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Role of Squash Cytology in Central Nervous System Lesions: A Cytomorphological Study

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Abstract

Background: Intra-operative cytology preparation was first introduced by Eisenhart and Cushing in early 1930s. With the advent of CT and MRI guided stereotactic biopsies, smear technique has gained importance because of technical simplicity and good preservation of cytological and nuclear details at low expense. *Aims:* 1. To study various patterns of central nervous system [CNS] lesions in squash cytology and histopathology. 2. To correlate cytological diagnosis with histopathology. 3. To evaluate the diagnostic utility of intra-operative squash cytology. *Settings and Study Design:* Analytical study at tertiary care hospital. *Material and Methods*: Hundred cases were analyzed. Squash smears were prepared from biopsy samples sent in isotonic saline. The smears were stained by Hematoxylin and Eosin [H &E] and Papaniculaou [PAP] stain. Histopathological evaluation was done subsequently from biopsy samples sent in formalin. Special stains were performed in selected cases. Cyto-morphological features were correlated with histopathology. *Statistical Analysis:* Frequencies, Chi-Square Test and Crosstabs were used for calculation. *Results:* Histopathological diagnosis of hundred cases included neoplastic lesions [90%] and non-neoplastic lesions [10%]. Correct diagnosis was achieved by squash cytology in 68 cases [73.11%] by complete correlation. However, diagnostic accuracy improved considerably [84.94%] after applying partial correlation criteria. For the detection of neoplastic lesions, squash cytology had sensitivity of 95.29%, specificity of 75% and efficacy of 93.54%. The p value, determining efficacy of squash cytology for detecting CNS neoplasms was statistically significant [$p<0.005$]. *Conclusions:* Squash cytology is a reliable, rapid and inexpensive intra-operative diagnostic tool. It has high sensitivity and is highly efficacious procedure.

Keywords: Neurosurgical; Squash Cytology; Histopathology; Intra-Operative; Diagnostic Accuracy.

Introduction

Space occupying lesions [SOLs] in the cranial cavity is known to mankind since 1774. Three decades ago, it was frequently stated that brain tumors were uncommon in Indians. With the development of recent investigative techniques, it has become obvious that brain tumors are as common in India as elsewhere[1].

In neurosurgical practice, rapid diagnosis of SOLs of central nervous system [CNS] helps neurosurgeon to confirm the presence of tumor in the minute tissue specimen, to plan the extent of surgery and modify it accordingly [2,3]. Smear technique has gained importance because of technical simplicity and good preservation of cytological and nuclear details at low expense [2,3,4].

Intra-operative squash cytological diagnosis is fairly accurate, safe, simple and reliable tool for rapid diagnosis of CNS lesions. It is preferred method as it offers a great detail of cellular morphology, avoiding distortion and ice artifacts often introduced by frozen section [2-9]. Cytological data from smear preparation are sufficient for intraoperative diagnosis and often

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provide complementary information to paraffin embedded tissue sections [10,11]. Hence, squash preparation technique has been employed universally in rapid diagnosis of CNS lesions [12].

The current study was undertaken to evaluate the utility of squash smear technique for intraoperative cytomorphological diagnosis, correlate with histopathological diagnosis and determine the diagnostic accuracy.

Material and Methods

All patients with space occupying lesions of CNS, admitted under neurosurgery were evaluated intra-operatively by squash cytology and subsequently by histopathological examination in the department of pathology. The study was conducted from January 2010 to June 2011 retrospectively and July 2011 to September 2012 prospectively for a total period of two years and eight months. The study was approved by Institutional Ethics Committee. One hundred cases were analysed.

Inclusion Criteria

All neurosurgical cases for which squash smears and subsequent histopathological examination were performed.

Exclusion Criteria

Those neurosurgical cases for which squash cytology was not satisfactory for interpretation, those cases in which subsequent histopathological examination was not performed and those cases in which tissues sent for squash cytology were not from the lesions of CNS or its covering.

Tissue sample sent in isotonic saline were utilized to make smears for cytological evaluation by squash technique as described by Adams et al [13]. Alcohol fixed smears were stained routinely by Hematoxylin and Eosin [H & E] and rapid Papanicolaou [PAP] method. In selected cases, air-dried smears were stained by May Grunwald Geimsa [MGG] stain. Special stains [Ziehl-Neelsen (ZN), Periodic Acid Schiff (PAS)] were employed in selected cases. Tissue samples sent in 10% formalin were allowed to fix for 24 hours. Gross examination was done and bits were given. Paraffin embedded sections were routinely stained by H & E. Special stains [PAS, reticulin] were employed in selected cases.

Cytomorphological features were compared with those of histopathology. All primary CNS tumors were histologically categorised according to 2007 WHO [World Health Organization] classification [14,15]. Those lesions not included under WHO classification were categorised as unclassified lesions. Available clinical details, radiological and laboratory investigation data were documented.

Statistical Analysis

Frequencies, Chi-Square Test and Crosstabs were used for calculation. Cytological diagnosis was compared with histopathological diagnosis and the efficacy of squash cytology was evaluated. All statistical calculations were done through SPSS 16.0 for windows.

Results

Out of 100 cases, 90 cases [90%] were neoplastic lesions constituting the majority and 10 cases [10%] were non-neoplastic lesions. CNS lesions ranged from 1 yr to 75 yr. Lesions were most common in third and fourth decade [mean = 44.58 yrs]. Females were affected in majority of cases [59%] with M:F ratio of 1:1.4. CNS lesions were more common in intracranial region [86%] than spinal cord region [14%]. The distribution of various neoplastic and non-neoplastic CNS lesions, WHO gradable primary neoplasms and their diagnostic accuracies are enumerated in Table 1 and Table 2. Statistical values were calculated by Galen and Gambino method. For the detection of neoplastic lesions, squash cytology had 81 true positive cases, two false positive cases, four false negative cases, six true negative cases, sensitivity of 95.29%, specificity of 75%, false positive error rate of 2.15%, false negative error rate of 4.3%, positive predictive value of 97.59%, negative predictive value of 60% and efficacy of 93.54%. The p value, determining efficacy of squash cytology for detecting CNS neoplasms was statistically significant [$p<0.005$].

Cytomorphological Features of Neoplastic Lesions of CNS

Neuroepithelial Tumors

Glioblastoma: Smears had a ragged, cellular edge that trailed off as a gradient of partially attached clumps [steel wool appearance]. Smears showed tumors cells in sheets, clusters and singles. Tumor cells were highly pleomorphic with hyperchromatic to vesicular nuclei with prominent nucleoli. Also seen were clustering of tumor cells around blood vessels,

bizarre tumor giant cells, fibrillary background, necrosis, endothelial proliferation and calcification [one case].

Gliosarcoma: Showed two populations of tumor cells composed of pleomorphic glial cells having scant fibrillary cytoplasm and hyperchromatic nuclei; and spindle cells having pleomorphic spindle shaped nuclei with moderate eosinophilic cytoplasm.

Pilocytic Astrocytoma: Smearing pattern was that of pulled cotton appearance [Figure 1]. Smears showed tumor cells in sheets, clusters and singles. Tumor cells had uniform elongated nuclei with piloid processes. Also seen were Rosenthal fibers [Figure 2]. Tumor cells were clustered around blood vessels. Background was fibrillary. Histopathological features are shown in Figure 3. One case showed calcific bodies. Eosinophilic granular body [EGB] was seen in two cases.

Fibrillary Astrocytoma: Showed tumor cells in sheets and clusters. Tumor cells had scant cytoplasm, perinuclear rim with ill-defined cell borders and enlarged hyperchromatic round to oval to elongated nuclei with coarse chromatin. Tumor cells were clustered around blood vessels.

Gemistocytic Astrocytoma: Showed sheets of polygonal tumor cells with abundant intensely eosinophilic cytoplasm and eccentric hyperchromatic nuclei. Background was fibrillary and short glial processes were seen.

Subependymal Giant Cell Astrocytoma: showed tumor cells in sheets, clusters and singly dispersed. Tumor cells were large, pleomorphic, round to polygonal with abundant fibrillary eosinophilic cytoplasm and eccentric nucleus with fine chromatin and prominent nucleoli. Also seen was clustering of tumor cells around blood vessels.

Anaplastic Astrocytoma: Smears showed tumor cells in sheets and clusters. Tumor cells were pleomorphic and had elongated, large oval nuclei with scanty cytoplasm. Also seen was clustering of tumor cells around blood vessels and few mitotic figures.

Medulloblastoma: Smears had smooth gradient with patchier edges. Nodular medulloblastomas showed tumor cells in sheets and scattered diffusely. Cells were pleomorphic round to oval with scant cytoplasm. [Figure 4] Also seen were apoptotic bodies, nuclear streaking and nuclear moulding. Histopathological features are shown in Figure 5. Anaplastic medulloblastoma showed large tumor cells with abundant cytoplasm and pleomorphic nuclei with prominent nucleoli.

Oligodendrogliomas: Showed loosely cohesive tumor cells in sheets and clusters in an ill-defined eosinophilic matrix. Tumor cells were monomorphic, small, round to oval with scant cytoplasm and round to oval lobulated nuclei. Also seen were many delicate vessels in fibrillary background. Anaplastic oligodendroglioma showed pleomorphic round to oval tumor cells having scant cytoplasm and round to oval hyperchromatic nuclei. Also seen was vascular proliferation and fibrillary background.

Oligoastrocytomas: Showed foci of oligodendroglial cells and astrocytes with enlarged hyperchromatic nuclei.

Ependymoma: Showed tumor cells in sheets, clusters and rosette-like pattern. Tumor cells were oval to elongated having round to oval nuclei with salt and pepper chromatin and fibrillary cytoplasm with tapering processes. Myxopapillary ependymoma showed tumor cells in sheets, clusters, papillary pattern and rosettes. Tumor cells were oval to elongated cells having round to oval nuclei with salt and pepper chromatin and fibrillary cytoplasm. Tumor cells were arranged around the myxoid globules. Background showed myxoid material.

Ganglioglioma: Showed tumor cells in sheets, clusters and in singes. One population of tumor cells were astrocytes while the other population was that of ganglion cells [Figure 6], some of which were dysplastic showing binucleation. Small round cells with hyperchromatic nucleus and scant cytoplasm were seen. Also seen were specks of calcification. Histopathological features are shown in Figure 7.

Meningeal Tumors

Smears had wide bridges of tissue spanning between the larger clumps of cells and spicules of cells radiate out from the cellular clumps.

Meningotheliomatous meningioma showed tumor cells in sheets, clusters and singles. Tumor cells were large, polygonal to round cells having round to oval pale nuclei with peripheral margination of chromatin and moderate amount of eosinophilic cytoplasm with ill defined cytoplasmic borders. Few cells showed intranuclear inclusions and mild nuclear atypia. Also seen were occasional psammoma bodies. Fibroblastic meningioma showed tumor cells in fascicles, whorls, clusters and singles. Tumor cells were large polygonal to spindle shaped showing nuclear and cytoplasmic features similar to that of meningotheliomatous meningiomas. Also seen were few psammoma bodies. Transitional meningioma showed features of both meningotheliomatous and fibroblastic meningioma. Psammmomatous meningioma showed features of

transitional meningioma with numerous psammoma bodies. Atypical meningioma showed tumor cells in sheets. Large Tumor cells with pleomorphic nuclei and ill defined cytoplasmic borders. Few mitotic figures were noted.

Hemangioblastoma showed tumor cells in clusters and singles. Tumor cells were large, polygonal having foamy cytoplasm and hyperchromatic uniform nuclei. Also seen were numerous endothelial cells and fusiform cells with oval nuclei.

Hemangiopericytoma showed tumor cells in fascicles, clusters and singles. Tumor cells were large spindle shaped having oval to elongated spindled hyperchromatic nuclei, mild nuclear atypia and moderate amount of eosinophilic cytoplasm with ill defined cytoplasmic borders.

Cranial and Paraspinal Nerve Tumors

Schwannoma: Smears had cellular fragments squished out, but heaps of tissue rolled out onto the edges of the smear. Smears showed tumors cells in fascicles and clusters. Fascicles showed right angled crisscrossing and frayed ends. Tumor cells were spindle shaped having oval to elongated hyperchromatic nuclei, nuclear streaking and eosinophilic cytoplasm.

Neurofibroma: Sparsely cellular smears showed occasional singly scattered spindle shaped tumor cells. Tumor cells had elongated hyperchromatic nuclei.

Pituitary Neoplasms

Smears produced even gradients of cells. Smears showed tumor cells in diffuse sheets, clusters and singles. Tumor cells were small, round to oval cells having moderate to scant eosinophilic cytoplasm and uniform round to oval central nuclei with inconspicuous nucleoli.

Adamantinomatous Craniopharyngiomas

Smears showed tumor cells in clusters, sheets and singles. Tumor cells were round to polygonal having moderate eosinophilic cytoplasm and round to oval vesicular nucleus. Also seen was eosinophilic acellular material.

Metastatic Carcinomas

Metastatic adenocarcinoma: Primary tumors were from intestine in one case and from breast in another case. However primary tumors could not be determined in other two cases. Smears showed tumor cells in clusters and glandular pattern. Tumor cells were large round to cells having eosinophilic vacuolated cytoplasm and pleomorphic vesicular nuclei and prominent nucleoli.

Metastatic squamous cell carcinoma: Showed tumor cells in clusters and sheets. Tumor cells were large polygonal to oval cells having abundant eosinophilic cytoplasm and pleomorphic vesicular nuclei and prominent nucleoli. However, the site of primary tumor could not be determined.

Primary CNS Lymphoma

Central region of smear showed an even gradient of tumor cells. Smears showed tumor cells in diffuse sheets and singles. Tumor cells were monotonous round to oval cells having round to oval hyperchromatic nucleus with scanty cytoplasm. Also seen were nuclear streaking, mitotic figures and apoptotic bodies.

Chordoma

Smears showed tumor cells in clusters. Tumor cells were polygonal to round having moderate eosinophilic vacuolated cytoplasm and pleomorphic nuclei with prominent nucleoli. Background showed myxoid material.

Cytomorphological Features of Non-Neoplastic Lesions of CNS

Epidermoid cyst: Poorly cellular smears showed sparse nucleated squamous cells and abundant acellular eosinophilic material. Also seen were few anucleated squamous cells.

Ratkes Cleft Cyst: Poorly cellular smears showed a cluster of cuboidal cells and abundant acellular material.

Tuberculoma: Poorly cellular smears showed scattered epithelial cells against a background of caeseous necrosis.

Acute Inflammatory Lesion: Showed acute inflammatory cell and few suspicious hyphal structures.

Arteriovenous Malformation: Showed reactive astrocytes against hemorrhagic background.

Necrotic Lesion: Showed necrotic material, reactive astrocytes and oligodendrocytes in loosely cohesive clusters and singles.

Diagnostic accuracy was good in meningeal tumors, cranial and paraspinal nerve tumors, pituitary neoplasms, metastatic tumors and haematopoietic tumor. It was intermediate for neuroepithelial

Table 1: Diagnostic accuracy of space occupying lesions of CNS

SL. No.	Histopathology	Cases [n=90]	Diagnosis Offered [n=85(94.44%)]	DACC	DAPC
	Neoplastic Lesions				
	Neoplastic Lesion	90	85	62[72.94%]	73[85.88%]
1.	Neuroepithelial Tumors	36	33	17[51.51%]	26[78.78%]
A	Astrocytoma	23	22	12[54.54%]	15[68.18%]
	Glioblastoma	10	9	5[55.55%]	6[66.66%]
	Pilocytic astrocytoma	4	4	2[50%]	2[50%]
	Fibrillary astrocytoma	3	3	3[100%]	3[100%]
	Gemistocytic astrocytoma	2	2	0	1[50%]
	Gliosarcoma	2	2	1[50%]	1[50%]
	Anaplastic astrocytoma	1	1	0	1[100%]
	Subependymal giant cell astrocytoma	1	1	1[100%]	1[100%]
B	Embryonal Tumors	4	3	3[100%]	3[100%]
	Anaplastic medulloblastoma	2	2	2[100%]	2[100%]
	Nodular medulloblastoma	1	1	1[100%]	1[100%]
	Primitive neuroectodermal tumor	1	0	NA	NA
C	Oligodendroglial tumor	3	3	0	3[100%]
	Anaplastic oligodendroglioma	2	2	0	2[100%]
	Oligodendroglioma	1	1	0	1[100%]
D	Ependymal tumor	3	2	2[100%]	2[100%]
	Ependymoma	2	1	1[100%]	1[100%]
	Myxopapillary ependymoma	1	1	1[100%]	1[100%]
E	Oligoastrocytic tumor	2	2	0	2[100%]
	Oligoastrocytoma	1	1	0	1[100%]
	Anaplastic oligoastrocytoma	1	1	0	1[100%]
F	Neuronal and mixed neuronal tumor	1	1	0	1[100%]
	Ganglioglioma	1	1	0	1[100%]
2	Meningial tumors	22	21	19[90.47%]	21[100%]
	Meningotheiomatous	9	9	9[100%]	9[100%]
	Fibroblastic meningioma	3	3	3[100%]	3[100%]
	Transitional meningioma	3	2	1[50%]	2[100%]
	Psammomatous	2	2	2[100%]	2[100%]
	Hemangiobastoma	2	2	2[100%]	2[100%]
	Atypical meningioma	1	1	1[100%]	1[100%]
	Angiomatous meningioma	1	1	1[100%]	1[100%]
	Hemangiopericytoma	1	1	0	1[100%]
3	Cranial and paraspinal nerve tumors	15	14	12[85.75%]	12[85.75%]
	Schwannoma	14	14	12[85.75%]	12[85.75%]
	Neurofibroma	1	0	NA	NA
4	Pituitary neoplasms	7	7	7[100%]	7[100%]
5	Metastatic	5	5	5[100%]	5[100%]
	Adenocarcinoma	4	4	4[100%]	4[100%]
	Squamous cell carcinoma	1	1	1[100%]	1[100%]
6	Sellar tumors	2	2	1[50%]	1[50%]
	Adamantinomatous craniopharyngioma	2	2	1[50%]	1[50%]
7	Lymphoma and haematopoietic tumors	1	1	1[100%]	1[100%]
	High grade lymphoma	1	1	1[100%]	1[100%]
8	Unclassified Tumors	2	2	0	0
	High grade tumor	1	1	0	0
	Chordoma	1	1	0	0
	Non-Neoplastic Lesions				
	Non-neoplastic Lesions	10	8[80%]	6[75%]	
1	Cystic	5	5	5[100%]	
	Ratkes cyst	1	1	1[100%]	
	Epidermoid cyst	4	4	4[100%]	
2	Inflammatory	3	1	1[33.33%]	
	Tuberculoma	2	0	NA	
	Acute inflammatory lesion	1	1	1[100%]	
3	Vascular	1	1	0	
	Arterio – venous malformation	1	1	0	
4	Unclassified	1	1	0	
	Necrotic	1	1	0	

DACC: Diagnostic Accuracy by Complete Correlation, **DAPC:** Diagnostic Accuracy after Considering Partial Correlation
The term "Complete correlation" was applied to the cases in which intra-operative diagnosis was exactly identical to the final histopathological diagnosis. "Partial correlation" was applied to cases in which +/- 1 grade of deviation in tumor grading was registered or when the diagnosis of the cell line of origin, mainly in glial tumor was not possible. "No correlation" was considered in cases where intra-operative diagnosis differed from the final histological diagnosis.

Table 2: Diagnostic accuracy in WHO grades of primary neoplastic lesions of CNS

Sl. No	Who Grade of Primary CNS Neoplasms	Cases [N=74]	Diagnosis Offered [N=69(93.24%)]	DACC [49(71.01%)]	DAPC [60(86.95%)]
1	GRADE I	44	42	35[83.33%]	37[88.09%]
2	GRADE II	10	9	5[55.55%]	9[100%]
3	GRADE III	4	4	0	4[100%]
4	GRADE IV	16	14	9[64.28%]	10[71.42%]

Table 3: Comparison of distribution of various space occupying lesions of CNS

Sl. No	Authors	Cases	Neuroepithelial Tumors	Meningial Tumors	Cranial and Paraspinal Nerve Tumors	Pituitary Neoplasms	Sellar Tumors	Hemopoetic Tumors	Metastatic	Non Neoplastic
1	Roessler K et al[18]	4172	1402 [33.60%]	559[13.4%]	199 [4.8%]	417 [10%]	40 [1%]	97[2.3%]	518[11.7%]	93 [2.3%]
2	Deshpande et al[2]	250	110 [44%]	45[18%]	25 [10%]	13 [5.46%]	8 [3.2%]	3 [1.2%]	6 [2.4%]	27 [10.8%]
3	Shah AB et al[12]	140	95 [67.85%]	21 [15%]	13 [9.28%]	15 [10.7%]	3 [2.1%]	1 [0.7%]	7 [5%]	2[1.4%]
4	Mitra S et al[17]	114	55 [48.24%]	26[22.80%]	15 [13.15%]	2 [1.8%]	1 [0.9%]	0	4 [3.6%]	2 [1.8%]
5	Kini JR et al[19]	100	47 [47%]	26 [26%]	13 [13%]	4 [4%]	3 [3%]	1 [1%]	1 [1%]	3[3%]
6	Malhotra V et al[20]	25	18 [72%]	0	0	0	0	0	1 [4%]	3 [12%]
7	Present study	100	36 [36%]	22 [22%]	15 [15%]	7 [7%]	2 [2%]	1[1%]	5 [5%]	10 [10%]

Table 4: Misdiagnosed cases in space occupying lesions of CNS

Sl. No.	Histopathology	Diagnosis Offered	Misdiagnosed	Cytology	Reason
	Neoplastic Lesions				
	Neoplastic	85	23[27.05%]		
1	Neuroepithelial Tumors	33	16[48.48%]		
A	Astrocytoma	22	10 [45.45%]		
	Pilocytic	4	2 [50%]	1.Glioblastoma	Pleomorphic glial cells, EGB
				2.Meningioma	Calcification, degenerative changes
	Gemistocytic astrocytoma	2	2 [100%]	1.Oligodendroglioma	Discohesive round cells, micro-calcifications.
				2. Necrosis	Non-representative
	Anaplastic	1	1 [100%]	1.Glioblastoma	Pleomorphic tumor cells, necrosis
	Glioblastoma	9	4 [44.44%]	1.No tumor	Mixed inflammatory cells
				2.Gliosarcoma	Spindle shaped cells
				3. Inflammatory lesion	Mixed inflammatory cells, necrosis
				4. Subependymal astrocytoma	Large tumor cells
	Gliosarcoma	2	1 [50%]	Schwannoma	Spindle shaped cells
B	Oligodendroglial tumor	3	3 [100%]		Pleomorphic cells
	Oligodendroglioma	1	1[100%]	1.Oligoastrocytoma	Surrounding normal astrocytes
	Anaplastic oligodendroglioma	2	2[100%]	1.Astrocytoma [III/1V]	Pleomorphic glial cells
				2.Glioblastoma	Pleomorphic glial cells, Necrosis
C	Oligoastrocytic tumor	2	2 [100%]		
	Oligoastrocytoma	1	1[100%]	1.Glioma [IV]	Pleomorphic astrocytes,
	Anaplastic oligoastrocytoma	1	1[100%]	2.Anaplastic astrocytoma	Pleomorphic astrocytes
D	Ganglioglioma	1	1[100%]	Dysembryoplastic neuroepithelial tumor	Morphological similarity lymphocytes
2	Meningial tumors	21	2[9.52%]		
	Hemangiopericytoma	1	1[100%]	Meningioma grade [II]	Morphological similarity

	Transitional meningioma	3	1[33.33%]	Atypical meningioma	Nuclear atypia and occasional mitoses
3	Cranial and paraspinal nerve tumors	14	2[14.28%]		
	Schwannoma	14	2[14.28%]	1.High grade glial tumor 2. Meningioma	Pleomorphic cells Morphologically similar spindle cells
4	Sellar tumors	2	1[50%]		
	Craniopharyngioma	2	1[50%]	Colloid cyst	Poor cellularity, eosinophilic acellular material
5	Unclassified Tumors	2	2[100%]		
	High grade tumor	1	1[100%]	Oligoastrocytoma	Reactive glial cells–non representative
	Chordoma	1	1[100%]	Craniopharyngioma	Polygonal cells
	Non-Neoplastic Lesions				
	Non-neoplastic Lesions	8	2[25%]		
1	Vascular	1	1[100%]		
	Arterio–venous malformation	1	1[100%]	Astrocytoma	Fibrillary background, endothelial cells
2	Unclassified	1	1[100%]		
	Necrosis	1	1[100%]	Oligoastrocytoma	Reactive gial cells

Table 5: Non-diagnostic cases in neoplastic and non-neoplastic space occupying lesions of CNS

Sl. No.	Lesions	Non-Diagnostic Cases [n=7 (7%)]	Reasons
1	**Neoplastic Lesions**	**5 [5.55%]**	
A	Neuroepithelial Tumors	**3[8.33%]**	
	Astrocytoma	1[4.34%]	
	Glioblastoma	1 [10%]	Large areas of necrosis
	Ependymal tumors	1 [33.33%]	
	Ependymoma	1 [50%]	Poor cellularity
	Embryonal tumors	1 [25%]	
	PNET	1 [100%]	Inflammatory cells and large areas of necrosis
B	**Meningial tumors**	**1 [4.54%]**	
	Meningothelial meningioma	1[11.11%]	Poor cellularity
C	**Nerve tumors**	**1 [6.66%]**	
	Neurofibroma	1 [100%]	Poor celluarity
2	**Non-neoplastic Lesions**	**2 [20%]**	
A	Infammatory Lesion	2[66.66%]	
B	Tuberculoma	2[66.66%]	Few lymphocytes and necrosis, scattered epitheliod cells. Special stain not contributory

Table 6: Comparison of diagnostic accuracy in space occupying lesions of CNS

Sl. No.	Authors	Cases	Complete	Partial
1	Roessler K et al[18]	4172	89.80%	95%
2	Ghosal N et al[22]	306	93%	
3	Deshpande et al[2]	250	91.10%	Increased
4	Asha T et al[25]	178	87.20%	
5	Iqbal M et al[24]	151	95.36%	
6	Sharma N et al[16]	149	89.30%	
7	Shah AB et al[12]	140	89.70%	
8	Mitra S et al[13]	114	88.50%	
9	Kini JR et al[19]	100	86%	98%
10	Sarvargoankar et al[26]	103	94%	
11	Qureshi IA et al[27]	94	93.30%	
12	Pawar NH et al[23]	50	88%	
13	Malhotra V et al[20]	25	92%	
14	Present Study	100	73.11%	84.94%

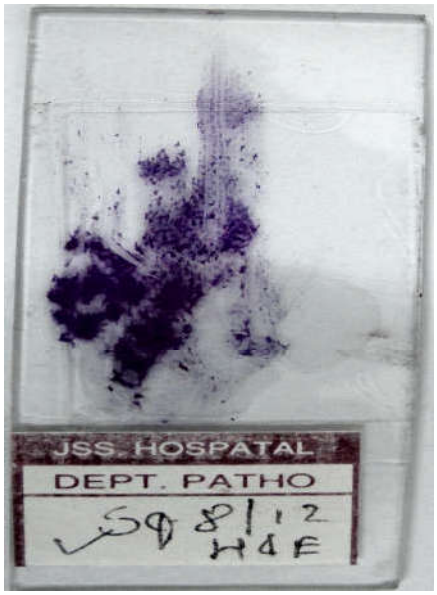

Fig. 1: Photograph of smear of pilocytic astrocytoma - showing pulled cotton appearance

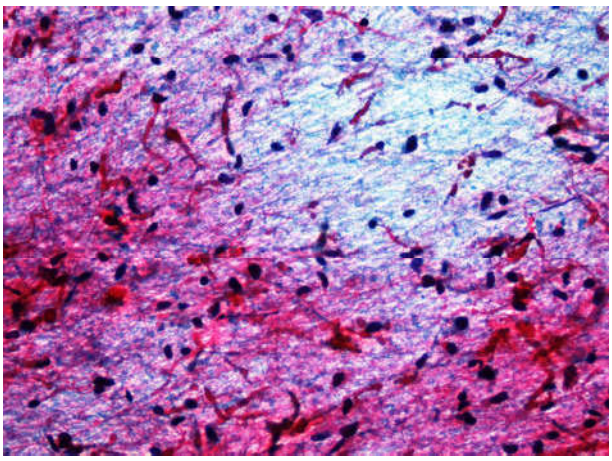

Fig. 2: Microphotograph of squash smear of pilocytic astrocytoma - showing piloid astrocytes and Rosenthal fibres in a fibrillary background. [PAP, X200]

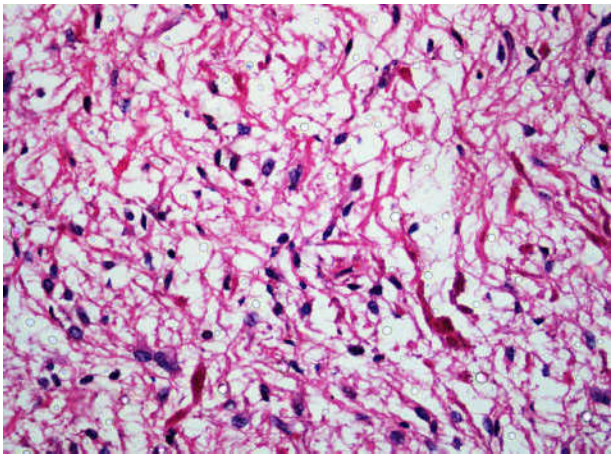

Fig. 3: Microphotograph of tissue section of pilocytic astrocytoma - showing piloid astrocytes and Rosenthal fibres in a fibrillary background. [H&E, X200]

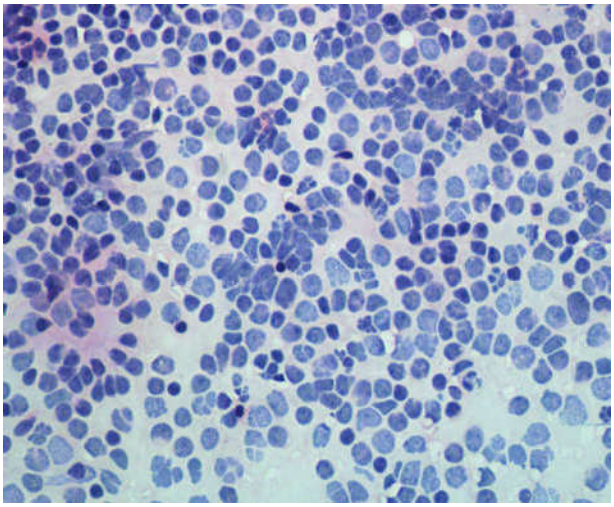

Fig. 4: Microphotograph of squash smear of medulloblastoma - showing tumor cells with ill defined cell borders and large hyperchromatic angulated nuclei. [H&E, X400]

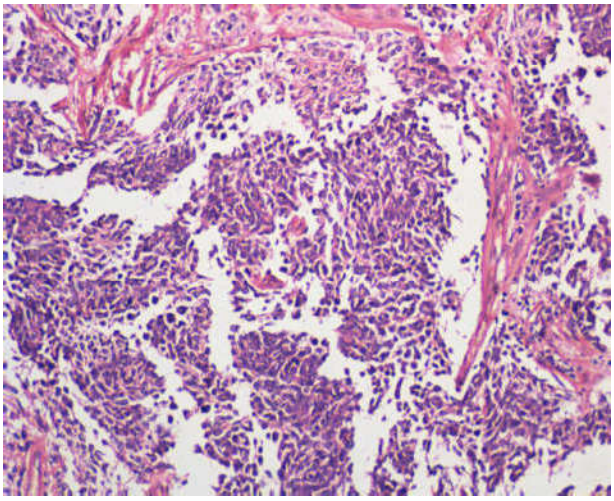

Fig. 5: Microphotograph of tissue section of medulloblastoma showing - tumor cells in nodules separated by fibrous septa. [H&E, X100]

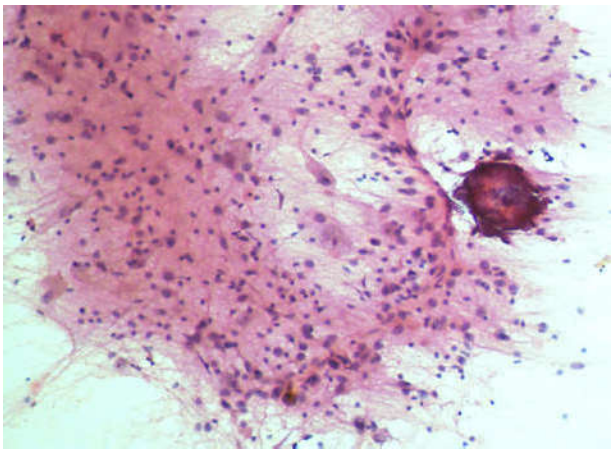

Fig. 6: Microphotograph of squash smear of ganglioglioma - showing dual population of cells composed of ganglion cells and glial cells in a fibrillary background with an area of calcification. [H&E, X200]

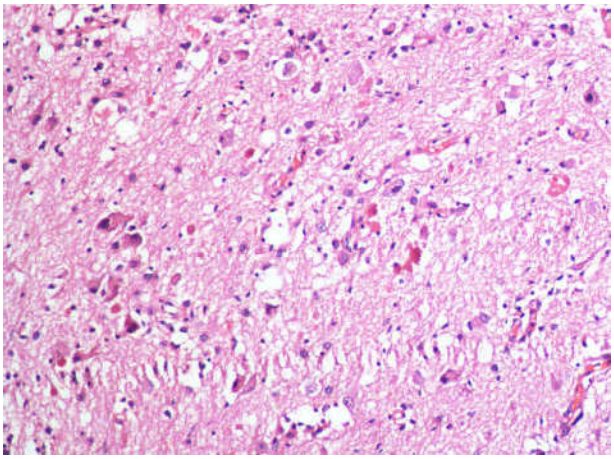

Fig. 7: Microphotograph of tissue section of ganglioglioma - showing mixed population of cells composed of ganglion cells and glial cells in a fibrillary background. [H&E, X100]

neoplastic lesions and sellar tumors and was poor in unclassified neoplasms.

Discussion

CNS lesions are the most challenging domains for the neurosurgeon. Making a diagnosis of CNS lesions is difficult on the basis of clinical and radiological findings only. Cytological and/ or histological diagnosis is required for confirmation and proper management [16]. Primary or metastatic neoplasia involving CNS is unique in being potentially life threatening due to rise in intracranial pressure and involvement of eloquent structures [17].

The distribution pattern of various CNS lesions in the present study was compared with other studies. In all the studies, neoplastic lesions were more common than non-neoplastic lesions. Among neoplastic lesions, neuroepithelial tumors were commonest followed by meningeal tumors. Haemopoetic tumors were least common in all the studies except for the study conducted by Rossler K et al [18] [Table 3]. Primary CNS lymphoma has been increasing in United States, typically in patients with AIDS.[21].

Out of 100 cases, diagnosis was offered on squash smears in 93 cases and 25 cases [26.88%] were misdiagnosed. Out of 90 cases of neoplastic lesions, diagnosis was offered in 85 cases and 23 cases [27.05%] were misdiagnosed. Out of 10 cases of non-neoplastic lesion, diagnosis was offered in 8 cases and 2 cases [25%] were misdiagnosed. Causes of all misdiagnosed cases were reasoned out [Table 4].

Ghosal N et al [22], Deshpande K et al [2], Kini JR et al [19], Pawar NH et al [23] and Iqbal M et al [24] had also misdiagnosed various CNS lesions and analysed pitfalls in their study.

Reasons for misdiagnosis were mainly reactive cells, non-representativeness of the sample, poor cellularity, morphological similarity of cells and the presence of necrosis and inflammation obscuring underlying pathology.

Diagnosis was not offered in seven cases. Reasons for non-diagnostic cases were mainly non-representativeness of the sample, poor cellularity and presence of necrosis and inflammation obscuring the actual underlying pathology[Table 5]. Deshpande et al [2] observed that common causes for inconclusive cases were increased fibrous component, biopsies from cyst wall, inflammation and necrosis obscuring morphology, lack of architecture on cytology, reactive changes and resistance to desegregation.

The diagnostic accuracy was 73.11% by complete correlation and increased to 84.94% on applying partial correlation. Other studies documented higher diagnostic accuracy by complete correlation. Diagnostic accuracy further increased by applying partial correlation criteria in the studies conducted by Rossler K et al [18], Deshpande et al [2] and Kini JR et al [19] [Table 6].

Accuracy rates comparing crush smear preparations with histopathology vary from 90% to 95%[24]. But Collaeo et al reported slightly lower [73%] correlation of solid lesions[28]. Accuracy of diagnosis on cytology depends upon familiarity with clinical history, tumor location, differential diagnosis in a particular location and cyto-histologic appearance of the potential aetiologies of CNS lesions. It also depends upon interest, enthusiasm and experience of neuropathologist. Furthermore, cytologic features of some rare CNS tumors are not well documented [24].

Consistency of the tissue affects diagnostic accuracy. Soft and friable tissues can be easily smeared, yielding good cellularity. Majority of gliomas, pituitary neoplasms, medulloblastomas and metastatic carcinomas yielded good cellularity and posed few diagnostic problems. Discrepancies observed between smears and histopathology were due to sampling error, under-grading or over-grading of gliomas and error in recognition of histologic cell type [13,29]. In the present study, neoplastic lesions were over-graded in 3 cases [4.54%] and under-graded in 2 cases [3.03%]. Disadvantage of smear technique lies with those lesions that are too firm to smear [13]. In contrast to other studies, in the present study, cell yield was good in schwannomas and meningiomas and posed no diagnostic difficulty except in two cases. But cell yield was poor in a case of neurofibroma as it

was difficult to smear. Despande K et al [2] and Iqbal M et al [24] had similar experience with neurofibroma cases.

Squash smear diagnosis was compared with histopathology with respect to WHO grades of primary CNS neoplasms. Diagnostic accuracy by complete correlation was 71.01% and increased to 86.95% on applying partial correlation criteria. There was discrepancy in 20 cases [28.98%]. Ghosal N et al [22] recorded diagnostic accuracy of 93% with discrepancy in 7% of cases.

In the present study, complete correlation was good in grade I and grade IV tumors, while this was poor in grade II and grade III tumors. Gliomas posed diagnostic problems despite good cell yield. Mixed gliomas are responsible for difficulties in smear interpretation due to presence of more than one histological cell type. Improper grading of astrocytic neoplasms occurs in cytologic preparations, as astrocytomas are known to vary significantly in grade from one area to another. Moriguard et al suggested that multiple biopsies from different areas might bring down false positivity and false negativity [2]. This suggestion was helpful in the present study to minimize diagnostic errors.

For detection of neoplastic lesions, sensitivity and specificity were 95.29% and 75% respectively. In contrast, Sharma N et al [17] documented lower sensitivity [90.6%] and higher specificity [87.5%]. Pawar NH et al [23] had also documented lower sensitivity [91.6%and higher specificity [100%]. This reflects failure to consider non-neoplastic lesion while offering squash smear diagnosis in the present study.

Potential pitfalls in intraoperative interpretation of CNS lesions include failure to consider that biopsy may not be representative of the lesion, failure to consider non neoplastic process and over-grading of neoplastic lesions [30]. Efficacy and sensitivity were high in the present study. But specificity was low, reflecting the failure to recognise the non-neoplastic lesions.

Conclusion

Squash cytology is a reliable, rapid and inexpensive intra-operative diagnostic tool. It is an important complementary tool to histopathological diagnosis. It may be considered as mirror image of histopathological features. Inadequate and improper samplings, reactive cells, poor cellularity, morphological similarity of cells, the presence of necrosis and inflammation obscuring the actual underlying pathology are important contributory factors for diagnostic pitfalls. It has a high sensitivity for detection of neoplastic lesions and is highly efficacious procedure. Hence, pathologists have to train themselves in interpreting cytomorphological features of various CNS lesions and overcome diagnostic pitfalls.

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