An Audit of Diagnostic Disparity between Intraoperative Frozen Section Diagnosis and Final Histopathological Diagnosis of Central Nervous System Lesions at a Tertiary Care Center

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Abstract

Introduction   Evaluation of intraoperative squash smear and frozen section (FS) in central nervous system (CNS) neoplasms is consistently practiced for rapid assessment and has several advantages to its credence. It is an invaluable tool to ensure adequacy of tissue obtained to establish the diagnosis. Moreover, it aids in guiding the surgeon for critical decisions regarding the extent of resection. Although molecular markers have been integrated with morphology in the revised 2016 World Health Organization classification of brain tumors, precise morphological assessment still remains the foundation for the diagnosis and rapid intraoperative assessment of morphological details is equally critical and rewarding.

Objective   This study aims to audit the diagnostic disparity between intraoperative diagnoses based on a combination of squash cytology and FS in cases of CNS lesions with gold standard, final diagnosis based on examination of formalin fixed paraffin embedded hematoxylin, and eosin-stained tissue sections.

Materials and Methods   All intraoperative squash cytology and FS reported for CNS lesions from January 2017 to December 2020 were reviewed. The cases were categorized into three groups—group 1: when diagnosis of intraoperative diagnosis based on a combination of squash cytology and FS was same as the final histopathological diagnosis (concordant), group 2: partially concordant, and group 3: discordant cases.

Statistical Analysis   Descriptive statistics was used to classify the data and diagnostic accuracy was calculated.

Results   Complete concordance was present in 69.96% (191/273) cases, 20.1% (55/273) cases showed partial concordance, and 9.89% (27/273) cases were discordant

Keywords
► frozen section
► squash smear
► central nervous system
► intraoperative diagnosis

with histopathological diagnosis. Out of the 27 discordant cases, misclassification of tumor type was the most common category (11 cases, 40%), followed by grading mismatch (7 cases, 25.9%), and misdiagnosis of tumor versus nontumor conditions (9 cases, 33.3%).

Conclusion   Our study shows that combination of intraoperative squash cytology and FS shows a high percentage of accuracy in arriving at intraoperative diagnosis in cases of intracranial lesions. Regular audits of discordant cases should be conducted by surgeons and pathologists as part of a quality assurance measure to sensitize themselves with the potential pitfalls, minimizing misinterpretation and helping in providing a more conclusive opinion to the operating surgeons.

Introduction

Intracranial tumors are a diverse group of malignancies that are broadly classified as primary or secondary (metastatic). The current World Health Organization (WHO) classification describes more than 130 different central nervous system (CNS) tumors, entities, or variants.[1]

Evaluation of intraoperative squash smear or frozen section (FS) in CNS neoplasms is consistently practiced at many centers for rapid assessment and has several advantages to its credence.[2] It is an invaluable tool to ensure adequacy of tissue obtained to establish the diagnosis. Moreover, it aids in guiding the surgeon for critical decisions regarding the extent of resection.[3,4] Although molecular markers have been integrated with morphology in the revised 2016 WHO classification of brain tumors,[1] precise morphological assessment still remains the foundation for the diagnosis and rapid intraoperative assessment of morphological details is equally critical and rewarding.[2] The usefulness and accuracy of this vital intraoperative technique in the assessment of CNS lesions have been confirmed in many studies.[5,6] Out of the two primary methods for evaluation of intraoperative biopsies, quash smear cytology has recently gained importance because of image-guided stereotactic biopsies. The soft and fragile texture of brain tumors aids in smear preparation providing exquisite nuclear details free of freezing artifact and distortion. Squash cytology is faster to prepare, easy to adopt, requiring minimal tissue, and is an inexpensive and reliable intraoperative technique allowing reliable diagnosis and guidance to neurosurgeon.[7] The FS technique is more reliable and superior in terms of characterization of architectural details and cytomorphology. It has the limitation of difficult section cutting due to soft consistency of tissue, costly equipment, and freezing artifacts due to ice crystal formation.[8]

Advantages and limitation of these techniques must be accepted by both the neurosurgeon and reporting pathologist.[9] The studies have considered combined use of FS and squash as complimentary procedures to assist neurosurgeons in accurate diagnosis as compared with single procedure only.[10] It is also recommended to evaluate intraoperative preparations in the context of clinical impression and neuro-imaging for an accurate intraoperative diagnosis.

The aim of the current study is to audit the diagnostic disparity between intraoperative diagnoses based on combination of squash cytology and FS in cases of CNS lesions with final histopathological diagnosis, which is taken as the gold standard.

Materials and Methods

This retrospective analytical study was performed at the department of pathology of a tertiary care hospital, in which all the consecutive intraoperative squash cytology and FSs reported for CNS lesions from January 2017 to December 2020 were reviewed. The squash smears and FS were prepared, examined, and reported intraoperatively for each case. CNS tumors were graded and classified according to WHO 2016 classification. The intraoperative diagnosis was correlated with gold standard, final formalin fixed paraffin embedded (FFPE) hematoxylin, and eosin stained (H&E) tissue sections. Where required, immunohistochemistry was performed on the FFPE tissue specimen for establishing the final diagnosis. Detailed demographic details, clinical history, and radiological findings were compiled for all the cases from the medical records.

Inclusion Criteria
All the intraoperative neurosurgical specimens which were evaluated by preparing both squash smears and FS and confirmed with histopathological examination of postsurgical biopsy tissue.

Exclusion Criteria

- Postsurgical biopsy tissue received without intraoperative squash or FS request from the neurosurgical department.
- Sample inadequate to perform all the above three procedures.

Intraoperative final diagnoses based on combination of squash cytology and FS were correlated with the histological diagnosis of the paraffin-embedded sections, the latter being regarded as the gold standard, and categorized into three groups. The discordant cases were recognized and the possible causes for the fallacies and shortcomings were analyzed.

The diagnostic compatibility and accuracy between the two diagnostic techniques was established on the basis of classification of CNS tumor and grading. The cases were categorized into three groups after correlation:

- Complete concordance was present when intraoperative final diagnosis based on a combination of squash cytology and FS was same as the final histopathological diagnosis (concordant).
- Intraoperative final diagnosis based on a combination of squash cytology and FS was not wrong but too broad to characterize it as fully concordant (partial concordance). In these cases, partial concordance did not affect the management of case.
- Intraoperative final diagnosis based on a combination of squash cytology and FS was not confirmed by final histopathological diagnosis (discordant).

Data collected were statistically analyzed using SPSS software (version 25) and compared between the intraoperative diagnostic procedures (squash smears and FS) and the final histopathological diagnosis.

Results

A total of 1,453 cases were received at our department for intraoperative evaluation during the study period, out of which 283 (19.5%) cases were CNS lesions. Out of the 283 cases, 10 cases were excluded from the study as tissue was exhausted after squash/FS preparation. Hence, a total of 273 cases were included in the study, of which 154 (56.4%) patients were male and 119 (43.6%) were female. The age of the patients ranged from 02 to 81 years, the mean age being 44.7 ± 18.4 years. Majority of the lesions were supra-tentorial, 250 (91.6%), and 23 (8.4%) were infra-tentorial. Most cases presented in the 4th to 7th decades (156/273, 57.1%). There were 24 (8.8%) pediatric cases. In the pediatric age group, medulloblastoma was the most frequent tumor, 11 (45.8%), and among the adults, glioblastoma (GBM; $n = 90$, 36.1%) was most frequently diagnosed tumor in the present study.

Histomorphological Spectrum of Central Nervous System Lesions

Out of the total 273 cases, 261 (95.6%) cases were found to be neoplastic lesions and 12 (4.4%) were nonneoplastic. Astrocytic and oligodendroglial tumors, ependymomas, meningiomas, tumors of sellar region, medulloblastoma, mesenchymal tumors, tumors of peripheral nerve, neuronal-glial tumors, lymphomas, and metastasis were the neoplastic lesions constituting 56.77% (155/273), 2.56% (7/273), 13.18% (36/273), 3.29% (9/273) 6.95% (19/273), 1.46% (4/273), 4.2% (10/273), 0.42% (1/273), 1.83% (5/273), and 6.22% (17/273), respectively. The nonneoplastic lesions composed of benign cysts, inflammatory lesions, reactive gliosis, and normal brain parenchyma constituting 0.84% (2/273) each, and vascular lesions and inflammatory lesions constituting 1.26% (3.273) each as shown in ►Table 1.

Degree of Correlation

Intraoperative final diagnosis based on combination of squash cytology and FS was correlated with the final histopathological diagnosis in each case. The overall diagnostic accuracy was 95%.

Complete correlation was present in 69.96% (191/273) cases, 20.1% (55/273) cases showed partial correlation, and 9.89% (27/273) cases were discordant with histopathological diagnosis as shown in ►Table 2.

Out of the 27 discordant cases, misclassification of tumor type was the most common category (11 cases, 40%), followed by grading mismatch (7 cases, 25.9%) and misdiagnosis of tumor versus nontumor conditions (9 cases, 33.3%) as shown in ►Table 3. In the misclassification category, the most distinct pitfall was in the diagnosis of lymphomas, which were misdiagnosed as high-grade gliomas by intraoperative techniques. Grading of astrocytic tumors was the major cause of discordance in the grading mismatch category in which five low grade astrocytomas were diagnosed as high grade and two high-grade astrocytomas diagnosed as low-grade gliomas. In the tumor versus nontumor category, five out of nine cases were diagnosed as normal or reactive tissues, which were confirmed as tumors on histopathological examination as shown in ►Table 3. In four cases, intraoperative diagnosis was of tumor; however, the final diagnosis turned out to be reactive or inflammatory.

The category of partial correlation lesions included those which were correctly categorized broadly as high grade or low grade but could not be further characterized or those tumors which were categorized broadly as round blue cell tumors or spindle cell neoplasm without further characterization.

Discussion

Intraoperative pathological preparations (cytology/FS) are an integral part of neurosurgical pathology.[11] The ideal intraoperative technique used should be rapid, accurate, and should allow the preservation of tissue for paraffin section study.[9] Although FSs give the best architectural details,[12,13] the innate soft nature and high water content of the brain tissue often render poor-quality FSs with freezing artifacts leading to interpretative errors. Hence, it is often complemented by alternative methods such as squash smears and touch imprint cytology.[14,15]

"Squash smears" are technically easier to prepare without necessity of special equipment and requirement of a very small amount of tissue. Variable smear thickness, crushing artifacts, and difficult smearing are some significant drawbacks of squash smears.[9] Soft lesions such as astrocytomas, oligodendrogliomas, and pituitary adenomas smear well. Lesions such as meningiomas and schwannomas are difficult to smear due to the increased fibrous component or calcification, often rendering smears of poor quality.[14–16] "Frozen sections" are better in diagnosing firmer lesions and offer better architectural features as compared with cytology. In the present study, the drawbacks of one technique were overcome by complementing with the other technique. In this study, squash smears yielded good cytomorphological details in cases of astrocytomas,

Table 1 Histomorphological spectrum of lesions with number of concordant, partially concordant, and discordant results

Type of lesion	Final diagnosis on HPE	N (%)	Concordant (n)	Partial correlation (n)	Discordant (n)
Diffuse astrocytic oligodendroglial tumors	Pilocytic astrocytoma WHO Gr I	6 (2.5)	3	3	0
	Pilomyxoid astrocytoma WHO Gr I	3 (1.3)	3	0	0
	Diffuse astrocytoma WHO Gr II	30 (1.8)	22	6	4
	Pleomorphic xanthoastrocytoma WHO Gr II	1 (0.42)	0	0	1
	Anaplastic astrocytoma WHO Gr III	9 (3.79)	7	1	1
	GBM WHO Gr IV	90 (37.9)	83	4	3
	Small cell GBM WHO Gr IV	1 (0.42)	0	1	0
	Giant cell GBM WHO Gr IV	1 (0.42)	0	1	0
	Gliosarcoma WHO Gr IV	6 (2.6)	4	2	0
	Oligodendroglioma WHO Gr II	4 (1.7)	2	2	0
	Anaplastic oligodendroglioma WHO Gr III	3 (1.3)	1	2	0
	Subependymal giant cell astrocytoma	1 (0.42)	0	0	1
Ependymomas	Classical ependymoma	3 (1.3)	2	1	0
	Myxopapillary ependymoma	2 (0.84)	1	1	0
	Anaplastic ependymoma WHO Gr III	2 (0.84)	0	2	0
Meningeal tumors	Angiomatous meningioma	1 (0.42)	1	0	0
	Atypical meningioma	8 (3.4)	2	6	0
	Anaplastic meningioma	1 (0.42)	0	1	0
	Fibroblastic meningioma WHO Gr I	3 (1.3)	3	0	0
	Psammomatous meningioma WHO Gr I	1 (0.42)	0	0	1
	Transitional meningioma WHO Gr I	7 (2.9)	7	0	0
	Meningothelial meningioma WHO Gr I	15 (6.3)	15	0	0
Tumors of sellar Region	Pituitary adenoma	5 (2.1)	3	0	2
	Pituicytoma	1 (0.42)	0	1	0
	Craniopharyngioma	2 (0.84)	2	0	0
	Granular cell tumor	1 (0.42)	0	0	1
Embryonal tumor	Medulloblastoma	17 (6.2)	4	12	1
Tumors of peripheral nerves	Schwannoma	10 (4.2)	7	3	0
Mesenchymal nonmeningothelial tumors	Hemangioblastoma	1 (0.42)	0	0	1
	Solitary fibrous tumor/hemangiopericytoma	1 (0.42)	0	1	0
	Ewing sarcoma	2 (0.84)	0	2	0
Neuronal and mixed neuronal–glial tumor	Ganglioglioma	1 (0.42)	1	0	0
Lymphoma	B cell-NHL	2 (0.84)	0	0	2
	DLBCL-AGC	1 (0.42)	1	0	0
	High-grade NHL	1 (0.42)	1	0	0
	Primary CNS lymphoma	1 (0.42)	0	0	1
Metastasis	Metastatic adenocarcinoma	6 (2.5)	5	1	0
	Papillary adenocarcinoma	1 (0.42)	1	0	0
	Metastatic carcinoma	8 (3.4)	6	2	0
	Poorly differentiated carcinoma	1 (0.42)	0	0	1
	Metastatic deposits of high-grade sarcoma	1 (0.42)	0	0	1

(Continued)

Table 1 (Continued)

Type of lesion	Final diagnosis on HPE	N (%)	Concordant (n)	Partial correlation (n)	Discordant (n)
Inflammatory lesions	Inflammatory granulation tissue	1 (0.42)	1	0	0
	Infarction	1 (0.42)	0	0	1
Cystic lesions	Epidermoid cyst	2 (0.84)	2	0	0
Vascular lesions	Cavernoma	2 (0.84)	0	0	2
	Benign vasoformative lesion	1 (0.42)	0	0	1
Reactive lesions	Exuberant reactive microglial proliferation	1 (0.42)	0	0	1
	Gliosis	2 (0.84)	1	0	1
Miscellaneous	Benign brain tissue	2 (0.84)	2	0	0
Total		273	191	55	27

Abbreviations: CNS, central nervous system; GBM, glioblastoma; HPE, holoprosencephaly; NHL, non-Hodgkin lymphoma; WHO, World Health Organization.

Table 2 Degree of agreement of intraoperative (squash + FS) diagnosis (gold standard being HPE diagnosis on final FFPE section) (N = 273)

Degree of agreement	Frequency (percentage)
Completely same diagnosis (concordant cases)	191 (70%)
Partial agreement in diagnosis	55 (20.1%)
No agreement in diagnosis (discordant cases)	27 (9.9%)

Abbreviations: FFPE, final formalin fixed paraffin embedded; FS, frozen section; HPE, holoprosencephaly.

meningothelial variant of meningioma, oligodendroglioma, medulloblastoma, metastatic carcinomas, and pituitary adenoma, while FSs fared well in fibroblastic meningioma and schwannoma. These findings were in accordance with studies by Rao S et al,[17] Shukla K et al,[18] and Savargaonkar and Farmer.[19] However, various limitations were still noted in both the techniques. Categories of discrepancies were noted in the study.

Problem in Tumor Grading in Astrocytoma
Incorrect assessment of grading of astrocytomas was seen in seven cases. Four cases of diffuse astrocytoma WHO Gr II and one case of subependymal giant cell astrocytoma WHO Gr I were diagnosed as high-grade glioma due to enhanced aniso-nucleosis, which was possibly a result of freezing artifact incorrectly resembling high-grade tumor. One case of GBM and one case of anaplastic astrocytoma were diagnosed as low-grade glioma probably due to sampling error from the peritumoral areas. This problem of either over-grading or under-grading of astrocytomas has also been reported by other authors as well.[14,20–22] Most of the studies have attributed this to the heterogeneity of astrocytomas. Improper grading of astrocytic neoplasms in cytologic preparations has also been documented in other studies.[15,16,23] According to some, it is inappropriate to attempt to grade CNS neoplasms on small

biopsy material whether by squash smear or frozen technique as astrocytomas are known for their heterogeneity. In some studies, paraffin sections showed that in under-graded cases, there were areas of both high- and low-grade types and cytological sampling might have failed to show the anaplastic areas.[15,16]

Differentiating the Coagulative Tumor Necrosis of Glioblastoma from Inflammation-Associated Necrosis
One case of GBM was misdiagnosed as necrotizing lesion due to sampling error. In this case, the necrotic areas were sampled and both the crush smears and FS yielded only necrotic tissue with very few viable cells. The case was diagnosed as GBM in subsequent FFPE sections where extensive areas of coagulative necrosis were seen, along with viable malignant cells. Chand et al, Mitra et al, and Plesec and Prayson also reported similar findings in their studies.[11,24,25]

Misclassification of Tumors
An evaluation of the 11 discordant cases in this category revealed various pitfalls, which led to misdiagnosis. One case of B-cell non-Hodgkin lymphoma (B-NHL) was diagnosed as high-grade glioma. In this case, the squash smear showed cellular clusters of atypical cells (►**Fig. 1A**) and FS showed cellular tumor with dispersed cell population (►**Fig. 1B**) thereby rendering a diagnosis of high-grade glioma. However, the final FFPE section revealed cellular tumor with cells arranged in sheets having prominent perivascular arrangement. (►**Fig. 1C, D**) These tumor cells were CD20-positive (►**Fig.1E**) with a K$_i$ 67 index of 60 to 70% (►**Fig. 1F**), finally confirming a B-NHL. Similarly, one case of primary CNS lymphoma was misinterpreted as high-grade glioma and one case of poorly differentiated carcinoma was reported as high-grade NHL. It may be hard to distinguish high-grade gliomas from lymphomas on FS having artifacts. Cytological smear preparation is recommended when suspecting a case of lymphoma. However, malignant cells in squash smear spread over an abundant gliofibrillary background to simulate a glioma.[26] In such scenarios, the background showing presence

Table 3 Categories of discrepancies between intraoperative and histopathological diagnosis

Category (%)	Intraoperative diagnosis	HPE diagnosis	No. of cases
Misclassification (40.7%)	High-grade glioma	B-NHL	1
	Poorly differentiated carcinoma	B-NHL	1
	Metastasis from poorly differentiated Ca	GBM WHO Gr IV	1
	Cystic lesion lined by endothelial cells	Cavernoma	1
	High-grade glioma	Medulloblastoma WHO Gr IV	1
	High-grade glioma	Psammomatous meningioma WHO Gr I	1
	Metastasis from poorly differentiated carcinoma	Metastatic high-grade sarcoma	1
	Meningioma	Pituitary adenoma	1
	Meningioma	Pleomorphic xanthoastrocytoma WHO Gr II	1
	High-grade NHL	Poorly differentiated carcinoma	1
	High-grade glioma	Primary CNS Lymphoma	1
Grading mismatch (25.9%)	Low-grade glioma	Anaplastic astrocytoma WHO Gr III	1
	High-grade glioma	Diffuse astrocytoma WHO Gr II	4
	Low-grade glioma	GBM WHO Gr IV	1
	High-grade glioma	SEGA WHO Gr I	1
Tumor vs. nontumor (33.3%)	Low-grade glioma	Cavernoma	1
	Low-grade glioma	Benign vasoformative lesion	1
	Metastasis favored over high-grade glioma	Exuberant reactive microglial proliferation	1
	Gliosis	Diffuse astrocytoma WHO Gr II	1
	Low-grade glioma	Granulomatous lesion	1
	Necrotizing lesion	GBM WHO Gr IV	1
	Normal brain tissue	Granular cell tumor	1
	Normal brain tissue	Hemangioblastoma	1
	Normal brain tissue	Pituitary adenoma	1
Total			27

Abbreviations: CNS, central nervous system; GBM, glioblastoma; NHL, non-Hodgkin lymphoma; WHO, World Health Organization.

of lymphoglandular bodies in squash cytology helps in lymphoma diagnosis.[27] A case of poorly differentiated carcinoma was also interpreted as high-grade glioma as squash smears showed cellular clusters of atypical cells (►**Fig. 2A**) and FS revealed a cellular tumor with dispersed atypical cell population and increased vascularity. (►**Fig. 2B**) The final paraffin section showed a cellular tumor in sheets and papillary pattern with fibrovascular cores, thereby confirming a poorly differentiated carcinoma. (►**Fig. 2C**, **D**). In this case the cellularity, atypia and vascularity lead to the misdiagnosis of high-grade glioma. Few other analytical errors included a case each of GBM and metastatic sarcoma diagnosed as metastatic deposits of poorly differentiated carcinoma. Medulloblastoma and psammomatous meningioma were diagnosed as high-grade gliomas. These interpretative errors resulting in misdiagnoses were attributed to increased vascularity and nuclear atypia with freezing artifacts compounding difficulties encountered. One case each of pituitary adenoma and pleomorphic xanthoastrocytomas was misinterpreted as meningioma The interpretative errors in these two cases were mainly due to

the freezing process that distorted the architecture, with variable section thickness and uneven staining obscuring the cytologic details and influencing subjective judgment.

Tumor versus Nontumor

One case each of hemangioblastoma, pituitary adenoma, and granular cell tumor were reported as normal brain tissue and one case of diffuse astrocytoma WHO Gr II was diagnosed as gliosis. Sampling error is one of the most common causes of this discrepancy. Thorough sampling and adequate smears and sections are recommended to reduce intraoperative discrepancies.[28] Similarly, exuberant reactive microglial proliferation in one case was misinterpreted as metastasis and a cavernoma and arteriovenous malformation (AVM) were diagnosed as low-grade glioma. The squash smears of AVM showed increased cellularity in a gliofibrillary background (►**Fig. 3A**) supported by mildly increased cellularity on FS (►**Fig. 3B**) misinterpreting it as low-grade glioma. The final FFPE section revealed a benign vascular lesion with varying sized blood vessels with
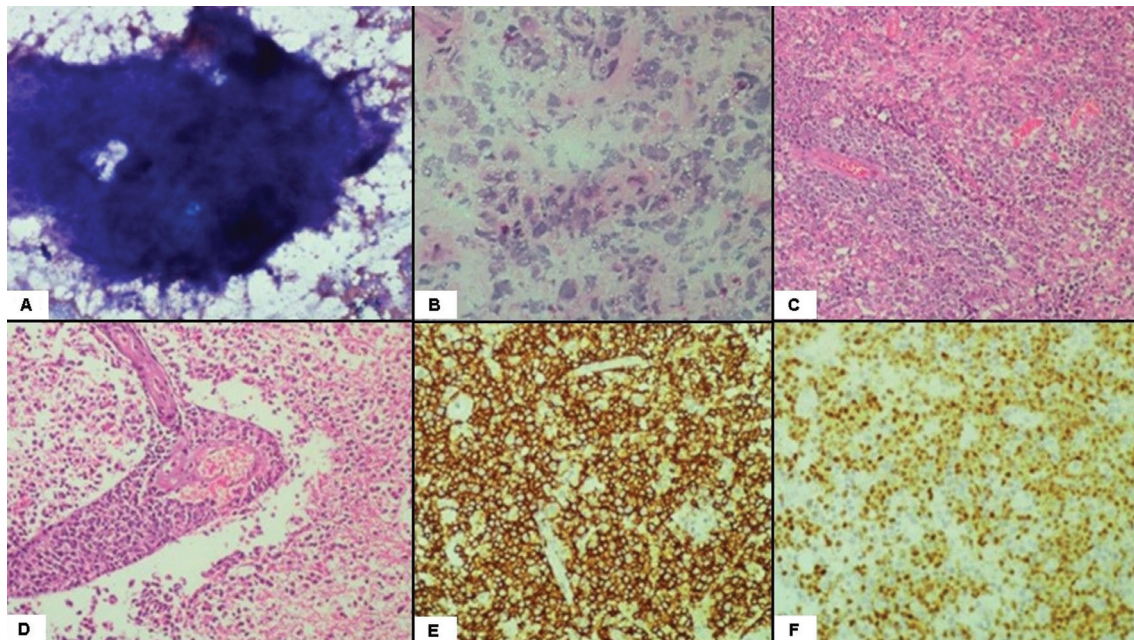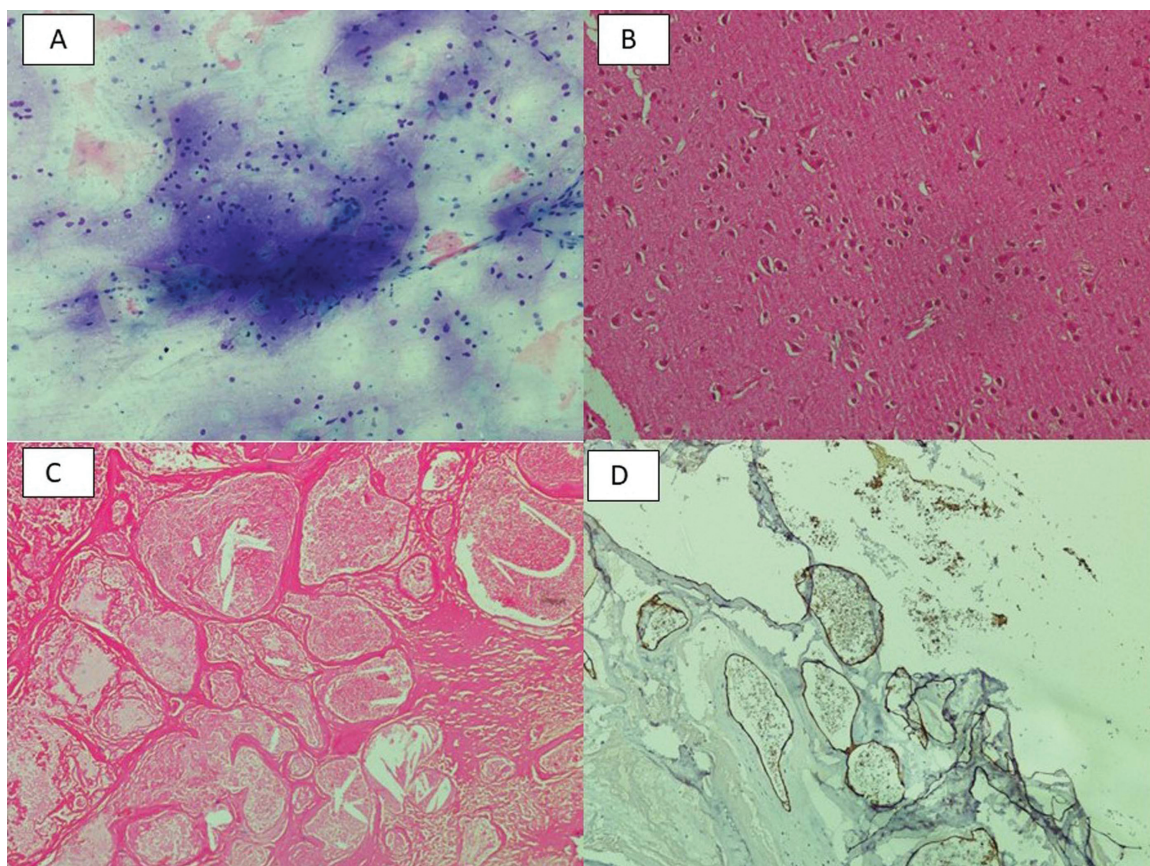
Fig. 1 Case of primary CNS lymphoma reported as high grade glioma; photomicrographs of squash smear, frozen section, and final paraffin section. (A) Photomicrograph of squash smear showing a cellular cluster of atypical cells (Leishman Giemsa stain, 40×). (B) Photomicrograph of frozen section showing a cellular tumor with dispersed cell population (hematoxylin and eosin [H&E] stain, 40×). (C, D) Photomicrograph of final paraffin section showing cellular tumor with cells arranged in a sheet (H&E stain, 10×) and prominent perivascular arrangement (H&E Stain, 20×). (E) Tumor cells showing diffuse membranous positivity for CD 20 immunostain (IHC, 20×). (F) K$_i$ 67 is high (60–70%); (IHC stain, 20×). CNS, central nervous system; IHC, immunohistochemistry.
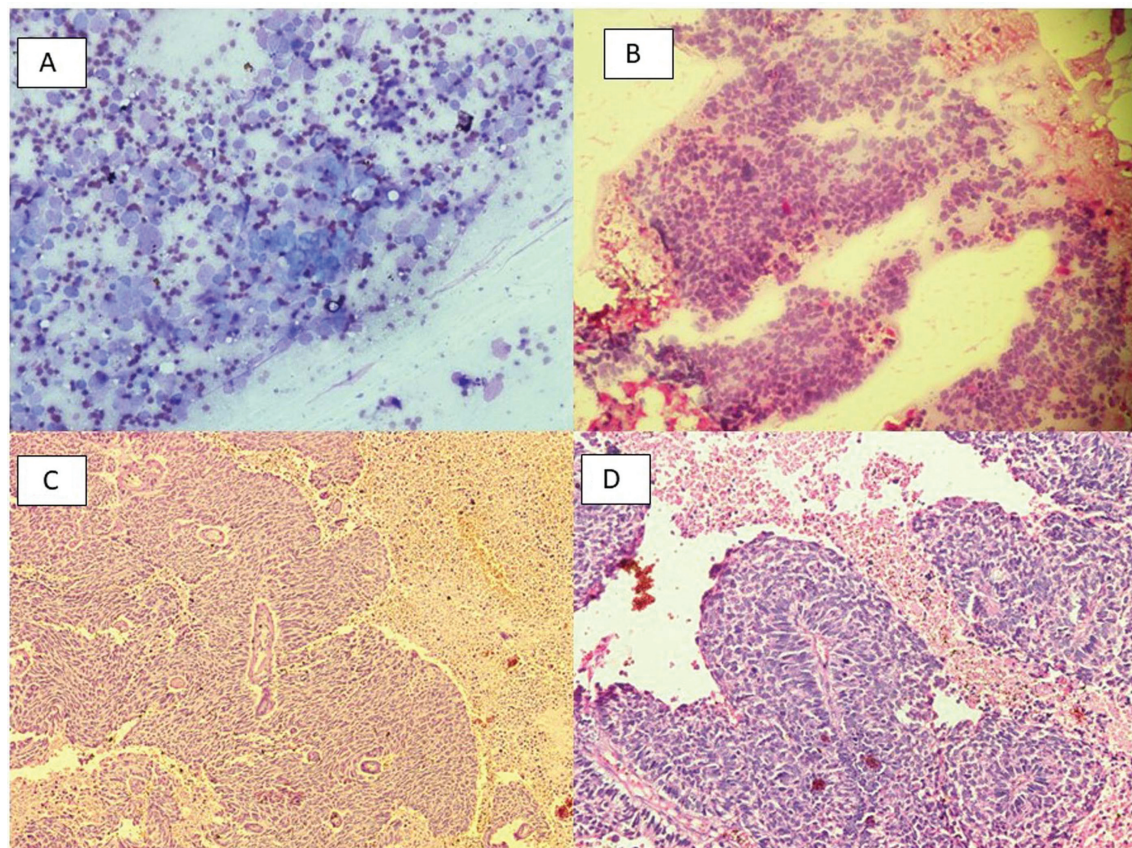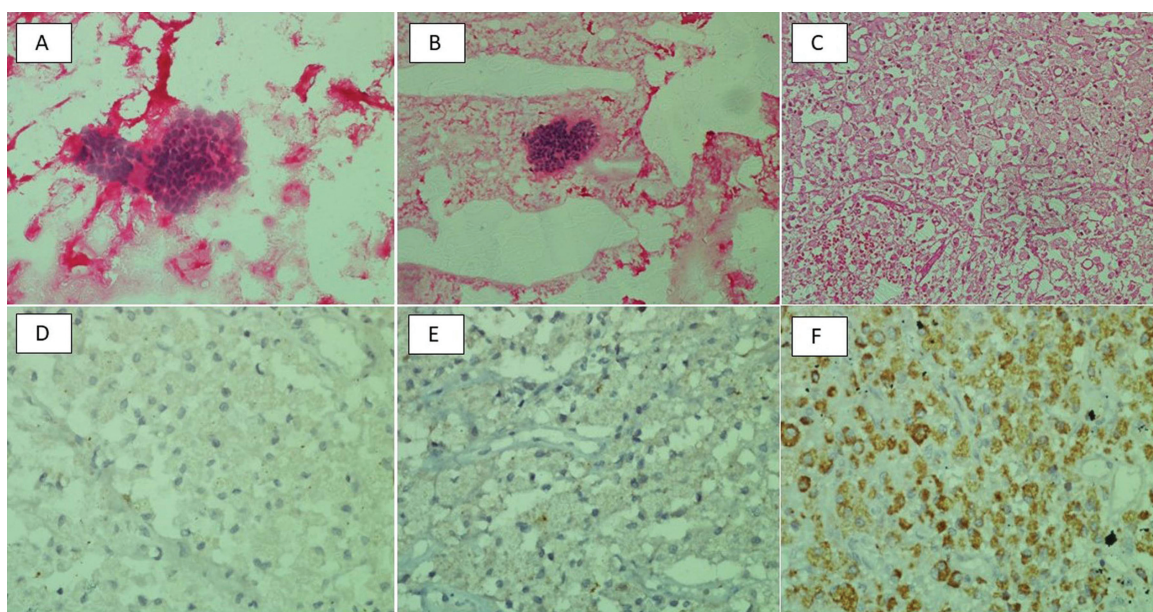

Fig. 2 Case of poorly differentiated carcinoma reported as high-grade glioma; photomicrographs of squash smear, frozen section, and final paraffin section. (A) Photomicrograph of squash smear showing a cellular cluster of atypical cells (Leishman Giemsa stain, 40×). (B) Photomicrograph of frozen section showing a cellular tumor with dispersed cell population (hematoxylin and eosin [H&E] stain, 40×). (C) Photomicrograph of final paraffin section showing cellular tumor with cells arranged in a sheet (H&E stain, 10×) and papillary architecture (H&E Stain, 20 ×). (D) High-power view showing a papillary architecture with well-defined fibrovascular core (H&E Stain, 40×).

Fig. 3 Case of AVM reported as low-grade glioma; photomicrographs of squash smear, frozen section, and final paraffin section. (A) Photomicrograph of squash smear showing cellular cluster of atypical cells arranged in a glial background suggestive of low-grade glioma (Leishman Giemsa stain, 40×). (B) Photomicrograph of frozen section showing a fragment of brain parenchyma with mild increase in cellularity (hematoxylin and eosin [H&E] stain, 40×). (C) Photomicrograph of final paraffin section showing lesion composed of varying sized blood vessels with surrounding reactive gliosis (H&E stain, 10×). (D) CD 34 immunostain highlights endothelial cells of these blood vessels (IHC Stain, 40×). AVM, arteriovenous malformation.

Fig. 4 A case of cerebral infract reported as metastatic deposit of carcinoma; photomicrographs of squash smear, frozen section, and final paraffin section. (A) Photomicrograph of squash smear showing a small cellular cluster of atypical cells with surrounding necrosis (H&E, 40×). (B) Photomicrograph of frozen section also showing a small cellular cluster of atypical cells with surrounding necrosis (H&E stain, 40×). (C) Photomicrograph of final paraffin section showing cellular tumor with cells arranged in sheets of large cells with abundant vacuolated cytoplasm (H&E stain, 10×). (D–F) These atypical cells were negative on GFAP immunostain (4D, IHC, 20×), negative on EMA immunostain (4E, IHC, 20×), and were positive for CD68 immunostain (4F, IHC, 20×).

surrounding reactive gliosis (►**Fig. 3C**). The endothelial cells were highlighted by CD34 immunostain (►**Fig. 3D**). A case of cerebral infarction was interpreted as metastatic deposits. A small cellular cluster of atypical cells with surrounding necrosis (►**Fig. 4A**) was noted on squash smears with similar observation on FS (►**Fig. 4B**). The final paraffin section shows sheets of large cells with abundant vacuolated cytoplasm (►**Fig. 4C**) which were immunonegative for Glial Fibrillary Acid Protein (GFAP) (►**Fig. 4D**), Epithelial Membrane Antigen (EMA) (►**Fig. 4E**), and immunopositive for CD68 (►**Fig. 4F**). A diagnosis of infarction was confirmed. In this case, the most probable explanation was interpreting histiocytes as epithelial cells due to their size and abundant cytoplasm.

Overall, the results of the present study were comparable with those of other studies. In present study intraoperative final diagnosis based on a combination of squash cytology and FS showed overall 95% diagnostic accuracy, which is comparable to other studies by Rao et al[17] and Di Stefano et al[20] where they achieved diagnostic accuracies of 94 and 95.29%, respectively, when both these procedures were used together. Other authors[14,29] also advocated the same and recommended the use of both squash preparation and FS for intraoperative diagnosis. While cytomorphology is preserved in crush smears, the FS, though lacking the cytological details, preserves the tissue architecture.

The strength of the study was the large sample size and the fact that all cases of discordance category were traced and re-evaluated to determine the possible cause for variation in the result or interpretation. The limitation of the study was inherent to its retrospective nature and unavailability of data on the progress of the patients after surgery.

Conclusion

The role of intraoperative consultation is of paramount importance and it remains a rapid and reliable tool for assessing CNS lesions intraoperatively. Communication between the surgeon and pathologist with complete clinical and neuroimaging details is imperative. Our study shows that squash cytology in conjugation with FS shows a high percentage of accuracy in arriving at intraoperative diagnosis for intracranial lesions. Regular audits of discordant cases should be conducted by surgeons and pathologists as part of a quality assurance measure to sensitize themselves with the potential pitfalls minimizing misinterpretation and helping in providing a more conclusive opinion to the operating surgeons.

Contributions
K.R. and P.S.M., specialist and epidemiologist, supervised the manuscript and performed the data analysis. V.S., pathologist, conceptualized and prepared the manuscript. M.Y., junior resident, collected data and prepared the draft manuscript. P.S., pathologist, conceptualized and prepared the manuscript. R.T., pathologist, conceptualized and supervised the manuscript. P.S.M., pathologist, supervised the manuscript.

Conflicts of Interest
The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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