Role of Squash Cytology in the Diagnosis of Pediatric Intracranial Tumors in a Tertiary Care Hospital

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Abstract

Intracranial tumors are a common cause of mortality in the pediatric population; therefore, accurate and timely diagnosis is crucial. This article highlights the role of squash cytology in diagnosing pediatric intracranial tumors and its correlation with histopathology. A retrospective analytical study was conducted from April 2022 to August 2023. A total of 70 samples were collected for both intraoperative squash cytology and histopathology. Rapid Papanicolaou and hematoxylin-eosin staining kits were used for cytology, while hematoxylin-eosin staining was used for histopathological examination. To reduce bias, the histopathology reports were generated by a separate team of pathologists. Among the 70 pediatric patients with intracranial space-occupying lesions, 41 cases were neoplastic and 29 were non-neoplastic. Of the 41 neoplastic cases, 22 were supratentorial and 19 were infratentorial. Pilocytic astrocytoma was the most common pediatric intracranial tumor, followed by medulloblastoma. Squash cytology demonstrated high diagnostic accuracy, with a sensitivity of 97.60%, specificity of 96%, positive predictive value of 97.60%, negative predictive value of 96%, and overall accuracy of 97.13%. A limitation of this study was the unavailability of immunohistochemistry for all cases. Squash cytology provides valuable information about the adequacy of the sample, the etiology, and tumor grade. As a sensitive diagnostic test, squash cytology can improve the prognosis for pediatric patients with intracranial tumors in India and globally.

Keywords: histopathology; pediatric intracranial tumors; squash cytology

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1. Introduction

Primary central nervous system (CNS) tumors are the second most common cancer in the pediatric population in India, surpassed only by hematological malignancies [1]. Patients often present at an advanced clinical stage due to delayed symptom recognition by parents or missed radiological detection. Intraoperative diagnosis plays a crucial role in managing these cases. While frozen section analysis offers a costly setup with freezing artifact limitations, squash cytology emerges as a rapid, cost-effective, and accurate intraoperative diagnostic tool. While radiological tools like CT scans and MRIs are valuable for pre-operative diagnosis, they can also lead to false-negative results.

The history of squash cytology dates back to 1930, pioneered by Eisenhardt and Cushing [2]. Its evolution saw significant advancements, including Russell, Krayenbuhl, and Cairns's use of toluidine blue in 1937, Morris's replacement of supravital stains with air-dried slides stained with eosin and methylene blue in 1947, and McMenemey's introduction of wet fixation in 95% alcohol in 1960.

Squash cytology empowers surgeons with immediate insights into specimen adequacy and potentially differentiating neoplastic from inflammatory etiologies. For neoplastic pediatric tumors, it can inform the feasibility of total resection and guide surgeons on resection limitations [1]. However, the technique is not without challenges. Inexperienced hands can introduce crushing artifacts, inappropriate smearing, and thickness management issues [3]. To mitigate these limitations, we adopted a protocol of reserving part of the tissue sent for squash samples to prepare additional smears if needed. Despite the valuable information provided by squash cytology, histopathological examination of adequately sampled tissue remains the gold standard.

This study aimed to assess the diagnostic accuracy of squash cytology compared to histopathology, further highlighting its role in managing pediatric intracranial tumors, an area with limited existing literature.

2. Methodology

A retrospective analytical study was conducted at the Department of Neuropathology, in collaboration with the Department of Neurosurgery, at a tertiary care hospital. Data were collected from April 2022 to August 2023, after receiving approval from the institute's ethical committee (Approval Number: IEC/2023/10098). Informed consent was obtained from each

guardian, following a thorough explanation of the study process in their local and understandable language.

The study aimed to 1) investigate cytomorphology, 2) correlate squash cytology findings with histopathological diagnoses, and 3) calculate the sensitivity, specificity, and diagnostic accuracy of squash cytology for diagnosing pediatric intracranial tumors using histopathology as the gold standard. Inclusion criteria comprised all pediatric patients (≤ 18 years) referred to the neurosurgery department with a clinical and radiological diagnosis of an intracranial space-occupying lesion, provided both squash cytology and histopathology specimens were available. Excluded were pediatric patients with spinal lesions, and cases with inadequate specimens. A total of 70 pediatric patients, clinically and radiologically suspected of having intracranial space-occupying lesions, were included in the study. Their clinical and radiological details were recorded in a dedicated dataset.

Tissue for squash cytology was received in saline-soaked pads. Upon arrival, it was immediately squashed, smeared, and fixed using a designated fixative. Three smears were stained with hematoxylin and eosin (H&E) stain, while three others were stained with Papanicolaou's stain. All smears were meticulously examined by a team of experienced pathologists, each possessing over 5 years of expertise in cytology and histopathology reporting. The neurosurgeon was informed of the report within 30-40 minutes of sample receipt.

Following surgery, tissue for histopathological examination was received, fixated overnight in 10% formalin, and subsequently processed. Slides were stained with H&E and interpreted by a separate team of pathologists to minimize bias. The final impression was formulated in accordance with the fifth edition of the World Health Organization (WHO) Classification of CNS Tumors [4].

Data were collected and stored in a Microsoft Excel spreadsheet and analyzed using SPSS software. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated as part of the analysis.

3. Results

Among 70 pediatric patients clinically and radiologically suspected of having intracranial space-occupying lesions, 41 (58.57%) were diagnosed with neoplastic tumors, while 29 (41.43%) had non-neoplastic lesions. The study included 51 males (72.85%) and 19 females (27.14%), with a male-to-female ratio of 2.6:1 (Table 1). Within the neoplastic group, 28 patients (68.29%) were male and 13 (31.71%) were female, with a ratio of 2.1:1 (Table 2).

The majority of patients with neoplastic lesions were in the 11– to 15-year age group. The age range at presentation spanned from 4 months to 18 years, with an average of 6.5 years. The youngest patient, a 4-month-old male, was diagnosed with choroid plexus papilloma (Figure 1A), while the oldest, an 18-year-old female, had medulloblastoma (Figure 1B).

Twenty-two cases (53.65%) involved supratentorial lesions, and 19 cases (46.35%) were infratentorial. The most common pediatric intracranial tumor in our study was pilocytic astrocytoma (Figure 2A) (7 cases, 17.07%), followed by medulloblastoma (6 cases, 14.63%), and craniopharyngioma (Figure 2B) (6 cases, 14.63%). Pediatric diffuse high-grade glioma (Figure 3A) accounted for 5 cases (12.19%), posterior fossa ependymoma (Figure 3B) for 4 cases (9.75%), and other tumors followed in that order.

Concordance between squash cytology and histopathology was observed in 67 cases (95.71%) out of the 70 patients studied, as shown in Table 3. Three cases (4.29%) exhibited discordance. One instance of craniopharyngioma was misclassified as a benign cystic lesion, while a case of reactive gliosis was overdiagnosed as a low-grade glial tumor, and a case of germ cell tumor was misinterpreted as an inflammatory lesion on squash cytology.

Table 4 details the true positive, true negative, false positive, and false negative cases. Based on histopathology as the gold standard, the sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of squash cytology for diagnosing pediatric intracranial tumors were calculated using a contingency table and found to be 97.60%, 96.00%, 97.60%, 96.00%, and 97.13%, respectively. Due to the retrospective nature of the study, we were unable to assess the clinical course of the patients through follow-up. Another limitation was the unavailability of immunohistochemistry results in all cases.

4. Discussion

According to the recent 5th edition of the WHO classification of CNS tumors, pediatric intracranial tumors are categorized into diffuse high-grade and low-grade types [4]. The WHO emphasizes molecular and immunohistochemistry markers for diagnosis. However, in developing countries where such facilities are scarce, morphological analysis becomes crucial for patient management.

Intraoperative diagnosis is particularly important in pediatric patients, guiding surgeons on the extent of resection, which significantly impacts outcomes [5]. Frozen sections have limitations due to freezing artifacts and disrupted tissue architecture, potentially leading to misinterpretation. Recently, with the availability of stereotactic biopsies, squash cytology has gained traction in intraoperative diagnosis [3]. It offers a rapid, cost-effective, and reasonably accurate method with minimal setup requirements.

Our study found pilocytic astrocytoma to be the most common pediatric tumor, similar to Lacruz et al.'s findings [1]. Males were disproportionately affected, with a 2.1:1 male-to-female ratio, aligning with Soon et al.'s observations [6]. On comparison between squash cytology and histopathology, our study revealed a diagnostic accuracy of 97.13%. This was higher than the accuracy of 94.67% reported by Jindal Arpita et al. in 2017 for a study of 150 pediatric patients [7]. Other studies found diagnostic accuracy ranging from 76% to 96% [8-20].

In our study, discordance was found in three cases (4.29%). One case of craniopharyngioma was initially misinterpreted as a benign cystic lesion, likely due to sampling from the tumor's cystic part. A germ cell tumor was mistaken for an inflammatory lesion due to a predominance of lymphocytes in the sample. Finally, a case of reactive gliosis was over-diagnosed as a low-grade glial tumor. This overlap can occur because astrocytic cells in reactive gliosis exhibit cell processes and mild atypia, morphologically resembling features of low-grade gliomas [21].

Table 1: Age and gender-wise distribution of cases.

Age group	Number of cases	Male	Female
0-5 years	12	11	1
6-10 years	15	13	2
11-15 years	22	14	8
16-18 years	21	13	8
Total	70	51 (72.85%)	19 (27.14%)

Table 2: Detailed breakdown of neoplastic cases.

MRI/CT location	Histopathological diagnosis	Total number of cases	<5 years		6-10 years		11-15 years		16-18 years	
			M	F	M	F	M	F	M	F
Supratentorial (22 cases, 53.65%)	Pediatric diffuse high-grade glioma	5	0	0	0	0	3	0	2	0
	Pediatric diffuse low-grade glioma	3	0	0	0	0	0	0	0	3
	Craniopharyngioma	6	0	0	2	0	4	0	0	0
	Germ cell tumor	2	0	0	1	0	1	0	0	0
	Choroid plexus papilloma	1	1	0	0	0	0	0	0	0
	Choroid plexus carcinoma	2	0	0	2	0	0	0	0	0
	Central neurocytoma	1	0	0	0	0	1	0	0	0
	Small round cell tumor	2	2	0	0	0	0	0	0	0
Infratentorial (19 cases, 46.35%)	Pilocytic astrocytoma	7	0	0	0	0	1	0	3	3
	Medulloblastoma	6	0	3	0	0	1	0	0	2
	Posterior fossa ependymoma	4	2	0	0	1	0	1	0	0
	Epidermoid tumor	2	0	0	1	0	1	0	0	0
Total		41	5	3	6	1	12	1	5	8

Table 3: Correlation of squash cytology with histopathological diagnoses.

Histopathology diagnosis		Number of cases (%)	Squash cytology diagnosis	
			Number of concordant cases (%)	Number of discordant cases (%)
Neoplastic lesions (41 cases, 58.57%)	Medulloblastoma	6 (8.57)	6 (8.57)	0 (0.00)
	Craniopharyngioma	6 (8.57)	5 (7.14)	1 (1.42)
	Pilocytic astrocytoma	7 (10.00)	7 (10.00)	0 (0.00)
	Pediatric diffuse high-grade glioma	5 (7.14)	5 (7.14)	0 (0.00)
	Germ cell tumor	2 (2.85)	1 (1.42)	1 (1.42)
	Ependymoma	4 (5.71)	4 (5.71)	0 (0.00)
	Pediatric-type diffuse low-grade glioma (diffuse astrocytoma)	3 (4.28)	3 (4.28)	0 (0.00)
	Choroid plexus carcinoma	2 (2.85)	2 (2.85)	0 (0.00)
	Choroid plexus papilloma	1 (1.42)	1 (1.42)	0 (0.00)
	Epidermoid cyst	2 (2.85)	2 (2.85)	0 (0.00)
	Small round cell tumor	2 (2.85)	2 (2.85)	0 (0.00)
	Central neurocytoma	1 (1.42)	1 (1.42)	0 (0.00)
Non-neoplastic lesions (29 cases 41.43%)	Tuberculosis	8 (11.42)	8 (11.42)	0 (0.00)
	Hydatid cyst	1 (1.42)	1 (1.42)	0 (0.00)
	Brain abscess	6 (8.57)	6 (8.57)	0 (0.00)
	Reactive gliosis	5 (7.14)	4 (5.71)	1 (1.42)
	Arachnoid cyst	3 (4.28)	3 (4.28)	0 (0.00)
	Arterio-venous malformation	1 (1.42)	1 (1.42)	0 (0.00)
	Focal cortical dysplasia	1 (1.42)	1 (1.42)	0 (0.00)
	Occipital meningocele	2 (2.85)	2 (2.85)	0 (0.00)
	Chronic non-specific inflammatory lesion	2 (2.85)	2 (2.85)	0 (0.00)
Total		70 (100)	67 (95.71)	3 (4.29)

Table 4: Diagnostic accuracy of squash cytology.

Diagnosis on squash cytology	Histopathology diagnosis		Total
	Positive	Negative	
Positive	41	1	42 (60.00%)
Negative	1	27	28 (40.00%)
Total	42 (60.00%)	28 (40.00%)	70

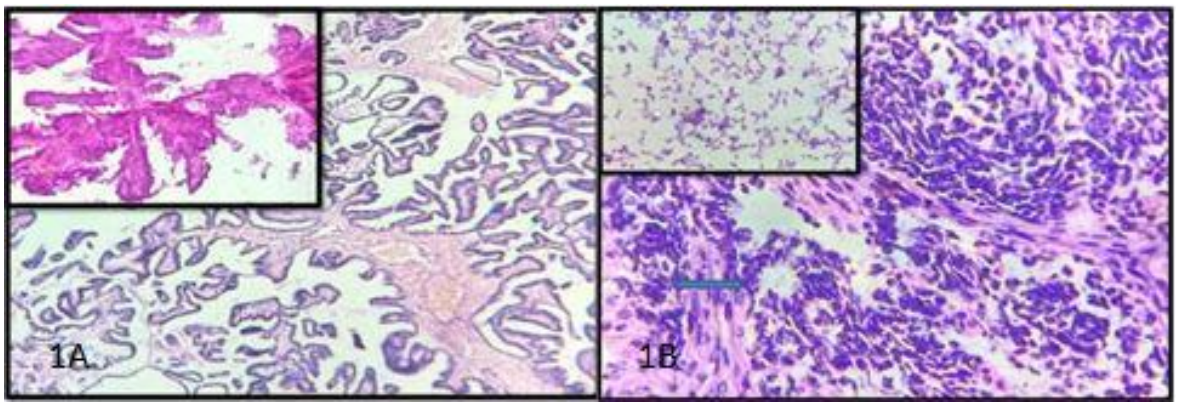

Figure 1A: (Left) The HE (40x) section reveals fibrovascular cores lined by a single layer of columnar to cuboidal cells. Pap (40x) inset smears demonstrate a papillary arrangement of neoplastic cells. Figure 1B: (Right) The HE (40x) section shows sheets of monotonous embryonal cells with scanty cytoplasm and Homer Wright rosettes (arrow). Pap (40x) inset smears reveal evenly distributed small round cells with scanty cytoplasm and rosette formation.

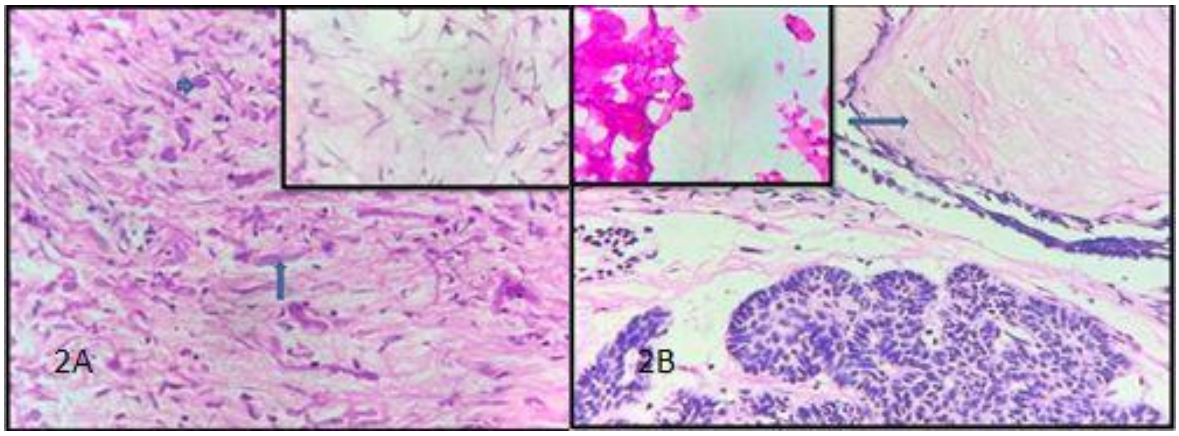

Figure 2A: (Left) The HE (40x) section reveals tissue with bipolar cells featuring long hair-like processes, Rosenthal fibers (arrow), and eosinophilic granular bodies (arrowhead). Pap (40x) inset smears showcase bipolar piloid cells. Figure 2B: (Right) The HE (40x) section shows peripheral palisading of epithelial cells surrounding wet keratin (arrow). The HE (40x) inset smear reveals cohesive sheets of flat epithelial cells alongside ghost cells.

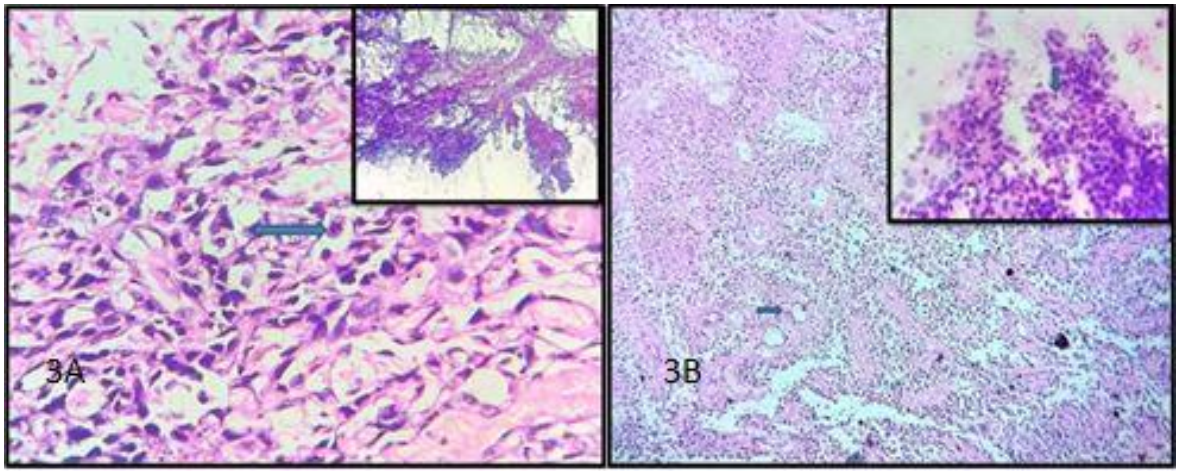

Figure 3A: (Left) The HE (40x) section shows pleomorphic neoplastic astrocytes with frequent mitosis (indicated by arrows). Pap (40x) inset smear reveals glomeruloid microvascular proliferation. Figure 3B: (Left-Ependymoma) The HE (10x) section reveals uniform cells with round to oval nuclei forming perivascular pseudorosettes (highlighted by arrows). The HE (40x) inset smear shows neoplastic cells within a fibrillary background, further emphasizing ependymal rosettes (marked by arrows).

Beyond technical limitations related to inexperienced users (e.g., crushing artifacts, inappropriate smearing, and inconsistent thickness), non-representative sampling remains another critical limitation of squash cytology. Applying additional staining methods and promptly assessing sample adequacy, with immediate communication to the surgeon for further sampling if needed, can help address these challenges. Misinterpretations, as seen in the germ cell tumor and reactive gliosis cases, can potentially lead to undertreatment or overtreatment, respectively [12].

Therefore, with a diagnostic accuracy of 97.13% and its potential to improve prognosis through intraoperative diagnosis, squash cytology remains a valuable tool for pediatric intracranial tumors. Proper sampling from representative areas can further enhance its accuracy and clinical utility. In developing countries, financial constraints and the unavailability of specialized tests like molecular markers and immunohistochemistry at every tertiary care hospital necessitate the use of sensitive tests like squash cytology for the benefit of patients. However, assessing the role of IHC markers in diagnosing spinal lesions could be a valuable area for future research.

Another limitation of our study is its retrospective, single-center, small-scale design without a comparison group. However, we have taken all necessary precautions to avoid selection bias. To overcome these limitations, we plan to conduct a prospective, multi-center, large-scale study with a comparison group for more reliable and generalizable results. Nevertheless, further research is necessary to clarify the role of squash cytology as a routine diagnostic tool for pediatric intracranial tumors.

5. Conclusion

Squash cytology offers a rapid, accurate, and cost-effective technique for intraoperative diagnosis of pediatric intracranial tumors. It provides valuable information on sample adequacy, tumor etiology (neoplastic, non-neoplastic, inflammatory, or infectious), and tumor grade, guiding the surgeon on the extent of resection. This can help prevent unnecessary wide excisions, which can significantly impact a child's development and overall outcome. Careful interpretation of squash cytology samples, along with proper correlation with clinical and radiological findings, has demonstrated a high degree of diagnostic accuracy, with sensitivity reaching 97.60%, specificity at 96.00%, and overall accuracy of 97.13%. Further improvement can be achieved through proper sampling techniques that target representative areas of the lesion.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions

Design conception, data collection, data analysis, and preparation of draft, Diya Bajaj (D.B.); Design conception, data collection, data analysis, and preparation of draft, A.G.; Data collection and data analysis, N.Y.; Design conception, data analysis, and preparation of draft, J.B.; Design conception and data analysis, S.R. All authors have read and agreed to the published version of the manuscript.

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