Original Article

Diagnostic Accuracy of Squash Preparations in Central Nervous System Tumors

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ABSTRACT

Background & Objectives: **The accurate assessment of the diseased tissue is fundamental to the diagnosis and management of disorders of the central nervous system (CNS). The 'squash' or 'crush' technique has been universally employed in the intraoperative diagnosis of CNS tumors. The aim of our study was to evaluate the accuracy of squash preparation in diagnosing CNS tumors by comparing with histopathology.**

Methods: **This was a descriptive study which included 63 patients with CNS tumors from whom most of the samples were collected by craniotomy. Squash smears were made and stained with H&E, Papanicolaou & May-Grunwald Giemsa stains. Paraffin sections were made from formalin fixed tissue sent separately.**

Results: **Of 63 cases, squash cytology diagnosis correlated with histopathology in 56 cases with a diagnostic accuracy of 88.9%. A 100% accuracy was seen in pilocytic astrocytoma, anaplastic astrocytoma, glioblastoma, ependymoma, anaplastic ependymoma, choroid plexus papilloma, schwannoma, hemangioblastoma, craniopharyngioma, prolactinoma and metastases. Of the 7 cases which did not correlate with histopathology, one was a sampling error, 4 were diagnostic errors and 2 were instances of grading discrepancy.**

Conclusion: **Squash preparations are a highly effective tool in the rapid intraoperative diagnosis of CNS tumors. It is a simple, reliable, cost effective procedure which in most cases can help the operating surgeon to come to a correct decision regarding the further management of the patients.**

Key words: **Central Nervous System, Tumor, Cytology**

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Introduction

The accurate assessment of the diseased tissue is fundamental to the diagnosis and management of central nervous system (CNS) tumors (1). The 'squash' or 'crush' smear technique has been universally employed in the intraoperative diagnosis of CNS tumors. With the advent of stereotactic biopsies, there has been a significant limitation in the amount of tissue available for the diagnosis.

The smear technique permits selective examination of multiple areas from a small biopsy specimen while conserving most of the tissue for the rapid and optimal preservation and processing needed for immunohistochemistry. "Smear preparations thus eliminate the need to perform intraoperative frozen sections on tiny irreplaceable specimens, and therefore abrogate the risk of distorting or losing valuable diagnostic material" (2). As an accurate and effective method for the intraoperative evaluation of CNS biopsies, smear techniques are well established and used in many neuro-oncologic centres. These preparations are also suitable for histochemical, immunohistochemical, and fluorescence in situ hybridization techniques (2).

The smear technique as a means for rapid diagnosis in neurosurgery had been introduced by Eisenhardt & Cushing in 1930, subsequently modified by Russell *et al*. which is the currently used method (3,4).

For rapid diagnosis the neuropathologists use two procedures including frozen section technique and smear technique.

Frozen section biopsy is a costly procedure, requiring more technical expertise as well as larger and firmer tissue, and sometimes leaving hardly any tissue for final histopathological confirmation, especially in case of stereotactic biopsy procedure; whereas, smear technique is a simple, rapid, cost effective and accurate method which requires less technical expertise and a very tiny piece of tissue, even less than 0.1 cm (5). Making crush/squash preparations of such specimens is a better way to prepare the tissue for diagnostic evaluation. Squash cytology is useful in parts of the world in which frozen sections are unavailable because of lack of electricity or gas, or of trained personnel. More recently, the technique has emerged as the preferred diagnostic tool over frozen technique. It is being used to confirm or exclude the presence of neoplasia, and also to define the neoplastic cell type and grade of the tumor (6). After 1980, several reports have been published on this technique from different centers of the world. In 2002, Karl Roessler *et al*. reported from Vienna, Austria an accuracy of 95% by squash cytology (6).

In the present study, we intend to correlate squash/crush cytology diagnosis with histopathology. This study would help for providing intraoperative tissue diagnosis and hence in the early management of patients.

Materials and Methods

The present study was conducted over a period of two years from August 2008 to July 2010. The study included 63 patients with central nervous system tumors, from whom samples were collected by craniotomy or stereotactic biopsy. The inclusion criteria included all the cases of CNS tumors confirmed by CT/MRI. Infective and non-neoplastic cystic lesions were excluded. Specimens were collected in two bottles containing normal saline and 10% phosphate buffered formalin respectively. The material in saline was used for making crush preparations, while that in formalin was routinely processed and paraffin embedded for histopathological examination. Specimens for cytology received

in normal saline varied from 0.5 mm to 2.5 mm diameter according to the type of procedures which were undertaken. Each specimen was cut into tiny pieces, examined and representative tissue chosen. Smears were made by taking a small piece of tissue with a scalpel blade, which was then placed on clean, grease free glass slide and subsequently smeared with another slide holding the second slide at right angles to the first and applying uniform pressure during smearing. The smear was immediately fixed in 95% ethanol and stained with H&E and Papanicolaou stains. Additional slides were air dried and stained with May-Grunwald Giemsa stain. Paraffin sections were made from the formalin fixed tissue samples and stained by H&E. Immunohistochemical markers such as Ki-67, GFAP were used wherever necessary.

Results

65.1% (41 cases) of the tumors were located in the supratentorium, 30.2% (19 cases) were in the infratentorium and 4.7% (3 cases) were in spinal cord.

The histological types of tumors in our study along with age distribution are given in Table 1.

Table 1- Histological types and age distribution of tumors in our study

Tumor	WHO grade	<12 years	13-40 years	>40 years	Total No. of cases
Pilocytic astrocytoma	I	1	3	1	5
Diffuse astrocytoma	II		2	1	3
Anaplastic astrocytoma	III		1		1
Anaplastic gemistocytic astrocytoma	III			1	1
Glioblastoma	IV		3	1	4
Giant cell glioblastoma	IV			1	1
Ependymoma	II	1			1
Anaplastic ependymoma	III	1			1
Choroid plexus papilloma	I		2		1
Atypical choroid plexus papilloma	II				1
Pineoblastoma	II		1	1	2
Medulloblastoma	IV	2	1		3
Schwannoma	IV		8	5	13
Meningothelial meningioma	I		3	4	7
Fibroblastic meningioma	I			4	4
Transitional meningioma	I		1	1	2
Angiomatous meningioma	I		2	3	5
Cavernous angioma	I	1	1		2
Hemangioblastoma	I			1	1
Craniopharyngioma	I	1	1		2
Prolactinoma	I		1		1
Metastatic tumors				2	2
Total		7	30	26	63

Schwannomas and meningiomas were the most common WHO grade I tumors whereas glioblastoma was the most common grade IV tumor. Tumors in adults comprised 88.9% of the cases while the remainder were in children <12 years of age. 66% of medulloblastoma and 100% of ependymoma were seen in children while all schwannomas and meningiomas were seen in adults. Astrocytomas were seen to be occurring in all age groups. Most tumors occurred equally in both sexes but meningiomas were significantly more common in women.

The primary purpose of this study was to analyze the diagnostic accuracy of squash cytology with histopathology as gold standard. The histopathological diagnosis and the number of cases diagnosed correctly by cytology are presented in Table 2. Of the 63 cases, cytology and histopathology diagnosis correlated in 56 cases. Consequently, the overall diagnostic accuracy of cytological diagnosis was 88.9% (Table 2). The 7 cytologically noncorrelating cases are listed in Table 3 along with the histopathology diagnoses.

Table 2- Diagnostic accuracy with regards to cytological diagnosis

Histopathology Diagnosis	No. of cases	No. of cases correctly diagnosed by squash cytology	Diagnostic Accuracy (%)
Pilocytic astrocytoma	5	5	100
Diffuse astrocytoma	3	2	66.7
Anaplastic astrocytoma	1	1	100
Anaplastic gemistocytic astrocytoma	1	0	0
Glioblastoma	4	4	100
Giant cell glioblastoma	1	1	100
Ependymoma	1	1	100
Anaplastic ependymoma	1	1	100
Choroid plexus papilloma	1	1	100
Atypical choroid plexus papilloma	1	0	0
Pineoblastoma	2	1	50
Medulloblastoma	3	2	66.7
Schwannoma	13	13	100
Meningothelial meningioma	7	7	100
Fibroblastic meningioma	4	4	100
Transitional meningioma	2	2	100
Angiomatous meningioma	5	4	80
Cavernous angioma	2	1	50
Hemangioblastoma	1	1	100
Craniopharyngioma	2	2	100
Prolactinoma	1	1	100
Metastatic tumors	2	2	100
Total	63	56	88.9

Table 3- Cases misdiagnosed in squash smears (with histopathology as gold standard) in our study

Site	Squash diagnosis	Histopathology diagnosis
Parietal Tumor	Anaplastic Astrocytoma	Diffuse Astrocytoma
Parasellar region	Gemistocytic Astrocytoma	Anaplastic Gemistocytic Astrocytoma
4th Ventricle	Ependymoma	Atypical Choroid Plexus papilloma
Cerebellum	Lymphoma	Medulloblastoma- Nodular
Pineal Region	Papillary tumor of pineal region	Pineoblastoma
Middle Cranial Fossa	Schwannoma	Angiomatous Meningioma
Intraconal Eye Lesion	Schwannoma	Cavernous angioma

On overall analysis, it was also noted in our squash smears that the cellular details and architectural details of many tumor types were well maintained. For example, certain features like papillae (in choroid plexus papilloma; Fig. 1) and whorls (in meningiomas; Fig. 2a & 2b) were well made out in our squash smears. Besides we noted that in schwannomas, the characteristic alternating hyper and hypocellular areas were well appreciated in squash smears similar to histopathological sections.

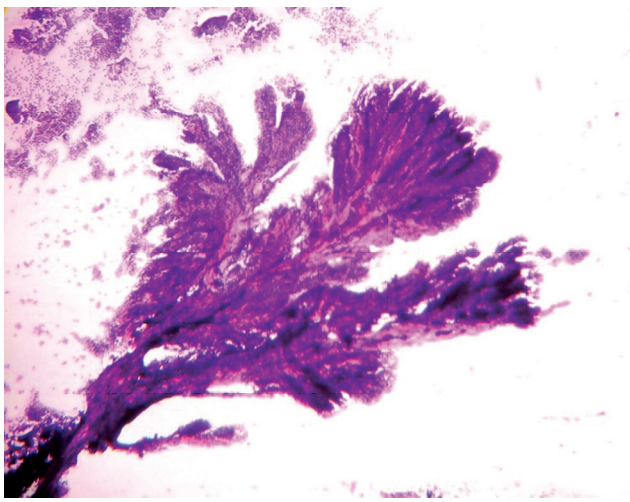

Fig. 1: Smear showing papillary fragment in choroid plexus papilloma (H&E, ×40).

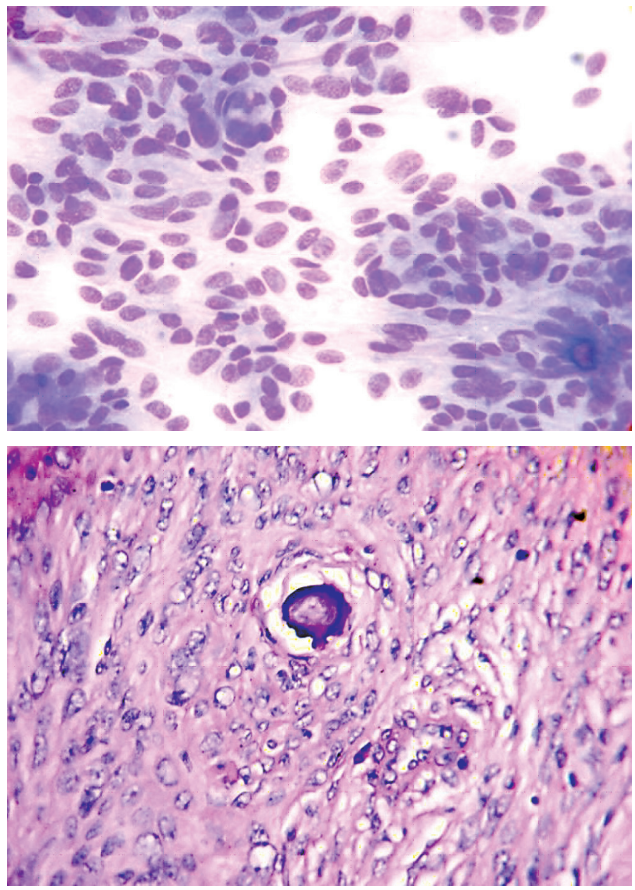

Fig. 2: A) Smear of meningothelial meningioma showing meningothelial whorls & a psammoma body (MGG, ×400), **B)** Section of meningothelial meningioma showing meningothelial cells with intranuclear inclusions & a psammoma body (H&E, ×400).

Discussion

Our diagnostic accuracy was similar to the results obtained in other standard studies as shown in Table 4 (6-12). The diagnostic accuracy (Table 2) was 100% in pilocytic astrocytoma, anaplastic astrocytoma, glioblastoma, ependymoma, anaplastic ependymoma, choroid plexus papilloma, schwannoma, hemangioblastoma, craniopharyngioma, prolactinoma and metastases which is comparable with the study by Shukla K *et al*. (7).

Table 4- Diagnostic accuracy of squash smear diagnosis (with histopathology as gold standard): A comparison of our study with other published studies

Author	No. of cases	Diagnostic Accuracy (%)
Roessler *et al*. (6)	4172	95
Shukla *et al*. (7)	278	87.76
Claude Mouriquand *et al*. (8)	292	87.5
Torres LFB *et al*. (9)	650	97.9
Firlik *et al*. (10)	595	90
Cappabianca *et al*. (11)	100	80
Malhotra *et al*. (12)	25	92
Present study	63	88.9

On squash smears, we had 15 astrocytomas, out of which 13(86.6%) correlated with histodiagnosis. Among the 2 cases of astrocytomas which did not correlate with histology (Table 3), one case was diagnosed as anaplastic astrocytoma (Grade III) on cytology due to the presence of moderate cellularity and moderate pleomorphism. However, the subsequent histopathology sections showed features of a diffuse astrocytoma with mild nuclear pleomorphism and a low proliferation index (Ki-67 <2%). The second case was diagnosed as gemistocytic astrocytoma on cytology but histology showed gemistocytic astrocytes along with nuclear pleomorphism, mitotic figures and Ki-67 was 9%, and hence a diagnosis of anaplastic gemistocytic astrocytoma was made. This discrepancy could probably be due to sampling error and difficulty in grading of astrocytic tumors on smears due to intratumoral variability of grade, variation in cellularity, pleomorphism and mitosis (6-8). Hence some authors conclude that it is not advisable to grade malignancies in small biopsy cytology (13, 14).

A case of intraventricular mass demonstrated a moderately cellular smear with perivascular rosette like structures and was labeled as ependymoma on cytology. But histology sections revealed that those rosette like structures were actually papillae, and it also showed mitotic figures (>2/10HPF), hence it was diagnosed as atypical choroid plexus papilloma. Shah AB *et al*. had reported a similar situation (13).

Two cases of medulloblastomas diagnosed on cytology correlated with histopathological diagnosis. But the third case, which was diagnosed as lymphoma on squash cytology based on the features of highly cellular smears with small round discohesive cells with high N/C ratio, nuclear hyperchromasia and structures appearing like lymphoglandular bodies, turned out on histopathology to be nodular type of medulloblastoma with typical nodular architecture, intra-nodular central neurocytoma like areas with perinodular collar of primitive hyperchromatic cells. Folkerth *et al*. have shown that medulloblastoma can simulate lymphoma on cytology (15).

One pineal gland lesion on cytology showed

features of papillary tumor of pineal region composed of tumor cells arranged in a papillary pattern, whereas histopathology showed tumor cells arranged in sheets with occasional pseudo rosettes and hence was reported as pineoblastoma. The other pineal lesion was correctly diagnosed as pineoblastoma.

One case of angiomatous meningioma was misinterpreted as schwannoma on cytology as has been pointed out earlier (16) .The absence of typical onion-shaped cell whorls and intranuclear inclusion bodies often simulated schwannomatous tissue (6). A cavernous angioma was erroneously reported as schwannoma on cytology. This is a typical example of sampling error where the nerve adjacent to the intraconal eye lesion (Table 3) was sampled for cytology which resulted in the misdiagnosis.

Conclusion

The overall diagnostic accuracy of squash cytology in our study was 88.9%. Hence, though cytology is an accurate method, it cannot replace histopathology in the diagnosis of brain tumors, but it can function as a valuable adjunct. It is an extremely useful tool in the rapid intra-operative diagnosis of CNS tumors as it is simple, reliable and cost effective which can help the operating surgeon to come to a correct decision regarding the further management of the patient. Along with accurate sampling to prevent sampling error, a highly trained neuropathologist in this area is essential. Knowledge of the various cytomorphological features is essential to give an accurate diagnosis on the tiny piece of tissue sample quickly.

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