RESEARCH ARTICLE

ROLE OF SQUASH SMEAR TECHNIQUE IN INTRAOPERATIVE DIAGNOSIS OF CNS TUMOURS

Bharti Jha, Viral Patel, Komal Patel, Anshul Agarwal
Government Medical College, Surat, Gujarat, India

Correspondence to: Bharti Jha (drbmjha@hotmail.com)

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ABSTRACT

Background: Neurosurgical practice frequently requires intraoperative consultation to optimize surgical procedure. Frozen section and Squash smear cytology can offer the same. As brain tissue is friable & predisposed to show ice crystal artefacts, frozen section is often difficult to interpret. Squash smear examination provides good cytological details to offer diagnosis in most cases except where anatomical correlation is needed. Present study was undertaken to evaluate the efficacy of Squash smear in absence of frozen section facility.

Aims & Objective: To evaluate the value of Squash smear cytology for rapid intraoperative diagnosis in CNS lesions and its correlation with final histomorphological diagnosis.

Material and Methods: Total 35 case of CNS tumours were examined by squash smear technique for cytomorphological analysis followed by histomorphological correlation on paraffin section.

Results: Complete correlation with histomorphological findings was observed in 82.35% of cases. Complete correlation was observed more with glial neoplasm.

Conclusion: Squash smear preparation proved to be a simple, inexpensive and rapid technique for intraoperative consultation of CNS tumours and can be effectively utilized as a diagnostic tool for intraoperative diagnosis in absence of frozen section facility.

Key-Words: Squash Smear Cytology; CNS Tumours; Intraoperative Diagnosis

Introduction

Primary CNS tumours are relatively infrequent in comparison with other malignant tumours.[1,2] It is estimated that the annual incidence of CNS tumours ranges from 10-17 per 1,00,000 persons for intracranial tumour and 1 to 2 per 1,00,000 persons for intraspinal tumours.[1] Neuro-epithelial tumours are proportionally more frequent in children.[3] The pathologist has two principal techniques for establishing a rapid tissue diagnosis – frozen section and squash smear examination. Frozen sections are the preferred methods especially when tissue is of firm consistency or if large piece of tissue has been obtained. However as brain tissue is predisposed to show ice crystal artefacts, its frozen section interpretation may prove difficult at times.

As most intrinsic brain tumours are of soft or gelatinous consistency, smear preparation can readily be made often revealing exquisite cytological details. The squash smear technique for brain biopsy is a very simple method whereby a rapid tissue diagnosis is possible within a few minutes of biopsy reaching the laboratory. The advantages of squash smear technology are (1) simple & rapid technique; (2) no technical expertise is required for preparation of smear; (3) provides both cytological and architectural features of CNS tumours; (4) background matrix and necrosis are easy to appreciate; (5) if sample taken from each of the small piece of tissue are smeared, the biopsy can be screened much more widely. The disadvantage of smear technique lies in the difficulty in identifying the tumour where architectural relationships are important or tumours those are too firm to smear.

In the present study effort was made to find out utility of squash smear technique for rapid intraoperative diagnosis of CNS lesions.

Materials and Methods

A total 35 clinically diagnosed and suspected cases of CNS tumours were selected. All the cases underwent neurosurgery at department of a tertiary care hospital. In each case, a detailed clinical and radiological history was taken. Tissue

obtained intraoperatively from all cases was subjected to squash technique.

In the operation theatre, tissue sample obtained by open biopsy from the different parts of the lesion was collected on a gel pad or wet cotton pad. Care was taken not to allow tissue to dry. Tissue was carefully inspected to see the presence of apparent necrosis or haemorrhage or both. Small 2-3 mm sized tissue bit was taken on one end of a glass slide. With the help of another glass slide pressure was applied over tissue to crush and was smeared on it. The amount of force required to smear was observed which varied depending on the consistency of tissue sample. In most cases it was possible to prepare 6-8 smears at least. Out of which half were immediately fixed in methanol for staining with H&E. Remaining smears were air-dried and were stained with MGG (May-Grunwad-Giemsa) stain. Smears were mounted with DPX.

Gross and naked eye examination was done to evaluate nature of spread. All smear were examined under scanner to evaluate cellularity and were further examined under low and high magnification for cytomorphological evaluation. All tissue were further processed for paraffin section method when possible.

Results

In all 35 cases intraoperative squash smear preparation, detail clinical history, gross examination findings, cytological findings of H&E and MGG stained smears and final histopathological diagnosis were recorded. The youngest patient was 1 year old and the oldest was 70 years old. The maximum no. of patients were in the age group of 31-40 years, 19.95% (7 cases) and 51-60 years, 19.95% (7 cases) followed by 21-30 years, 17.10% (6 cases). The male: female ratio observed was almost 1:1 (51% males and 49% females)

In majority of the cases (85.7%) lesions encountered were situated intracranially, while in 5 cases (14.29%) were of intraspinal origin. Among 35 cases, 21 cases (60%) were solid, 3 cases (8.5%) were cystic and 11 cases (31.5%) were having solid & cystic areas together.

In the present study (Table 1) tumours of neuroepithelial tissue were most common (42.85%), followed by 17.14% of meningeal tumours, 14.29% of tumours of cranial and spinal nerves, 11.42% cystic lesions, 5.7% local extensions from regional tumours, 2.85% germ cell tumour , 2.85% tumour of sellar region and 2.85% tumour of uncertain histogenesis .

Considering histological diagnosis, meningioma (20%) was most common tumour followed by high grade astrocytoma (14.27%) and glioblastoma (14.27%). Out of 35 cases, 48.6% were grade 1 tumours, 25.7% grade 4 tumours, 11.4% grade 2 tumours and 14.27% grade 3 tumours.

Histopathological correlation was possible in all cases except one case in which biopsy material was insufficient for processing. Out of 34 cases correct diagnosis (complete correlation) was offered in 28 cases (82.35%). In 4 cases (11.76%), cytological diagnosis partially correlated with histopathological examination. In 2 cases (5.88%) correlation was not possible. Complete correlation was observed more frequently with glial neoplasms. Benign conditions like meningioma, schwannomas offered least complete correlation as compared to other.

Table-1: Case Distribution among Various WHO Categories of CNS Tumours

WHO Classification			No.	%
Neuroepithelial Tumours	Astrocytic Tumour	Low grade Astrocytoma	2	5.85
		High grade Astrocytoma	5	14.27
		Glioblastoma	5	14.27
	Ependymal Tumours (Ependymoma)		1	2.85
	Embryonal Tumours (Primitive Neuroectodermal Tumours)		1	2.85
Tumours Meninges	Meningioma		7	20
	Atypical Meningioma		1	2.85
Tumours of Cranial and Spinal Nerves	Schwannoma		4	11.42
	Germ cell Tumour		1	2.85
Tumours of Sellar Region (Craniopharyngioma)			1	2.85
Cystic Lesions	Epidermoid Cyst		1	2.85
	Mucocele		1	2.85
	Arachnoid Cyst		1	2.85
	Hematoma		1	2.85
Local Extension of Regional Tumours	Rhabdomyosarcoma of skull		1	2.85
	Squamous cell Carcinoma		1	2.85
	Follicular Carcinoma of Thyroid		1	2.85

Discussion

In the present study (Table 1) tumours of neuroepithelial tissue were most common

comprising of 42.85%, followed by 17.14% of meningeal tumours, 14.29% of tumours of cranial and spinal nerves, 11.42% cystic lesions, 5.7% local extensions from regional tumours, 2.85% germ cell tumour, 2.85% tumour of sellar region and 2.85% tumour of uncertain histogenesis. Considering histological diagnosis, meningioma (20%) was most common tumour followed by high grade astrocytoma (14.27%) and glioblastoma (14.27%). Out of total 35 cases, 48.6% were WHO grade 1 tumours, 25.7% WHO grade 4 tumours, 14.27% WHO grade 3 tumours and 11.4% WHO grade 2 tumours.

Surgical techniques for diagnosis of brain lesions have evolved over the past few decades. Intra operative consultations about the pathology of CNS lesions are requested to differentiate neoplastic lesion from reactive, to differentiate metastatic neoplasm from primary, to grade malignancy, to determine tumour margins (e.g. in low grade astrocytoma) and to obtain tissue for culture or other special procedures. The variable thickness of the smear offers an advantage. In the peripheral thin portion the cytological details of the whole cell can be visualized, while the central thick portion provides the general architectural detail especially the tumour cell relation with the vessel and the vascular change.[6] Squash smear technique can be effectively utilized for intra operative consultation baring few conditions where anatomical correlation is required e.g. differentiation from reactive gliosis.

In the present study, 80% of CNS tumours encountered were between the age of 16 and 59 years which was comparable with Korean study who observed about 70% in the same age group.[7] Glioblastoma was the common tumour in older patients while germ cell tumours showed a definite predilection for children. Male: Female ratio in the present study was found 0.75:1 which was comparable with Korean group (0.9:1) and Amarati et al (0.75:1). In the present study 85.29% tumours were intracranial and 14.21% were spinal. The Andrews et al and KNP study found 88.66% intracranial, 13.34% spinal and 93.5% intracranial, 6.5% spinal respectively. Meningioma was the commonest (22.85%) tumour encountered in our study. Comparable findings were reported by KNP study[7] (24.1%) and CBTRUS study(24%).[9] Astrocystic tumours were the 2nd most common tumour observed in the present study (20.12%). Jerzy et al also reported it as 2nd most common tumour in his study. For craniopharyngioma and embryonal tumours, results were comparable to KNP study who reported 3.7% and 2.7% respectively.

Astrocytic tumours were completely correlated histologically in present study with an average of 95%. Differentiation of low grade astrocytoma and normal cerebral tissue on cytology is difficult sometimes because the astrocytes display minimal to no anaplasia and are loosely dispersed in a delicate fibrillary background. Out of 10 malignant glial tumours 5 cases (50%) were reported as glioblastoma, 4 cases (40%) were reported as high grade astrocytoma and in one case suspicion of anaplastic meningioma was offered which was confirmed on paraffin section. It was noted that presence of necrosis and marked endothelial proliferation were in favour of the diagnosis of glioblastoma rather than high-grade astrocytoma. In one case, cytological diagnosis of high-grade astrocytoma was offered which on received biopsy was from the surrounding tissue showing reactive gliosis with possible non-representative lesion. The cytological diagnosis of the reactive gliosis should be made when there is higher cellularity than normal with a predominance of the astrocytic cells, some of which have slightly irregular nuclei but delicate chromatin. It is important to note that in gliotic smears there will be other glial cells, such as oligodendrocytes, between proliferative and reactive astrocytes. This type of gliotic smears may be seen in periphery of metastatic carcinoma (as seen in one of our case), Craniopharyngioma and brain abscess. Therefore, the presence of a gliotic smear points to abnormal tissue, making it desirable to ask neurosurgeon to send a new and larger specimen in case of clinical deference.

In present study benign tumours were completely correlated in 75% (12 cases) while partial correlation was obtained in 12.5% (2 cases). Significant partial correlation was noted in benign tumours like meningioma and schwannoma. One case was reported as nonglial tumour that was confirmed as angiomatous meningioma on final diagnosis. Another case reported as nonglial

tumour with neural elements was confirmed as schwannoma on final diagnosis. Thus reporting a squash smear as "glial" or "nonglial" appears to be quite justifiable. Correlation with data like clinical history and imaging study helps in narrowing down the diagnosis. It might be possible that clear cut differentiation between types of meningioma may not be possible on cytology (as in our case).

Germ cell tumour and vascular tumour like hemangioblastoma showed complete correlation in our case in present study. This was comparable with other studies.

Conclusion

Squash smear technique proves to be an equally helpful technique for intraoperative consultation of CNS tumours if clinical and radiological findings are properly correlated in the absence of frozen section facility. And it may prove to be an adjuvant technique to frozen section for guiding the clinician to optimize surgery.

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