Intraoperative Squash Cytology and Histopathological Correlation of Glial Tumors at a Tertiary Care Hospital

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Abstract

Introduction Central nervous system (CNS) tumors account for around 1 to 2% of all neoplasms, commonest of them being gliomas. Gliomas constitute a large, heterogenous group of tumors known for a wide variation in clinical presentation, gross and microscopic features, and biologic behavior. Squash cytology can be a great asset in the intraoperative diagnosis of CNS pathology. In this article, we correlate it with the histopathology of gliomas.

Methods A prospective analytical study was conducted at the Department of Neuropathology, Super-Speciality Hospital, NSCB Medical College, Jabalpur, India. A total of 75 samples were collected for intraoperative squash cytology. The biopsy samples were collected subsequently after surgery for histopathological correlation. Statistical analysis was done using SPSS software to calculate the sensitivity, specificity, and diagnostic accuracy of squash cytology.

Results Of the total 75 patients clinically and radiologically suspected of having gliomas, 43 (57.33%) were males to give a male-to-female ratio of 1.34:1. The mean age at presentation was 36.50 ± 16.87 years. Right-sided tumors were more common. The most common location was the frontal lobe (46.66%). Concordance with squash cytology was found in 81.33% of cases. Sensitivity, specificity, and diagnostic accuracy of squash cytology in the diagnosis of gliomas were found to be 98.61, 66.66, and 97.33%, respectively.

Conclusion Squash cytology is a rapid, inexpensive, and accurate diagnostic method for intraoperative diagnosis of gliomas that can guide the surgeon on the extent of tumor resection.

Keywords
- glioma
- cytology
- histopathology
- intraoperative
- squash

Introduction

Gliomas comprise a large and heterogenous group of tumors and are notorious for a wide variation in clinical presentation, gross and microscopic features, and biological behavior.[1] Intraoperative cytology and frozen section play important roles in the diagnosis of neurosurgical samples and help in approaching the central nervous system (CNS) lesions. Squash cytology has been shown to be of great value in intraoperative consultation of CNS pathology.[2] The history of intraoperative

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cytology dates back to 1930 when Eisenhardt and Cushing introduced the technique by using supravital staining.[3] Squash smears are inexpensive and no special skill is required to obtain a sample.[1] No particular supplies are needed and minute tissue pieces can be employed for processing.[1] However, failure to manage thickness, crushing artifacts, and inappropriate smearing are the limitations of squash smears.[1] The frozen section provides better structural details, but freezing artifacts compromises quality. The characteristic soft nature and high water content of nervous tissue result in poor quality of a frozen section.[1] Rapid immunocytochemistry is limited to some antibodies well documented in the literature such as keratins.[4,5] Immunohistochemistry is helpful in further typing of tumors; however, it may produce nonspecific background staining.[6] Morphology cannot be an accurate prognostic indicator where pathologists receive small biopsy samples, but the gold standard still remains the histopathological examination of an abundantly sampled tissue.[7] This study aims to correlate intraoperative squash cytology with histopathological examination.

Materials and Methods

A prospective analytical study was conducted in the department of neuropathology at a tertiary care center. This cross-sectional study was conducted from March 2022 to October 2022. The study was started after approval from the institute's ethical committee. Informed consent was obtained from each patient/guardian to collect and process the samples for the study.

The objectives of the study were the following:

- To study the cytomorphological features of gliomas.
- Correlation of the cytological diagnosis with the histopathological diagnosis of gliomas.
- To assess the sensitivity and specificity of intraoperative squash cytology in the diagnosis of gliomas taking histopathology as the gold standard.

Inclusion criteria: All the patients who came to the neurosurgery department with clinical and radiological signs of glioma and in which both squash cytology and histopathology samples were available were included in the study.

Exclusion criteria: Patients with inadequate squash cytology or histopathology sample were excluded from the study.

A total of 75 cases clinically and radiologically suspected of having gliomas were included in the study. Clinical and radiological details were collected in a dataset while receiving samples for intraoperative squash cytology. Tissue received in saline-soaked pads was squashed and smears were prepared immediately and fixed in ethanol. Half of the smears were stained with hematoxylin and eosin (H&E) and the remaining smears were stained with Papanicolaou's stain. All the smears were examined by two separate pathologists and the report was conveyed to the neurosurgeon within 30 minutes of receiving the sample. The diagnostic criterion for low-grade glioma was increased cellularity and atypia, while the criterion for high-grade glioma was the presence of mitosis, necrosis, and/or microvascular proliferation.[8] Tissue for histopathological examination was received in 10% formalin after surgery and fixed overnight. After processing, slides were stained with H&E stain and interpreted by two different pathologists to reduce the bias and a final diagnosis was generated as per the World Health Organization (WHO) Classification of CNS Tumors, fifth edition.[9] Data were recorded in a Microsoft Excel sheet and statistical analysis was done using SPSS 22.0 software. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated.

Results

A total of 75 patients clinically and radiologically suspected of glioma were studied. Out of these, 72 cases were diagnosed as glioma on histopathology and 3 turned out to be reactive gliosis. Forty-three (57.33%) patients were males and 32 (42.66%) were females to give a male-to-female (M:F) ratio of 1.34:1. The age range was 13 to 78 years with the mean age being 36.50 ± 16.87 years. The youngest patient was a 13-year-old adolescent boy diagnosed with pediatric-type diffuse high-grade glioma (CNS WHO grade 4) and the eldest was a 78-year-old man diagnosed with glioblastoma NOS (CNS WHO grade 4). Gliomas were on the right side in 39 cases (52%), left side in 24 cases (32%), and midline in 12 cases (16%). The most common location for gliomas was the frontal lobe ($n = 35$, 46.66%), followed by the temporal lobe ($n = 18$, 24%), parietal lobe ($n = 14$, 18.66%), occipital lobe ($n = 4$, 0.05%), and midline and posterior fossa ($n = 4$, 0.05%). Intraoperative squash smears were reported as shown in ►**Table 1**.

High-grade gliomas constituted 43 cases (57.33%) and low-grade gliomas constituted 23 cases (30.66%). On histopathological analysis, gliomas were found to be CNS WHO grade 1 in 8 cases (10.66%), grade 2 in 14 cases (18.66%), grade 3 in 14 cases (18.66%), and grade 4 in 36 cases (48%). On histopathological examination, the most common tumor was glioblastoma NOS CNS WHO grade 4 ($n = 32$, 42.66%; ►**Fig. 1A**), followed by astrocytoma ($n = 22$,

Table 1 Distribution of cases on squash cytology

Squash cytology diagnosis	No. of cases	Percentage
High-grade glioma	43	57.33
Low-grade glioma	23	30.66
Glial tumor—ependymoma	2	2.66
Astrocytoma with piloid features	4	5.33
Hypercellular astroglial tissue-reactive gliosis	2	2.66
Necrosis only	1	1.33
Total	75	100

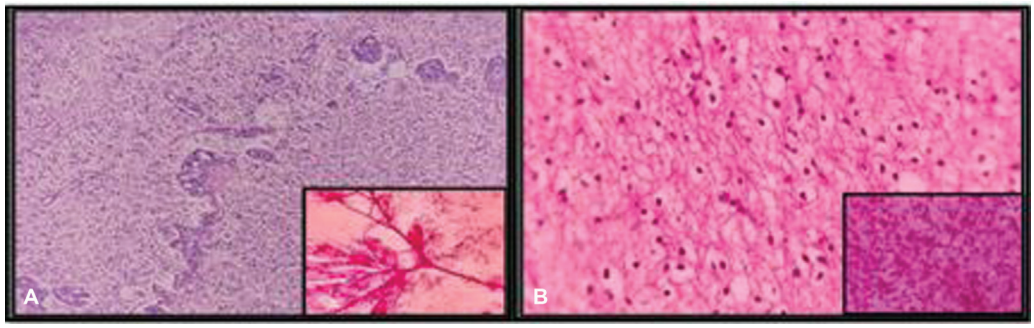

Fig. 1 (A) Hematoxylin and eosin (H&E) 10X view. The section shows glomeruloid microvascular proliferation, while the inset shows squash cytology of the same patient showing the morphology of a high-grade glioma along with microvascular proliferation. (**B**) H&E 10X view. The section shows round to oval anaplastic astrocytes showing atypia, while the inset shows squash cytology of the same patient showing the morphology of a low-grade glioma.

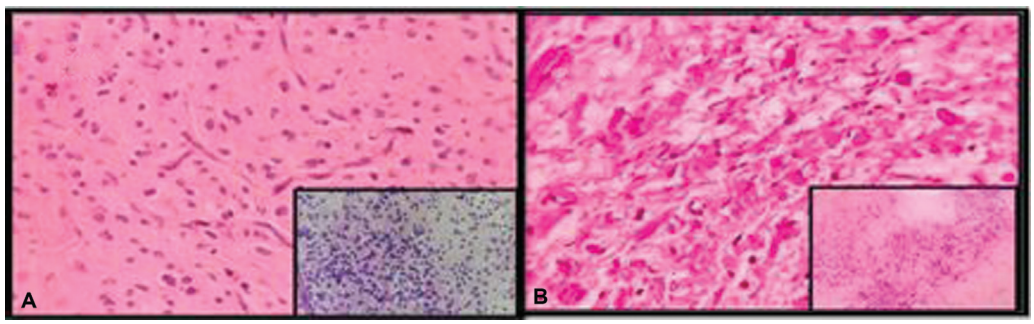

Fig. 2 (A) Hematoxylin and eosin (H&E) 10X view. The section shows oligodendrocytes with a perinuclear halo and chicken wiring of vessels in the background, while the inset shows the squash cytology of the same patient showing round cells in the fibrillary background. (**B**) H&E 10X view. The section shows round to oval bipolar neoplastic cells with long hairlike processes along with eosinophilic granular bodies and Rosenthal fibers, while the inset shows the squash cytology of the same patient showing bipolar cells showing low-grade morphology with piloid features.

29.33%; ►**Fig. 1B**), oligodendroglioma ($n = 4$, 5.33%; ►**Fig. 2A**), pilocytic astrocytoma ($n = 4$, 5.33%; ►**Fig. 2B**), and ependymoma ($n = 2$, 2.66%; ►**Fig. 3**). Concordance was found between squash cytology and histopathology in 61 cases (81.33%) as shown in ►**Table 2**. Discordance was seen in 14 cases (18.66%), and it was mainly with respect to grading of the tumors.

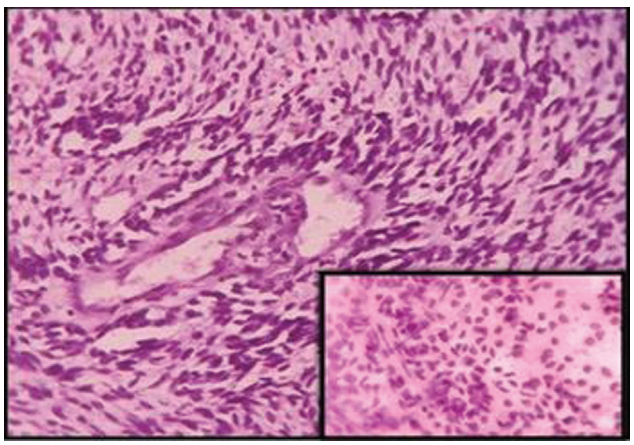

Fig. 3 Hematoxylin and eosin (H&E) 10X view. The section shows neoplastic cells arranged in sheets with a perivascular pseudorosette, while the inset shows the squash cytology of the same patient showing rosettes.

True-positive, true-negative, false-positive, and false-negative cases were as shown in ►**Table 3**. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of squash cytology to diagnose gliomas taking histopathology as the gold standard were found to be 98.61, 66.66, 98.61, 66.66, and 97.33%, respectively. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of squash cytology to diagnose low-grade gliomas taking histopathology as the gold standard were found to be 86.36, 79.24, 63.33, 6.66, and 81.33%, respectively. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of squash cytology to diagnose high-grade gliomas taking histopathology as the gold standard were found to be 80.00, 88.00, 93.02, 31.25, and 82.66%, respectively. Diagnostic accuracies were not similar for low- and high-grade gliomas in our study and the detection rate for gliomas was more in the histopathological examination than in squash cytology.

Discussion

Gliomas have been difficult to describe with respect to causes, pattern of occurrence, and prognosis. The WHO classification of CNS tumors, fifth edition, has mainly stressed on the molecular and immunohistochemistry diagnosis of brain tumors. In resource-poor countries, where molecular and immunohistochemistry setup is not

Table 2 Correlation of squash cytology with histopathological diagnoses

Histopathology diagnosis	No. of cases, *n* (%)	Squash cytology diagnosis	
		No. of concordant cases, *n* (%)	No. of discordant cases, *n* (%)
Angiocentric glioma (grade 1)	2 (2.66)	1 (50)	1 (50)
Pilocytic astrocytoma (grade 1)	4 (5.33)	4 (100)	0 (0)
Dysembryoplastic neuroepithelial tumor (grade 1)	2 (2.66)	2 (100)	0 (0)
Astrocytoma (grade 2)	10 (13.33)	8 (80)	2 (20)
Astrocytoma (grade 3)	12 (16)	8 (66.67)	4 (33.33)
Oligodendroglioma (grade 2)	2 (2.66)	2 (100)	0 (0)
Oligodendroglioma (grade 3)	2 (2.66)	2 (100)	0 (0)
Glioblastoma (grade 4)	32 (42.66)	26 (81.25)	6 (18.75)
Pediatric diffuse high-grade glioma (grade 4)	4 (5.33)	4 (100)	0 (0)
Ependymoma (grade 2)	2 (2.66)	2 (100)	0 (0)
Reactive gliosis	3 (4)	2 (66.67)	1 (33.33)
Total	75 (100)	61 (81.33)	14 (18.67)

Table 3 True-positive (TP), true-negative (TN), false-positive (FP), and false-negative (FN) cases for gliomas

Diagnosis on squash cytology	Histopathology diagnosis		**Total**
	Positive	Negative	
Positive	71 (TP)	1 (FP)	72
Negative	1 (FN)	2 (TN)	3
Total	72	3	75

readily available, morphological diagnosis plays an important role in the diagnosis and management of neurosurgical cases. An intraoperative diagnosis of CNS lesions using squash cytology and frozen section helps in a better neurosurgical approach to these cases. Chand et al observed several technical errors in frozen sections and freezing artifacts that disturb the architecture, leading to misinterpretation.[10] Intraoperative squash cytology has recently gained importance because of the availability of stereotactic biopsies.[1] It is a rapid, inexpensive, and fairly accurate diagnostic method for intraoperative diagnosis of gliomas. There are limitations in both the frozen section and squash cytology techniques, but understanding the pitfalls and errors may increase diagnostic accuracy. The most common age group for occurrence of gliomas was the fourth to fifth decade of life in our study with a male predominance. In a clinicopathological study of 115 patients by Khanna et al, M:F ratio was 2.3:1.[11] Ghosal et al found a male preponderance with M:F ratio of 1.5:1 in a study of 306 patients.[12] In our study, glioblastoma was the commonest tumor that is similar to that found in a study done by Shrestha et al[13] and Ohgaki and Kleihues.[14] On comparing squash cytology and histopathology, our study revealed diagnostic accuracy of 97.33%. Concordance was seen between both methods in 61 cases (81.33%). This was in accordance with the studies done by Das et al,[15] Patil et al,[16] and Mitra et al.[17] Discordance was found in 14 cases (18.66%). The reason for discordance was mainly due to sampling from the necrotic part of the lesion, from the periphery of the lesion, or due to similar cytomorphology of the lesions. One case of reactive gliosis was misinterpreted as low-grade glioma. The astrocytes of reactive gliosis show cell processes and a mild degree of cytological abnormality that overlaps with a slight degree of abnormality seen in low-grade astrocytomas.[18] The biopsy from the central part of the tumor may be more representative, but a biopsy taken from a peripheral area can be misdiagnosed cytologically due to sampling error.[19] Diagnostic accuracy can be increased with proper sampling from the representative area of the lesion.

Conclusion

Intraoperative squash cytology is a widely accepted technique for rapid and accurate intraoperative diagnosis of gliomas. It is an inexpensive test that requires no special supplies, and adequately sampled minute tissue can provide a fairly accurate intraoperative diagnosis. Intraoperative squash cytology primarily helps assess for adequacy of representative specimen. But along with that, as it offers

diagnosis about etiology like infection or neoplasm and about grades (low or high), which helps the surgeon to decide about the extent of resection. Careful examination of squash cytology samples provides a diagnostic accuracy of approximately 97.33%, which can be further improved with proper sampling from the representative area of the lesion. It is beneficial for the patient and helps the surgeon to decide on the extent of tumor resection during surgery, which can reduce postoperative complications related to wide excision.

Conflict of Interest
None declared.

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