

2.6 Statistical analysis

Categorical data was presented in terms of frequencies and percentages, while the continuous variables were presented in terms of mean and standard deviation. All comparisons were made against the histopathology as the gold standard. The overall agreement between squash cytology and pre-operative radiology and histopathology was presented as percentages with a 95% Wilson score confidence interval (CI). Cohen's kappa coefficient was calculated to assess the agreement that exists between each modality and histopathology; these kappa coefficients were then categorized based on the Landis and Koch criteria [13] with a kappa coefficient range of 0.81 to 1.00 corresponding to almost perfect agreement. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated for each category of lesion. Some tumour categories comprised only a small number of cases, with two categories (Choroid plexus papilloma and pineal parenchymal tumor of intermediate differentiation) containing a single case each. Therefore, the estimates derived at the individual category level should be interpreted with caution because of the limited sample size. Similarly, the number of lesions within certain

categories was small; consequently, statistical comparisons and p-values were not calculated separately for individual categories where the sample size was insufficient. Statistical analysis was performed using IBM SPSS Statistics version 26.0, and a p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Demographic and clinical profile

Patients' mean age was 33.74 years (range 3 months to 85 years, with predominance in the 2nd decade). There were 52 males and 48 females (ratio 1.08 to 1). Headache was the most common symptom and most common site of involvement was cerebrum (Table 1).

Table 1. Demographic and clinical profile of the study population (n = 100).

Variable	Category	n (%)
Age group (years)	0 to 10	10 (10)
	11 to 20	21 (21)
	21 to 30	15 (15)
	31 to 40	17 (17)
	41 to 50	20 (20)
	51 to 60	9 (9)
	61 to 85	8 (8)
Sex	Male	52 (52)
	Female	48 (48)
Presenting feature	Headache	52 (52)
	Vomiting	16 (16)
	Paraparesis	15 (15)
	Seizures	15 (15)
Lesion site	Cerebrum	59 (59)
	Spine	19 (19)
	Cerebellum	15 (15)
	Cerebellopontine angle	7 (7)

3.2 Distribution of lesions

Among 100 lesions, 86 (86%) were neoplastic and 14 (14%) non-neoplastic were observed in all three modalities. In histopathological diagnosis, glioma was the most common lesion 33 cases (33%) followed by meningioma 19 cases (19%),

inflammatory 11 cases (11%) and schwannoma 9 cases (9%). Most of the glial neoplasms were glioblastoma 11 cases and pilocytic astrocytoma 5 cases, of which the first was of the high grade while the latter was of the low grade type (Table 2).

Table 2. Distribution of lesions by final histopathological diagnosis (n = 100).

Histopathological diagnosis	n	%
Glioma	33	33
Meningioma	19	19
Inflammatory lesion	11	11
Schwannoma	9	9
Craniopharyngioma	5	5
Pituitary adenoma	5	5
Metastasis	5	5
Neurofibroma	3	3
Medulloblastoma	3	3
Epidermoid cyst	3	3
Haemangioblastoma	2	2
Pineal parenchymal tumour (ID)	1	1
Choroid plexus papilloma	1	1
Total	100	100

ID, of intermediate differentiation. Neoplastic lesions, 86; non-neoplastic lesions, 14.

3.3 Cytomorphological spectrum

Squash smears of choroid plexus papilloma were hypercellular with papillary fronds lined by a single layer of monomorphic cuboidal to columnar cells (Figure 5) and the corresponding histological sections revealed a similar papillary formation lined by a single layer of cuboidal to columnar cells (Figure 6). Squash smears of meningotheial meningioma revealed whorls of meningotheial cells (Figure 7) while squash smears of psammomatous meningioma variant showed cellular smear with psammoma bodies (Figure 8). Histopathological section of psammomatous meningioma showed psammoma bodies (Figure 9). A dense infiltrative population of inflammatory cells, mainly lymphocytes with necrosis (constituted by dead cells and coagulative necrosis (Figure 10) was seen on smears taken from inflammatory lesions, while corresponding histological sections revealed a lymphocyte-rich infiltrate with the presence of Langhans-type giant cells (Figure 11). Squash smear and histopathology of pineal parenchymal tumor revealed medium to large sized monomorphic tumour cells with vesicular chromatin and conspicuous nucleoli (Figures 12 & 13). Smears revealed pleomorphic and bizarre astrocytic cells, vascular

proliferation and giant cells in glioblastoma (Figures 14 and 15), and histological sections revealed marked pleomorphism, vascular proliferation and necrosis with palisading of cells of the tumour (Figures 16 and 17). In ependymoma both in the squash smear (Figure 18) and in the related histological section (Figure 19) the perivascular pseudorosettes were detected. Squash smears of neurofibroma featured spindle cells with tapering nuclei in a myxoid stroma (Figure 20) while on histology it was composed of wavy bundles of spindle cells in myxoid collagenous matrix (Figure 21). Squash smears of Pilocytic Astrocytoma commonly demonstrated bipolar piloid cells with elongated processes, in addition to cells bearing short processes against a fibrillary background. Comparable bipolar piloid cells were also identified in the corresponding histological sections (Figures 22 and 23). In schwannoma, squash smears revealed coherent groupings of spindle cells (Figure 24) while the histology shows dense interlacing fascicles of spindle cells (Figure 25). Squash cytology and histology of Pituitary adenoma shows monomorphic cells with round nuclei and salt-and-pepper chromatin (Figure 26) and cells are arranged in trabecular pattern in histology (Figure 27).

3.4 Cytohistological correlation

Among the 100 cases evaluated, the squash cytology diagnosis was concordant with the final histopathological diagnosis in 95 cases, while five cases showed discordance, giving an overall cytohistological concordance of 95.0% (95% CI: 88.8–97.8), with histopathology considered the reference standard. The overall diagnostic accuracy of 95.0% was calculated by considering cases with agreement at the broader diagnostic-category level as concordant, even when there was a difference in the specific subtype. Thus, partial concordance in certain lesions such as gliomas was considered complete concordance for the purpose of the overall analysis. Cohen's kappa demonstrated almost perfect agreement between squash cytology and histopathology.

On category-wise analysis, the concordance was 90.9% for gliomas (30/33; 95% CI: 76.4–96.9), 94.7% for meningiomas (18/19; 95% CI: 75.4–99.1), and 80.0% for craniopharyngiomas (4/5; 95% CI: 37.6–96.4). Complete concordance (100%) was observed for inflammatory lesions (11/11; 95% CI: 74.1–100), schwannomas (9/9; 95% CI: 70.1–100), pituitary adenomas (5/5; 95% CI: 56.6–100), metastases (5/5; 95% CI: 56.6–100), neurofibromas (3/3; 95% CI: 43.8–100), medulloblastomas (3/3; 95% CI: 43.8–100), epidermoid cysts (3/3; 95% CI: 43.8–100), haemangioblastomas (2/2; 95% CI: 34.2–100), choroid plexus papilloma (1/1; 95% CI: 20.7–100), and pineal parenchymal tumour of intermediate differentiation (1/1; 95% CI: 20.7–100).

Although 100% concordance was observed in these categories, the number of cases in several categories was small, resulting in wide confidence intervals. Therefore, these apparently complete concordance rates should be interpreted cautiously and should not be taken as definitive estimates of diagnostic performance. The category-wise results are presented in Table 3.

In addition, a one-versus-rest analysis was performed to assess the ability of squash cytology to distinguish each diagnostic category from all other categories combined. The corresponding diagnostic accuracy measures are also presented in Table 3.

Table 3. Diagnostic performance of squash cytology against histopathology, on a one-versus-rest basis (n = 100).

Lesion	N	Concordant, n/N (%)	Sensitivity % (95% CI)	Spec. %	PPV %	NPV %	Acc. %
Glioma	33	30/33 (90.9)	90.9 (76.4 to 96.9)	97.01	93.75	95.59	95.0
Meningioma	19	18/19 (94.7)	94.7 (75.4 to 99.1)	100	100	98.78	99.0
Craniopharyngioma	5	4/5 (80.0)	80.0 (37.6 to 96.4)	100	100	98.96	99.0
Schwannoma	9	9/9 (100)	100 (70.1 to 100)	98.90	90.00	100	99.0
Neurofibroma	3	3/3 (100)	100 (43.8 to 100)	98.97	75.00	100	99.0
Medulloblastoma	3	3/3 (100)	100 (43.8 to 100)	98.97	75.00	100	99.0

Pituitary adenoma	5	5/5 (100)	100 (56.6 to 100)	100	100	100	100
Metastasis	5	5/5 (100)	100 (56.6 to 100)	100	100	100	100
Haemangioblastoma	2	2/2 (100)	100 (34.2 to 100)	100	100	100	100
Choroid plexus papilloma	1	1/1 (100)	100 (20.7 to 100)	100	100	100	100
Pineal parenchymal tumour (ID)	1	1/1 (100)	100 (20.7 to 100)	100	100	100	100
Inflammatory lesion	11	11/11 (100)	100 (74.1 to 100)	100	100	100	100
Epidermoid cyst	3	3/3 (100)	100 (43.8 to 100)	100	100	100	100
All lesions	100	95/100 (95.0)	95.0 (88.8 to 97.8)	NA	NA	NA	95.0

CI, confidence interval; Spec., specificity; PPV, positive predictive value; NPV, negative predictive value; Acc., one-versus-rest accuracy; ID, of intermediate differentiation; NA, not applicable.

Of the five discordant cases, three were gliomas (two ependymomas and one diffuse astrocytoma), and the other two were meningioma and craniopharyngioma (Table 4). In all five cases, the discrepancy arose mainly from morphological overlap and limited tissue sampling, which led to the initial misclassification (Figures 28 and 29).

Table 4. Discordant squash cytology cases and reasons for discrepancy.

Histopathological diagnosis	Squash cytology diagnosis	Reason for discordance
Ependymoma	Neurofibroma	Sampling error; absence of pseudorosettes
Meningioma	Ependymoma	Rosette-like arrangements misinterpreted as whorls and vascular proliferation
Ependymoma	Schwannoma	Absence of vascular proliferation and pseudorosettes
Craniopharyngioma	Pilocytic astrocytoma	Florid reactive gliosis with fibrillary background
Diffuse astrocytoma	Medulloblastoma	Sampling error; darkly stained round to oval nuclei; absent fibrillary background

3.5 Radiological correlation

Preoperative radiological diagnosis matched with histopathology in 94 of 100 cases with an overall chance-corrected agreement of almost 100% (94.0% (95% CI 0.875 to 0.972)) and an accuracy of 94.0% (95% CI 0.875 to 0.972). The rate of agreement

between the lesion category and the imaging diagnosis (category-specific sensitivity) was 87.9% for gliomas (29/33), 94.7% for meningiomas (18/19) and 50.0% for haemangioblastomas (1/2). The remaining lesion categories showed complete radiological agreement with the final histopathological diagnosis; however, the number of cases in these categories was small, and the findings should therefore be interpreted with caution. The per-lesion radiological performance is presented in Table 5, and representative imaging findings are shown in Figures 1–4. The radiological diagnosis differed from the final histopathological diagnosis in nine cases (Table 6). In three of these cases, the radiological diagnosis differed from the specific glial subtype identified histopathologically; however, the broader diagnosis of a glial tumour was correctly assigned. These cases were therefore considered concordant at the broad diagnostic-category level but discordant at the subtype level. The remaining six cases showed discordance at the lesion-category level, with the radiological diagnosis belonging to a different tumour category from the final histopathological diagnosis. These discrepancies were mainly observed in lesions with similar anatomical locations and overlapping radiological characteristics.

Table 5. Diagnostic performance of radiology against histopathology, on a one-versus-rest basis (n = 100).

Lesion	N	Sensitivity % (95% CI)	Spec. %	PPV %	NPV %	Acc. %
Glioma	33	87.9 (72.7 to 95.2)	98.51	96.67	94.29	95.0
Meningioma	19	94.7 (75.4 to 99.1)	100	100	98.78	99.0
Haemangioblastoma	2	50.0 (9.5 to 90.5)	98.98	50.00	98.98	98.0
Schwannoma	9	100 (70.1 to 100)	98.90	90.00	100	99.0
Neurofibroma	3	100 (43.8 to 100)	98.97	75.00	100	99.0
Craniopharyngioma	5	100 (56.6 to 100)	100	100	100	100
Pituitary adenoma	5	100 (56.6 to 100)	100	100	100	100
Metastasis	5	100 (56.6 to 100)	98.95	83.33	100	99.0
Medulloblastoma	3	100 (43.8 to 100)	98.97	75.00	100	99.0
Choroid plexus papilloma	1	100 (20.7 to 100)	100	100	100	100
Pineal parenchymal tumour (ID)	1	100 (20.7 to 100)	100	100	100	100
Inflammatory lesion	11	100 (74.1 to 100)	100	100	100	100
Epidermoid cyst	3	100 (43.8 to 100)	100	100	100	100
All lesions	100	94.0 (87.5 to 97.2)	NA	NA	NA	94.0

Table 6. Discordant radiological cases and cause of misdiagnosis.

Histopathological diagnosis	Radiological diagnosis	Cause of misdiagnosis
Anaplastic astrocytoma	Glioblastoma	Similar radiological features (within glial category)
Pleomorphic xanthoastrocytoma	Diffuse astrocytoma	Similar radiological features (within glial category)
Gliosarcoma	Glioblastoma	Similar radiological features (within glial category)
Ependymoma	Neurofibroma	Similar site and radiological features
Ependymoma	Schwannoma	Similar site and radiological features
Diffuse astrocytoma	Medulloblastoma	Similar radiological features
Gliosarcoma	Haemangioblastoma	Absence of oedema and necrosis
Haemangioblastoma	Pilocytic astrocytoma	Same site and similar radiological features
Atypical meningioma	Metastasis	Peritumoural oedema and necrosis

3.6 Immunohistochemical correlation

Immunohistochemistry was performed in three cases (Table 7) and helped resolve the cytohistological discrepancy in two of them, accounting for two of the five discordant cases. A lesion reported as neurofibroma on squash cytology was reclassified as ependymoma based on GFAP positivity and a Ki-67 index below 1%. A second lesion, reported as medulloblastoma on squash cytology, was positive for GFAP, S-100 and neurofilament, with a Ki-67 index below 10%, and was reclassified as diffuse astrocytoma. In the third case, immunohistochemistry confirmed the cytological diagnosis of diffuse astrocytoma.

Table 7. Correlation of squash cytology with immunohistochemistry.

Squash cytology diagnosis	Immunohistochemical diagnosis	Immunohistochemical markers
Neurofibroma	Ependymoma	GFAP positive; Ki-67 less than 1%
Medulloblastoma	Diffuse astrocytoma	GFAP positive; S-100 positive; Ki-67 less than 10%; NF positive
Diffuse astrocytoma	Diffuse astrocytoma	GFAP positive; S-100 positive

GFAP, glial fibrillary acidic protein; NF, neurofilament.

4. DISCUSSION

Intraoperative squash smear cytology was performed in 100 CNS lesions and showed a 95.0% concordance with histopathological diagnosis. Preoperative radiological assessment achieved correct categorization in 94.0% of cases. The agreement between the squash cytology and radiology with the final histopathological diagnosis was nearly perfect, with kappa values of 0.94 and 0.93, respectively. In a study of 150 cases of space occupying lesions of CNS, Jindal et al. (2017) [2] found the sensitivity of squash smear cytology to be 94% compared to the 95.0% as observed in the present study. Kumarguru et al. (2022) [11] also found that cytology and radiographic diagnosis complement each other in arriving at a diagnosis, albeit the rate of agreement depended on the criteria used to compare with the histopathology. Demographic characteristics in the current cohort such as the maximum number of cases observed in age group of 2nd decade, marginal predominance of the male sex, and headache as the principal presentation are similar to that of previous Indian and neighbouring series [2,3,11]. The profile features described in this study are broadly similar to the features reported by Jindal et al. (2017) [2] and Kumarguru et al. (2022) [11] in their clinical studies. In contrast, other studies, such as that by Ghanghoria et al. (2014) [17], reported a predominance of meningiomas over gliomas in their series. Conversely, Jaiswal et al. (2012) [18] and Poonia et al. (2021) [19] reported a relatively lower proportion of meningioma cases. The observed variation in the predominant lesion type across studies may be attributed to differences in the study populations, referral patterns, and case mix among different centres.

The overall concordance of 95.0% achieved in this study is akin to the 92.5% accuracy reported by Mitra et al. (2010) [4] and 89.8% accuracy reported by Shrestha et al. (2017) [20]. In the same vein, the accuracy of the squash cytology in the study by Govindaraman et al 2017 [5] when compared to in-situ diagnosis by histopathology came out to be 90.67% and 92% by Patil et al 2016 [6], 85.7% by de Souza Balsimelli et al 2019 [21], and 89.2% by Nanarng et al 2015 [22]. The lower concordance observed for gliomas in the present study (90.9%) is in agreement with the difficulties reported in previous studies in diagnosing and grading glial tumours on cytological preparations. The limited amount of tissue available in squash smears can make it difficult to determine the exact tumour type or grade, particularly in heterogeneous lesions. Jaiswal et al. [18] reported good diagnostic accuracy for glioblastoma, whereas the accuracy was comparatively lower for oligodendroglial lesions, highlighting the difficulty in diagnosing certain glial tumour subtypes. Differences between cytological and histological diagnoses have also been reported more often in the grading of gliomas than in their overall classification [16].

In the present study, all nerve-sheath tumours were correctly diagnosed on squash cytology. Similar high diagnostic accuracy for schwannoma has been reported by Jindal et al. (2017) [2], Govindaraman et al. (2017) [5], Kumarguru et al. (2022) [11], Tejaswi et al. (2020) [16], and Acharya et al. (2016) [23]. The lower diagnostic accuracy reported by Kar et al. [24] could be related to differences in sampling, the composition of cases, and the anatomical site of the lesions.

Florid reactive gliosis is another well-recognised pitfall of squash cytology [5,25]. Govindaraman et al 2017 [5] suggested that reactive gliosis may have contributed to the misclassification of the single craniopharyngioma case in the present series.

Table 8. Comparison of diagnostic accuracy of squash cytology of various studies

Authors	Number of Cases	Diagnostic Accuracy
Patil et al ⁽⁶⁾	50	92%
Govindraman et al ⁽⁵⁾	75	90.67%
Acharya et al ⁽²³⁾	220	83.78%
Nanarng et al ⁽²²⁾	75	89.2%
De Souza Balsimelli et al ⁽²¹⁾	113	85.7%
Jindal et al ⁽²⁾	150	94%

Tejaswi et al ⁽¹⁶⁾	52	95.9%
Kumarguru et al ⁽¹¹⁾	50	90%
Mitra et al ⁽⁴⁾	96	88.5%
Present study	100	95%

The cytological discrepancies observed in the present study could not be attributed solely to technical problems such as inadequate staining or failure to prepare satisfactory smears. Most of the discrepancies appeared to be related to overlapping morphological features and inadequate or unrepresentative tissue sampling. Mitra et al. (2010) [4] identified reactive changes, limited tissue, low cellularity, difficulty in obtaining firm tissue fragments, and overlapping cytomorphological features as important factors contributing to diagnostic errors. Similar factors have also been reported by Patil et al. (2016) [6], Nanarng et al. (2015) [22], and Pawar et al. (2009) [26].

The findings of the present study therefore emphasise the importance of interpreting squash cytology in conjunction with the clinical and radiological findings, particularly in lesions showing overlapping cytological features. The consistency and ease with which tissue can be smeared also play an important role in intraoperative cytology. Soft and friable lesions, such as gliomas, pituitary adenomas, medulloblastomas, and metastatic carcinomas, generally yield satisfactory smears more readily than firm or fibrous lesions.

One limitation of the present study is the possibility of sampling bias, as the adequacy and representativeness of tissue available for squash cytology may vary depending on the size, consistency, location, and heterogeneity of the lesion. Lesions that are difficult to sample or those with marked morphological heterogeneity may therefore be underrepresented or less accurately characterised on cytology. This may have influenced the diagnostic concordance observed in the present series.

In the present series, radiological information provided useful complementary information to squash cytology, and the lesions were correctly classified in 94.0% of cases. The relatively lower diagnostic performance observed in subtyping glial tumours and haemangioblastoma is comparable with the findings reported by Kumarguru et al. (2022) [11] and Boni et al. (2019) [27]. Pant et al. (2015) [28], while evaluating the histological diagnosis of various tumours, particularly those of astrocytic origin, in relation to their radiological characteristics, also highlighted the diagnostic difficulties caused by overlapping imaging features.

In the present study, some imaging discrepancies occurred in lesions that belonged to different tumour entities but shared similar anatomical locations and/or radiological appearances. Immunohistochemistry was required in three cases and helped resolve two of the five cytological discrepancies. This finding is in agreement with the study by Philip et al. (2023) [12], in which squash cytology was evaluated in combination with immunohistopathology and radiological findings. Their results suggested that the combined use of these modalities can be particularly helpful in distinguishing morphologically similar lesions of the central nervous system.

The utility of frozen section examination in the intraoperative diagnosis of CNS lesions has been evaluated in previous studies. Yadav et al. (2022) [29] assessed the diagnostic disparity between the intraoperative diagnosis based on a combination of squash cytology and frozen section and the final histopathological diagnosis. In contrast, Jain et al. (2022) [30] directly compared squash smear cytology with frozen section examination in the intraoperative diagnosis of CNS tumours. In their study, both techniques were correlated with the final histopathological diagnosis, allowing their diagnostic performance to be compared directly.

Frozen section examination was not performed in the present study. Therefore, a direct comparison of squash cytology with frozen section in terms of diagnostic accuracy, turnaround time, and cost could not be undertaken. The findings of Yadav et al. [29] and, particularly, the direct comparative study by Jain et al. [30] can therefore only provide contextual information for interpreting the results of the present study.

4.1 Strengths and limitations

The consecutive series of 100 cases and the systematic correlation of three diagnostic modalities with a single reference standard, which is histopathological diagnosis, represents the major strengths of this study. Additionally, representative cytomorphologic findings are described in detail. There are some photographic limitations to be taken into consideration when interpreting results. First, the reporting pathologist evaluated the squash smears with knowledge of the patients' clinical and radiological findings. This access to ancillary information may have introduced incorporation bias and potentially contributed to the relatively high level of concordance observed in the study. Secondly, all cases examined were those with sufficient tissue and histopathological diagnosis, which may have led to verification bias. Third, as the study was performed at a single tertiary-care center, the findings may have limited generalizability to smaller or lower-level healthcare facilities, where patient profiles and referral patterns may differ. Fourth, for several categories only one to three cases were observed, and their point estimates (as well as the 100% estimates) do have wide confidence intervals and are therefore volatile. Finally, interobserver variation was not assessed.

4.2 Clinical and health-system implications

Squash cytology is a quick intraoperative diagnosis which does not require a continuous power supply and a cryostat. This enables to pass the critical information to the surgical team in time about their level of resection and the necessity of further tissue sampling. When establishing a basic IOP service, this may prove easier to establish than a conventional frozen section service, especially in secondary and tertiary care hospital settings, where such services are not available. These potential benefits were not measured in terms of cost effectiveness or turnaround-time in the present study, however, so they should be confirmed in further studies.

4.3 Future directions

The large confidence intervals reported here could be made more precise with larger multicentre studies, preferably prospective, that included sufficient numbers of rare tumours. Further studies could focus on determining the degree of interobserver agreement in squash smear interpretation and evaluating emerging molecular and deep-learning-based diagnostic classifiers [8,9]. Their findings should also be examined in the context of the WHO CNS5 molecular classification framework [7]. In addition, prospective studies involving the same patient cohort could directly compare squash cytology with frozen-section examination in terms of diagnostic performance, turnaround time, and overall cost, thereby providing a more comprehensive assessment of their practical utility. In routine practice, the greatest value of squash cytology lies in being used in conjunction with the clinical and radiological findings and for final confirmation or disbelief to be made using paraffin section histopathology and if necessary, immunohistochemistry.

5. CONCLUSION

Squash smear cytology demonstrated a 95.0% overall concordance with histopathological findings, with a kappa value indicating almost perfect chance-corrected agreement. Although diagnostic accuracy was comparatively limited for uncommon lesions because of the small number of cases within individual categories, the technique provided rapid and clinically valuable information for the more frequently encountered lesions. Thus, integration of squash smear findings with the clinical presentation and radiological features can facilitate a reliable intraoperative diagnosis. The cytological assessment should, however, be regarded as complementary to definitive histopathological examination, with immunohistochemistry used when further confirmation or classification is required.

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Figures



Figure 1. Sagittal T1-weighted contrast-enhanced MRI showing a heterogeneous lesion with a central hypointense area surrounded by an irregular contrast-enhancing ring, suggestive of glioblastoma.

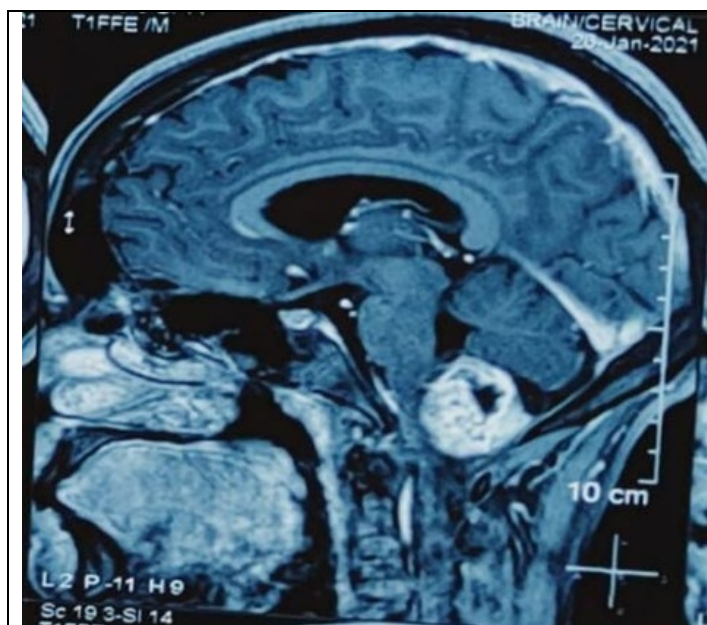


Figure 2. Sagittal T1-weighted post-contrast MRI showing a cyst with a heterogeneously enhancing mural nodule in the posterior fossa, suggestive of pilocytic astrocytoma.

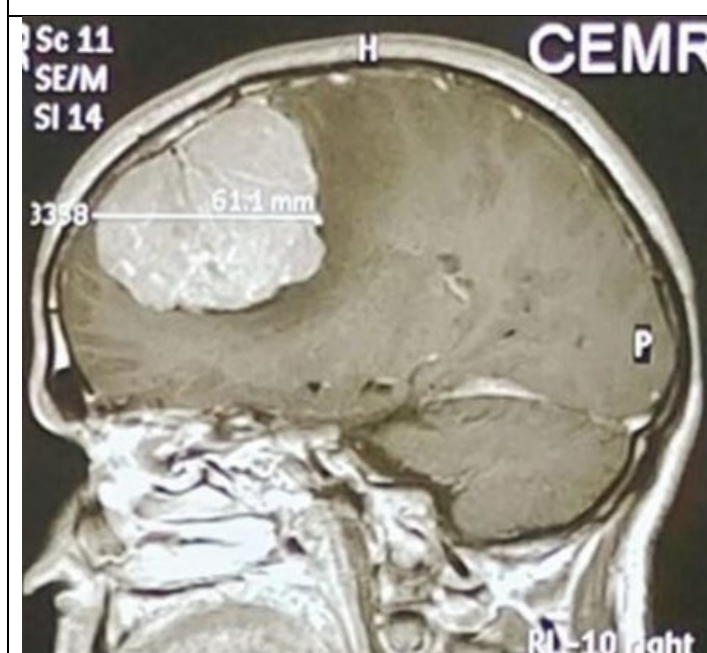


Figure 3. Sagittal T1-weighted contrast MRI showing a well-defined enhancing lesion with surrounding oedema in the right frontal region, suggestive of meningioma.



Figure 4. T1-weighted contrast-enhanced MRI showing a well-defined contrast-enhancing lesion, suggestive of choroid plexus papilloma.

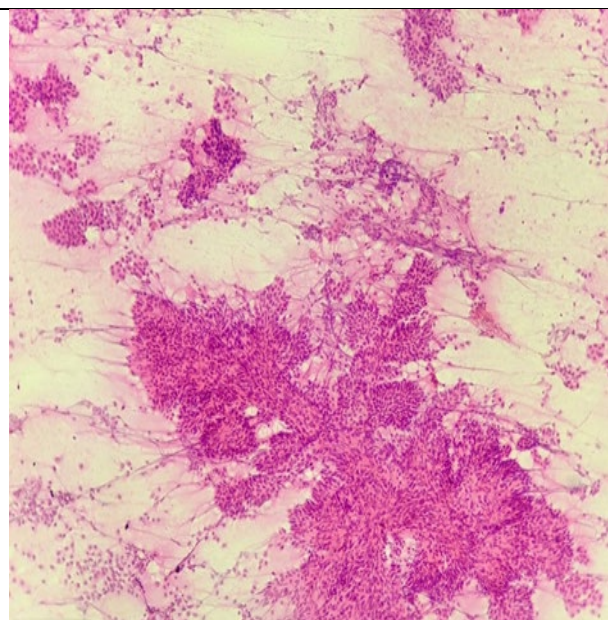


Figure 5. Squash smear of choroid plexus papilloma showing a hypercellular smear with a papillary pattern lined by single-layer cuboidal to columnar monomorphic cells (H&E, x100).

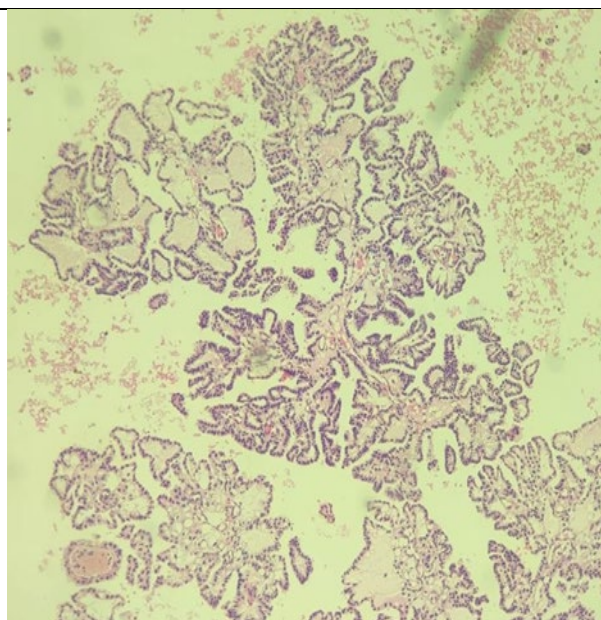


Figure 6. Histopathological section showing papillary architecture lined by monomorphic cuboidal to columnar cells (H&E, x100).

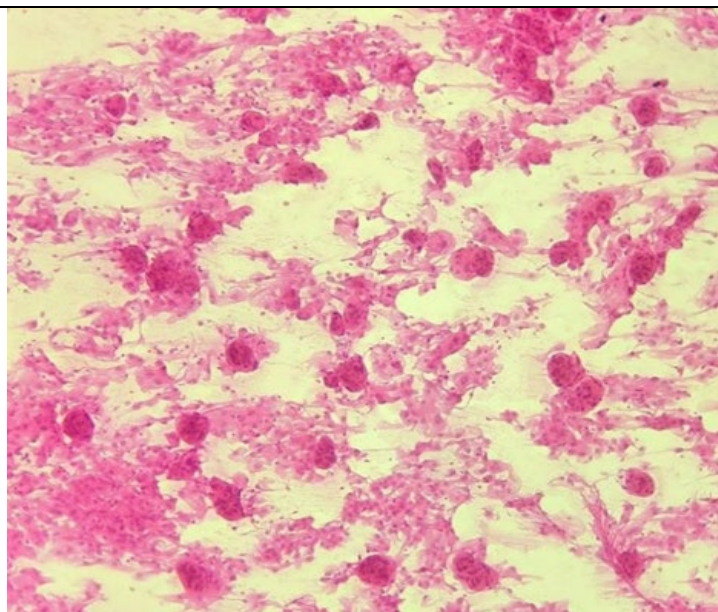


Figure 7. Squash smear of meningothelial meningioma showing a cellular smear with a whorled pattern of meningothelial cells (H&E, x100).

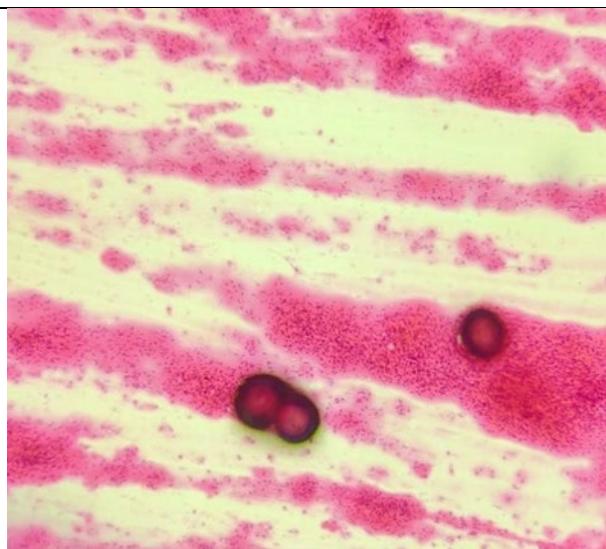


Figure 8. Squash smear of psammomatous meningioma showing a cellular smear with psammoma bodies (H&E, x100).

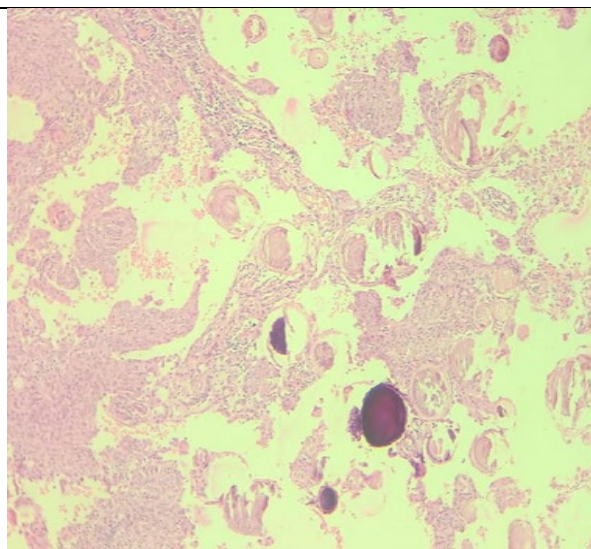
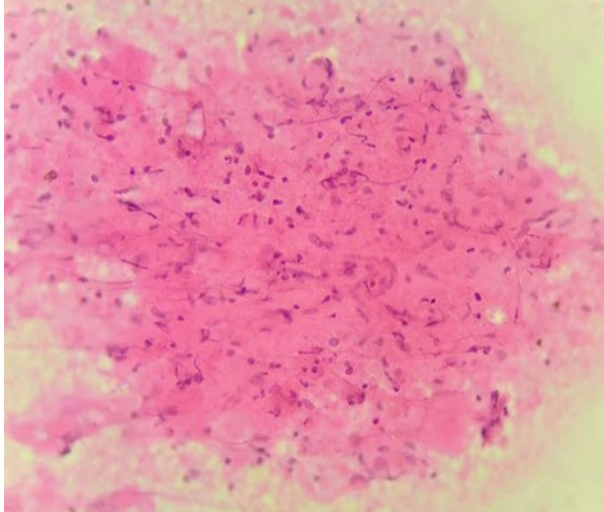
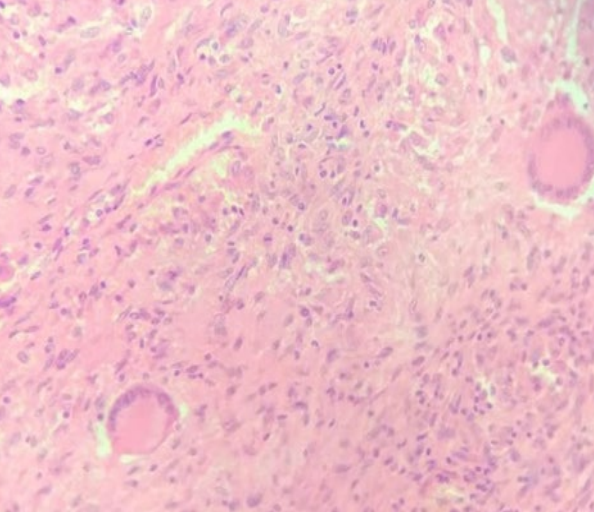
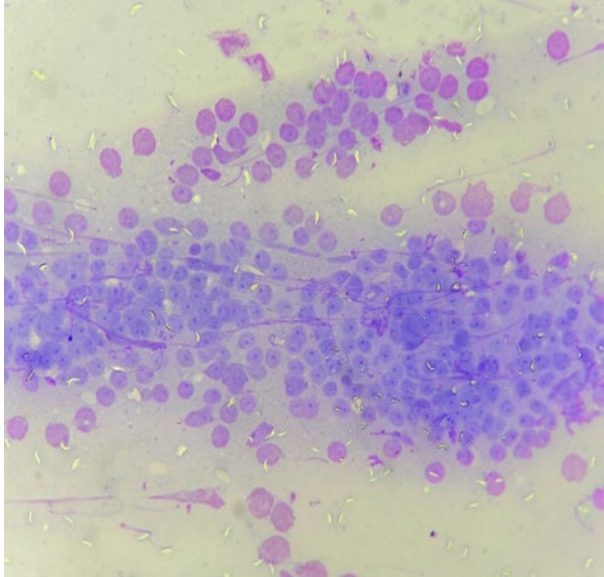
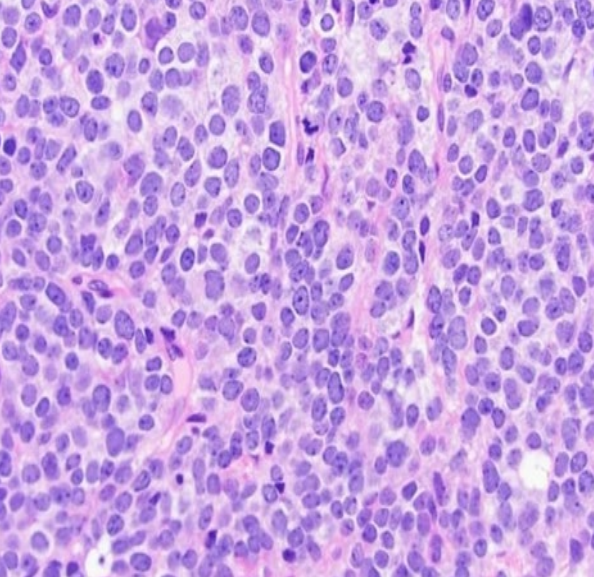


Figure 9. Histopathological section of meningioma showing high cellularity with psammoma bodies (H&E, x100).

	
Figure 10. Squash smear showing a dense cluster of inflammatory cells, predominantly lymphocytes, with necrosis (H&E, x400).	Figure 11. Histopathological section showing Langhans giant cells along with predominantly lymphocytic inflammatory infiltrate (H&E, x400).
	
Figure 12. Squash smear of pineal parenchymal tumour composed of medium- to large-sized monomorphic tumour cells with vesicular chromatin and conspicuous nucleoli (MGG, x400).	Figure 13. Histopathological section of pineal parenchymal tumour of intermediate differentiation showing sheet-like architecture, hypercellularity and small pseudorosettes (H&E, x400).

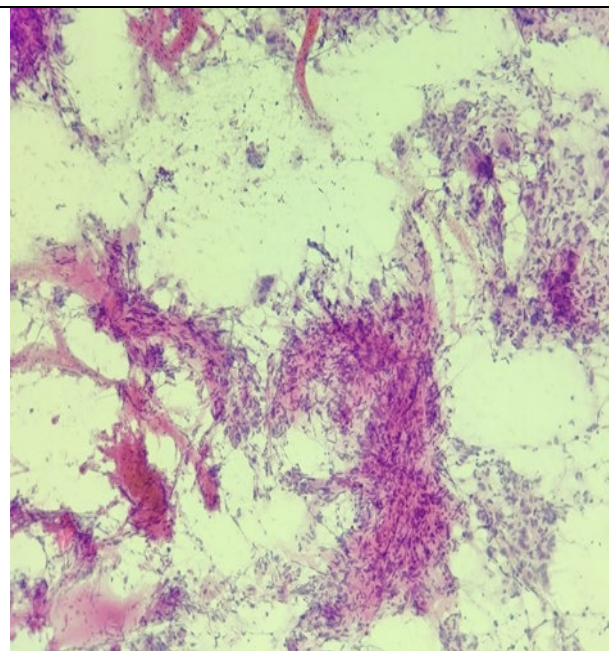


Figure 14. Squash smear of glioblastoma multiforme showing pleomorphic astrocytic cells with vascular proliferation and giant cells (H&E, x100).

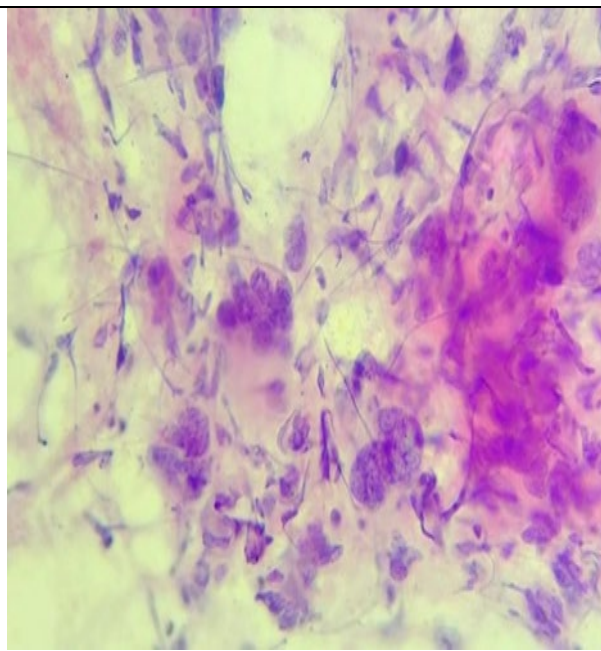


Figure 15. Squash smear showing bizarre astrocytic cells with marked pleomorphism and occasional giant cells (H&E, x400).

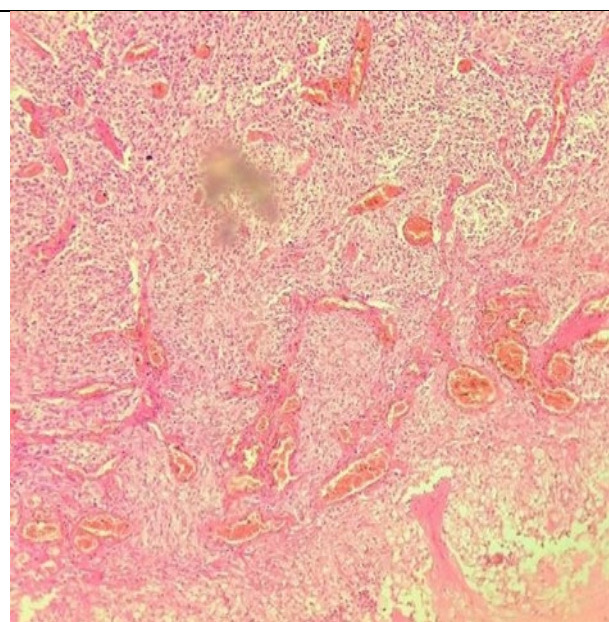


Figure 16. Histopathological section showing markedly pleomorphic tumour cells, necrosis and endothelial cell proliferation (H&E, x100).

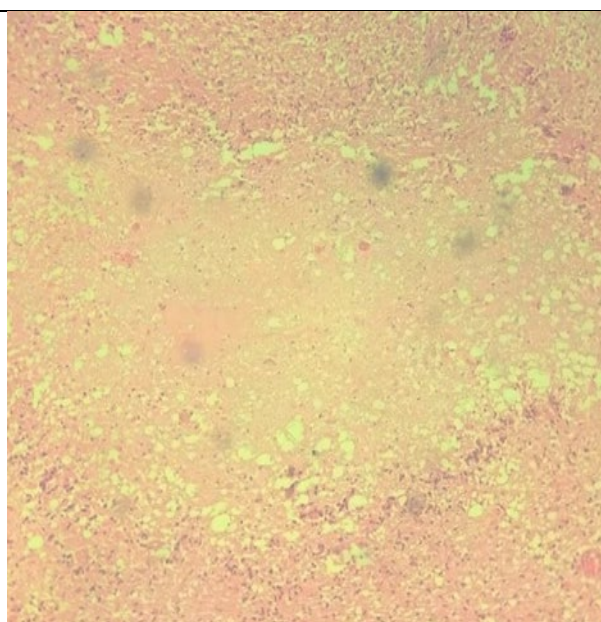


Figure 17. Histopathological section showing a central area of necrosis surrounded by palisading tumour cells (H&E, x400).

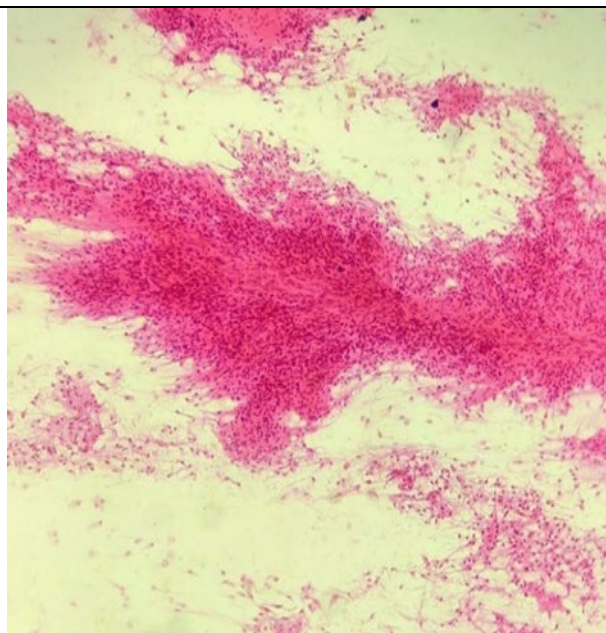


Figure 18. Squash smear of ependymoma showing perivascular pseudorosettes composed of ependymal cells around a central mass of fibrils (H&E, x100).

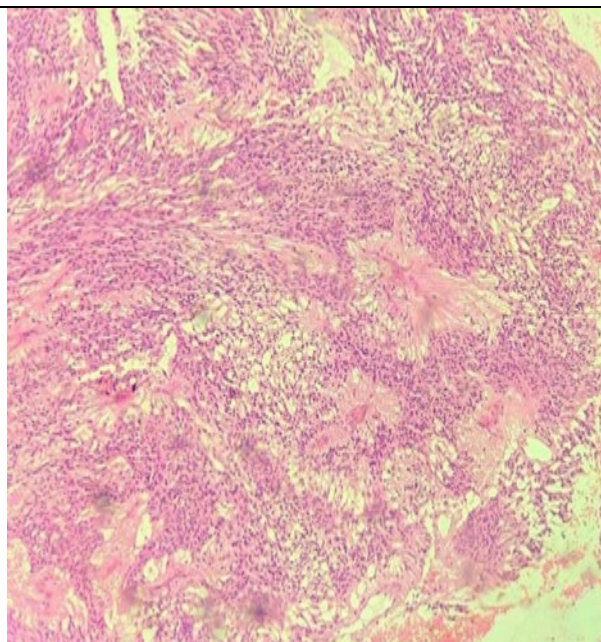


Figure 19. Histopathological section of ependymoma showing perivascular pseudorosettes (H&E, x100).

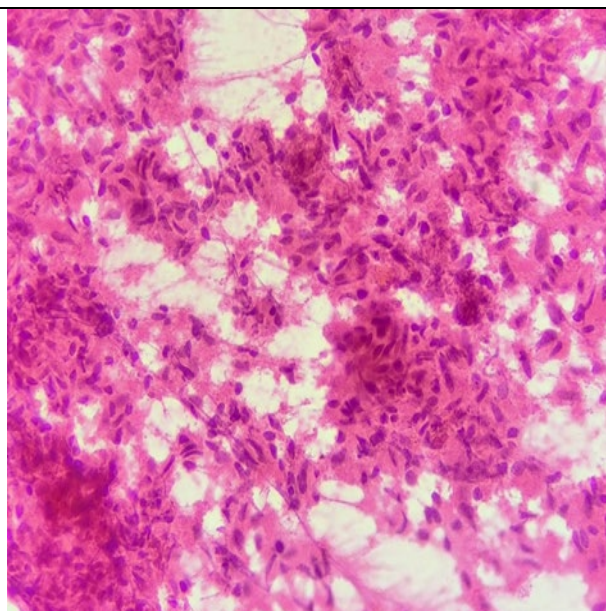


Figure 20. Squash smear of neurofibroma containing spindle cells embedded in abundant myxoid stroma with tapered nuclei (H&E, x400).

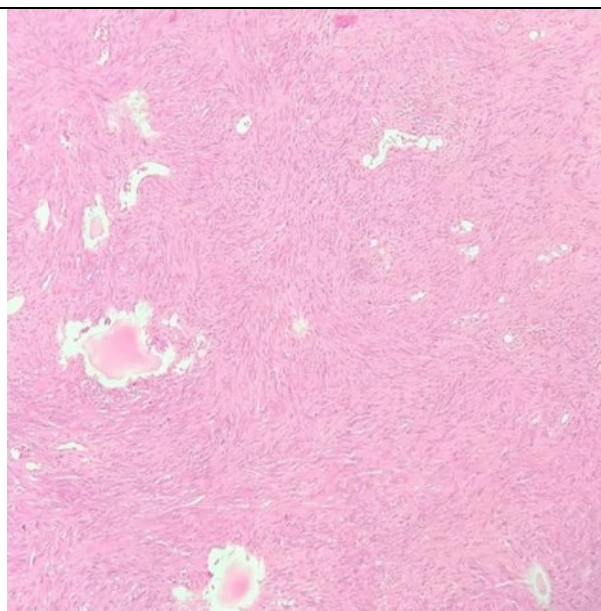


Figure 21. Histopathological section showing wavy bundles of spindle cells arranged in a myxoid collagenous matrix (H&E, x100).

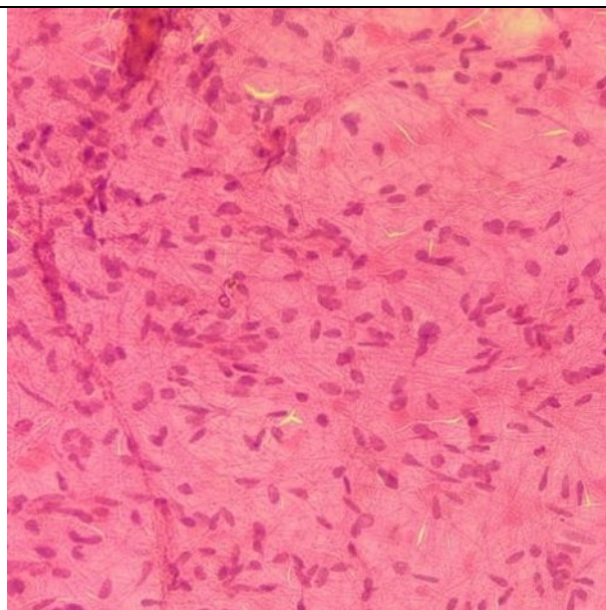


Figure 22. Squash smear of pilocytic astrocytoma showing bipolar piloid cells with long processes and bland nuclei with smooth contours against a fibrillary background (H&E, x400).

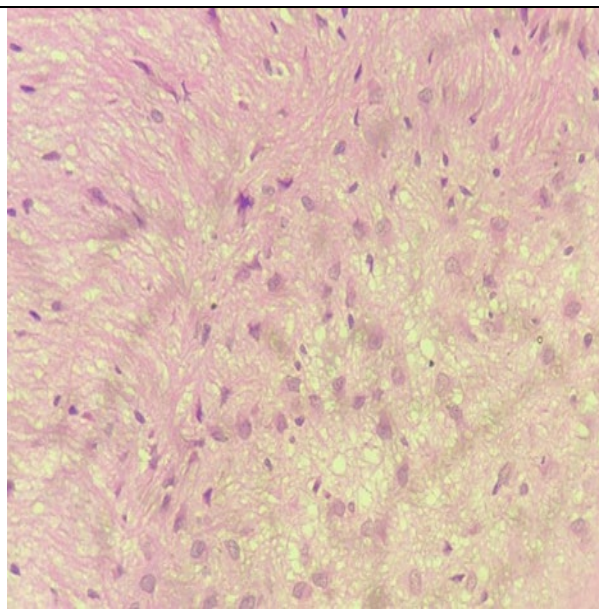


Figure 23. Histopathological section of pilocytic astrocytoma showing tumour cells arranged in a diffuse growth pattern with elongated nuclei and fibrillary processes (H&E, x400).

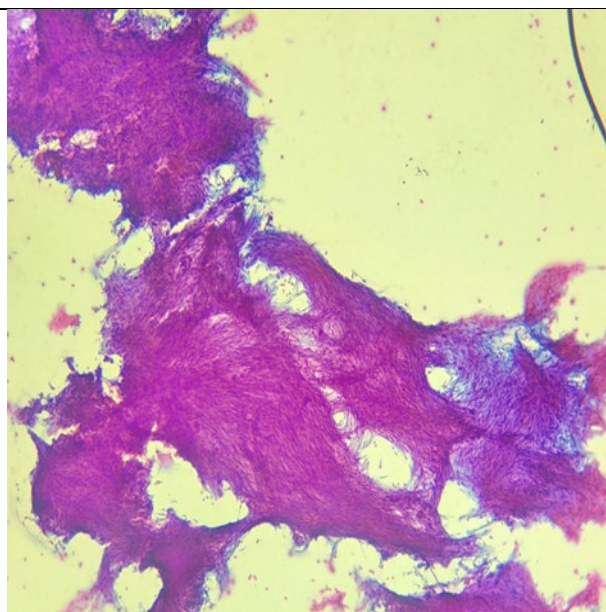


Figure 24. Squash smear of schwannoma showing a cohesive lesion with sharp borders composed of spindle-shaped nuclei (MGG, x100).

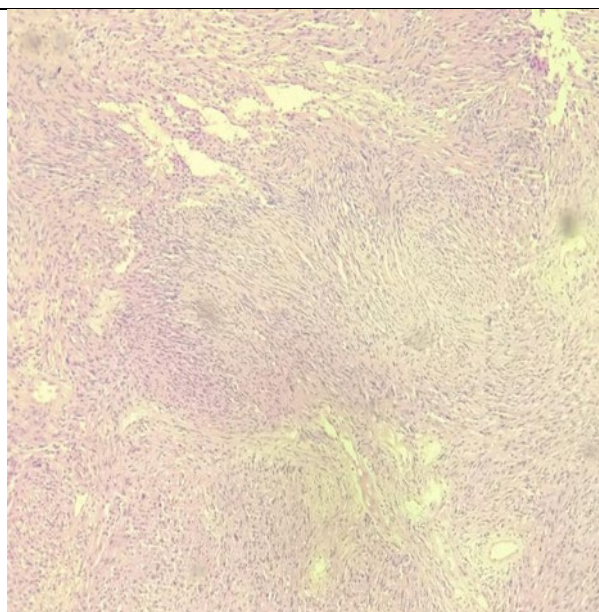


Figure 25. Histopathological section of schwannoma showing dense interlacing bundles of spindled Schwann cells arranged in a linear pattern with hypocellular areas (H&E, x100).

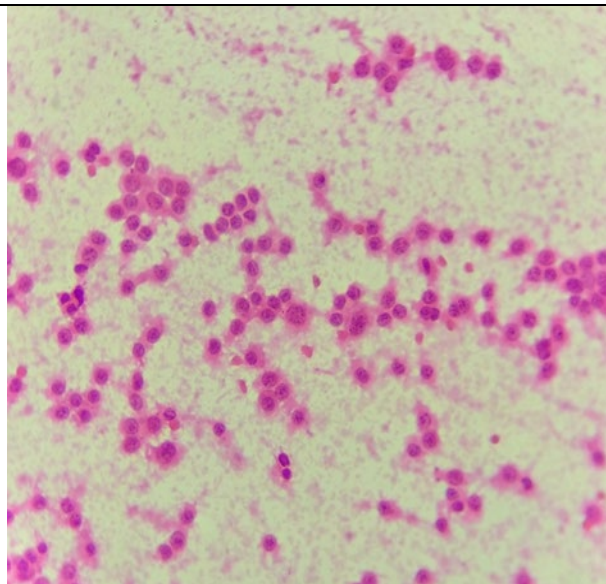


Figure 26. Squash smear of pituitary adenoma showing monomorphic tumour cells with round nuclei, salt-and-pepper chromatin and moderate cytoplasm (H&E, x400).

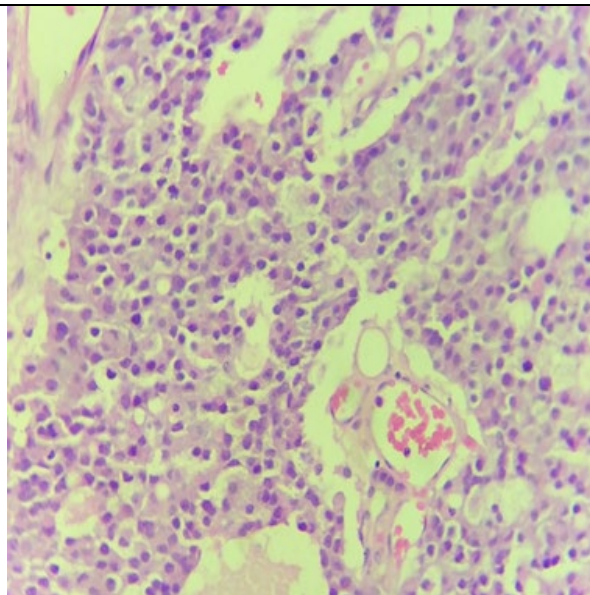


Figure 27. Histopathological section of pituitary adenoma showing a trabecular pattern of tumour cells with round to oval nuclei and moderate cytoplasm (H&E, x400).

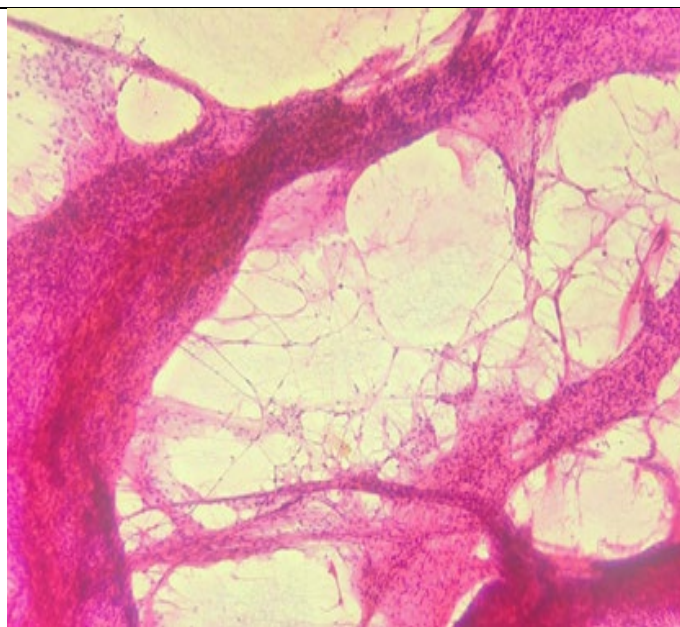


Figure 28. Squash smear of craniopharyngioma misdiagnosed as pilocytic astrocytoma owing to florid reactive gliosis and prominent fibrillary processes (H&E, x100).

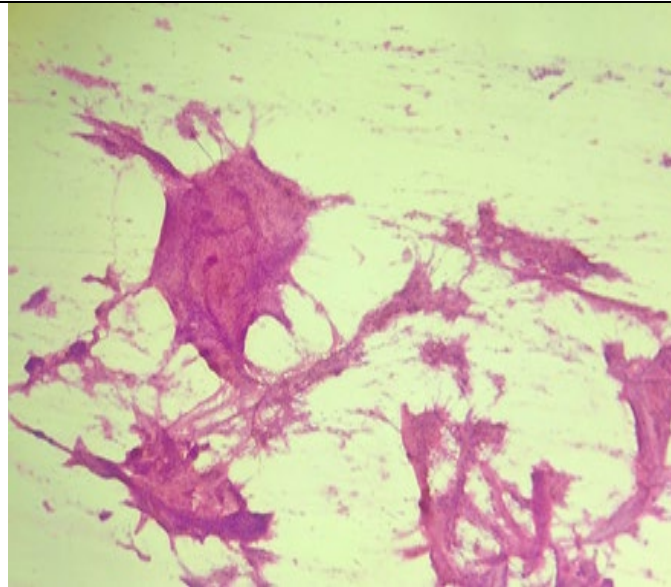


Figure 29. Squash smear of astrocytoma misdiagnosed as schwannoma owing to three-dimensional tissue clusters and spindle-shaped nuclei (H&E, x40).