

Cytohistopathological Study of Central Nervous System Neoplasms

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Abstract: ***Introduction:** In the neurosurgical practice, a rapid pathological diagnosis of the space occupying lesions of the nervous system is an asset and helps the neurosurgeons to plan the extent of surgery and modify it accordingly. The squash smear preparation is fairly accurate, simple and reliable tool for intraoperative diagnosis of neurosurgical biopsies. The current study was undertaken to assess the diagnostic accuracy of intraoperative cytomorphological diagnosis by squash smear technique and its correlation with histopathological diagnosis, which has been considered as 'gold standard.'* ***Aims:** 1. Study of squash / imprint cytological smears of freshly excised CNS neoplasms biopsies, after staining with hematoxylin & eosin, MGG & methylene blue stain. 2. To correlate with histopathological findings and study the diagnostic accuracy of squash smear vs paraffin sections. Settings and Design: Prospective, observational study. Subjects and methods: The prospective study was conducted in the Department of Pathology in collaboration with the Department of Neurosurgery of Vivekananda polyclinic and institute of medical sciences, Lucknow from May 2009 to May 2011. One hundred and two samples with symptomatic and radiologically assessed CNS neoplasms were included in the study. Intraoperative squash smears were stained by rapid methylene blue stain, hematoxylin and eosin and MGG stain. Cytological diagnoses were correlated with histopathological diagnosis which was taken as gold standard, keeping in view the clinicoradiological diagnosis as well. Diagnostic accuracy of cytospreads was calculated. Results: The overall diagnostic accuracy among the central nervous system neoplasms in our study was 93.1 % (95/102). Conclusion: Squash smear technique is a fairly accurate, simple, rapid and reliable tool for rapid intraoperative diagnosis of neurosurgical biopsies.*

Keywords: intraoperative squash smears, CNS neoplasms, diagnostic accuracy

1. Introduction

In the neurosurgical practice, a rapid pathological diagnosis of the space occupying lesions of the nervous system is an asset and helps the neurosurgeons to plan the extent of surgery and modify it accordingly. High resolution and specialized neuroimaging techniques combined with the use of stereotactic biopsies help in the rapid and definitive intraoperative diagnosis on minute and diminutive tissue specimens. The squash smears preparation is fairly accurate, simple and reliable tool for rapid intraoperative diagnosis of neurosurgical biopsies¹. The current study was undertaken to assess the diagnostic accuracy of intra-operative cytomorphological diagnosis by squash smear technique and its correlation with histopathological diagnosis, which has been considered as 'Gold standard'.

2. Materials and Methods

This prospective study was carried out in the Department of Pathology in collaboration with the Department of Neurosurgery, Vivekananda Polyclinic and Institute of Medical Sciences, Lucknow from May 2009 to May 2011. One hundred and two cases of central nervous system tumors were sent for squash smear cytology. Pre-operative clinicoradiological assessment of the cases was done.

Freshly biopsied, unfixed specimens were collected in a clean glass container from the operation theater and were brought to the cytology laboratory immediately. When some delay was inevitable few drops of normal saline were put in the bottle to prevent desiccation. The specimen was

inspected thoroughly by naked eye for the presence of normal brain tissue, necrotic, hemorrhagic or firm part of the biopsy. The viable tissue, whether normal or abnormal was firm in consistency and translucent in appearance. The necrotic tissue appeared white, gray or yellow and opaque. The hemorrhagic tissue appeared red usually variegated and friable. After thorough naked eye examination of the specimen, squash smears were prepared, from the representative areas of the biopsy specimens. Apparently viable tissue was used for making squash smears. The material was placed on a centre of a labeled glass slide. Another slide was placed over the first slide on top of the tissue fragment. Then, a sufficient pressure was applied depending upon the consistency of sample to crush the specimens and then spread smoothly. One of the wet smears (unfixed) was stained immediately supravitaly with methylene blue. A drop of 1% methylene blue was kept and then covered by a cover glass. It was left on the bench for few seconds so that the cell may be stained. The smear was thoroughly examined under the microscope. An impression of cytodagnosis was conveyed to the neurosurgeon while the patient was still in the operation theatre. An immediate microphotograph was taken for permanent records for future reference. In each instance, rest of the smears except one or two were immediately wet fixed in 95% alcohol for haematoxylin and eosin staining. One or two smears were kept air dried for May Grunwald Geimsa stain. The remaining part of the biopsy material was fixed in 10% neutral formal saline for subsequent histopathological examination for which routine paraffin blocking was done. The slides were stained by Haematoxylin and Eosin stain. Cytomorphological diagnosis was jotted down on the same

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day. The histopathological examination of the corresponding material was performed side by side for final diagnosis on histopathology sections.

The diagnoses arrived by the two different techniques were made independently by me and confirmed by consultant pathologist. Where the diagnosis tallied there was no problem but if there was disagreement than the histopathological diagnosis was considered as final and ultimate, keeping in view the clinico-radiological diagnosis as well. Diagnostic accuracy of cytospreads was calculated.

3. Observations and Results

In the present study 102 cases of central nervous system tumors were included .The most common tumor were gliomas followed by meningiomas. Age of presentation of different central nervous system tumors was in between 5yrs and 83yrs. Slight male preponderance was noted with male:female ratio 1.6:1 [table 1]

According to the WHO grading system, we encountered 35 cases of gliomas of which 12 were low grade and 23 were high grade. Low grade tumors included pilocytic astrocytomas grade I, diffuse astrocytoma grade II, pleomorphic xanthoastrocytoma grade II and ependymoma grade II.

Table 1: Age and Sexwise Distribution of Central Nervous System Tumors (N=102)

Lesions	Age in Years										Total (%)
	< 20		21-40		41-60		>61		Total		
	M	F	M	F	M	F	M	F	M	F	
GLIOMAS	4	3	5	3	10	6	3	1	22	13	35(30.7)
CHOROID PLEXUS PAPILLOMA	-	-	-	-	1	-	-	-	1	-	01(0.87)
CENTRAL NEUROCYTOMA	-	-	1	-	-	-	-	-	1	-	01(0.87)
EMBRYONAL TUMOR	3	1	1	1	-	-	-	-	4	2	06(5.26)
SCHWANOMMA	-	1	3	4	1	1	2	-	6	6	12(10.52)
MENINGIOMAS	1	-	5	4	7	7	4	1	17	12	29(25.4)
HAEMANGIOBLASTOMA	1	-	-	-	-	-	-	-	1	-	01(0.87)
PITUITIARY ADENOMA	-	-	2	1	3	-	1	-	6	1	7(6.14)
METASTATIC TUMOURS	-	-	1	4	2	3	-	-	3	7	10(8.77)
TOTAL	9	5	18	17	24	14	10	2	61	41	102(89.47)

Four cases of Pilocytic astrocytomas were characterized by moderate cellularity with characteristic bipolar astrocytic cells (Fig. 1a), Rosenthal fibers (Fig1a inset) and fibrillary background with occasional endothelial proliferation (Fig.1b). Mild nuclear pleomorphism was found in few cases. No evidence of necrosis or mitosis was seen. In a single case of diffuse astrocytoma the histopathological diagnosis of diffuse astrocytoma was missed in the smears

and was diagnosed as reactive gliosis in cytospreads. Single case of pleomorphic xanthoastrocytoma revealed high cellularity with fibrillary background. The cells revealed vacuolated cytoplasm along with moderate nuclear pleomorphism. In smears, it was diagnosed as low grade astrocytoma.

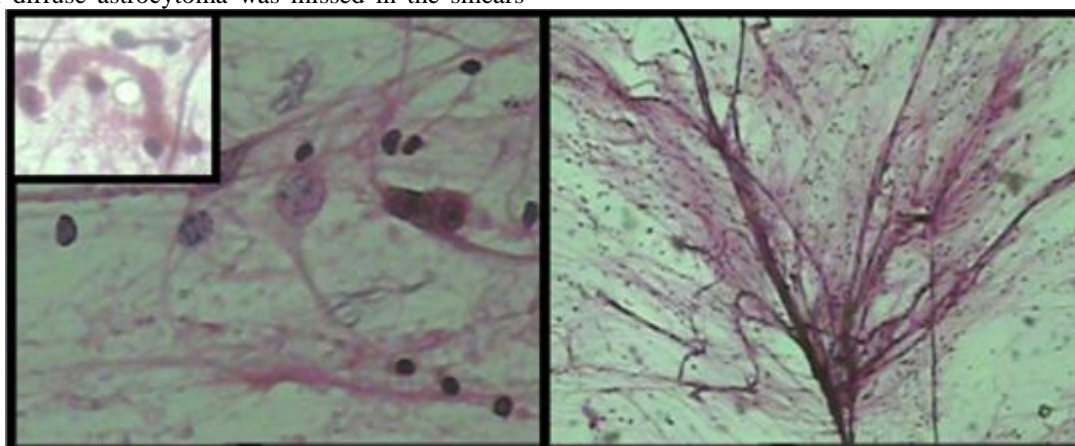


Figure 1 (a)

Figure 1 (b)

Fig.1a Smear of Pilocytic astrocytoma showing bipolar astrocytic cells (H: E 40X). Inset shows Rosenthal fibre. Fig. 1b showing endothelial proliferation

Seventeen cases of Glioblastomas multiforme revealed fibrillated background with necrotic debris alongwith endothelial proliferation (Fig.2a) and perivascular hypercellularity(Fig.2b). Gemistocytes and tumor giant cells were also seen. Four cases of Gliosarcoma revealed glial cells alternating with tight and thick fascicles of spindly cells (Fig.3). Single case of Anaplastic oligodendroglioma showed granular background and cells with pale eosinophilic cytoplasm and round nuclei and evenly distributed chromatin

High grade gliomas included Anaplastic oligodendroglioma grade III, Anaplastic ependymoma grade III, Glioblastoma multiforme grade IV, Gliosarcoma grade IV. All of these showed high cellularity, nuclear pleomorphism and mitotic figures.

and small nucleoli. Few mini- gemistocytes were also seen (Fig.4).

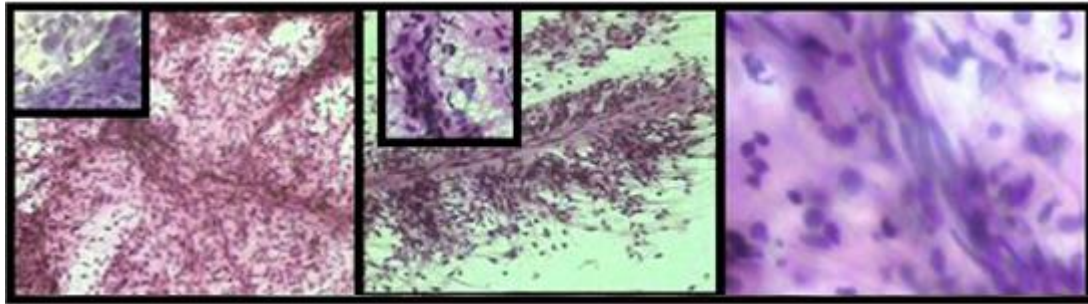


Figure 2 (a)

Figure 2 (b)

Figure 3

Fig.2a Smear of G.B.M showing marked endothelial proliferation (H: E10X).Inset shows mitotic figure (M.G.G, 40X). Fig.2b shows perivascular arrangement of tumor cells (H: E, 10X). Inset shows bizarre & pleomorphic cells (H: E, 40X).

Fig.3 Smear of Gliosarcoma showing thick fascicles of spindle cells alternating with pleomorphic glial cells (M.G.G, 40X)

The ependymal tumors included six cases of Ependymoma, these were fibrillated and well vascularised with round to oval cells with scant, ill-defined cytoplasm with nuclear pleomorphism. Perivascular pseudorosettes were prominent (Fig.5a). In addition, single case of Anaplastic Ependymoma revealed marked nuclear pleomorphism and endothelial proliferation. Mitotic figures and necrosis were also seen (Fig.5b).

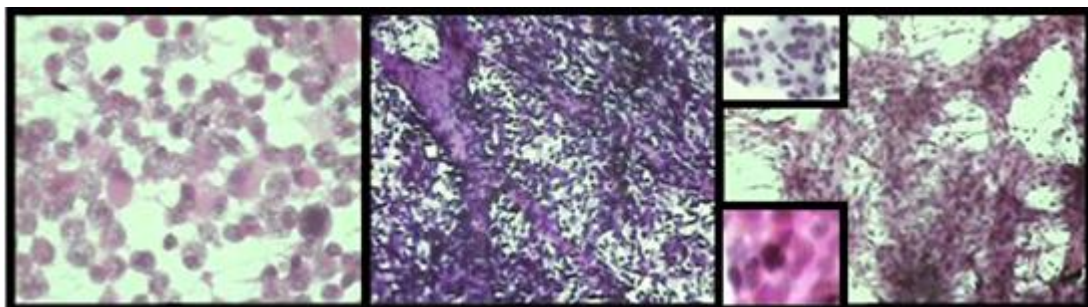


Figure 4

Figure 5 (a)

Figure 5 (b)

Fig. 4 Smear of Anaplastic oligodendroglioma with hypercellularity, mild pleomorphism & minigemistocytes (H: E, 40X).

Fig.5a Ependymoma showing hypercellularity, endothelial proliferation, perivascular arrangement of cells (H: E, 10X).

Fig. 5b Anaplastic Ependymoma, inset 1 shows pleomorphic, hyperchromatic cells(M.G.G,40X) .Inset 2 shows mitotic figure(H:E 40X)

The choroid plexus tumors included grade I tumor Choroid plexus Papilloma which showed high cellularity and uneven distribution of cells with a clean background. The cells were arranged in cohesive papillary clusters along a fibrovascular core (Fig.6). The cells were cuboidal to columnar resting on a basement membrane and nuclei were uniform in size and shape with fine chromatin and inconspicuous nucleoli Fig.6 (inset). No atypia, mitosis or necrosis was seen.

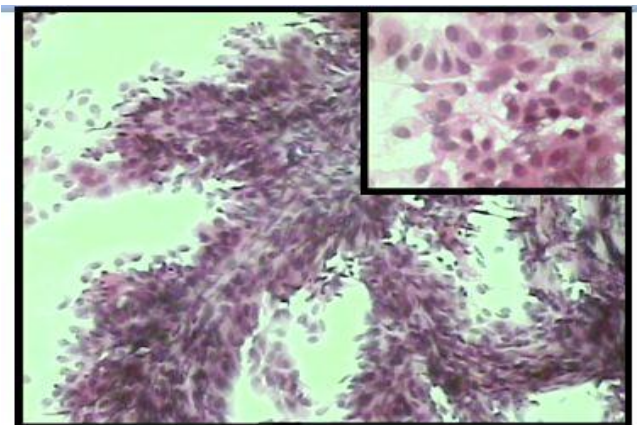


Figure 6: Choroid plexus papilloma –Smear showing papillary arrangement of orderly cuboidal epithelial cells resting on fibrovascular cores (H:E,10X). Inset shows columnar cells(H:E,40X)

The neuronal tumor included grade II tumor Central neurocytoma showed smears with high cellularity and evenly distributed monomorphic population of cells and finely granular background. The cytoplasm was scant eosinophilic with perinuclear clearing and round to oval nuclei with finely speckled chromatin. Delicate, branching capillaries were present in the fibrillary areas (fig.7).

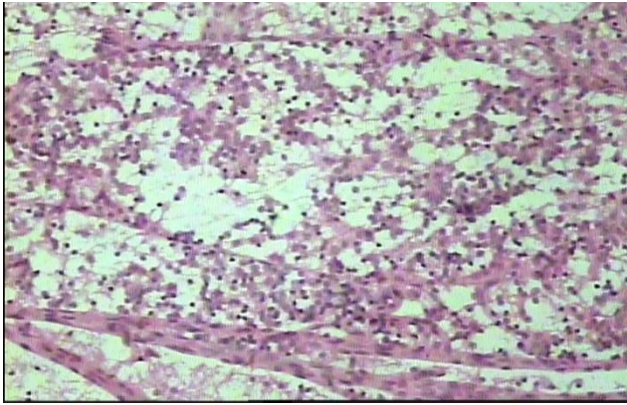


Figure 7

Smear of Central neurocytoma showing monotonous areas of small, round cells alternating with fibrillar zones. Capillary proliferation also seen (H: E, 10X).

Embryonal tumors grade IV included 6 cases of medulloblastoma, which were hypercellular and easily spread and showed evenly distributed cells with scant cytoplasm and round to oval nuclei with coarse punctate chromatin and granular background. In few cases, rosettes and fibrillary background were also found (Fig.8).

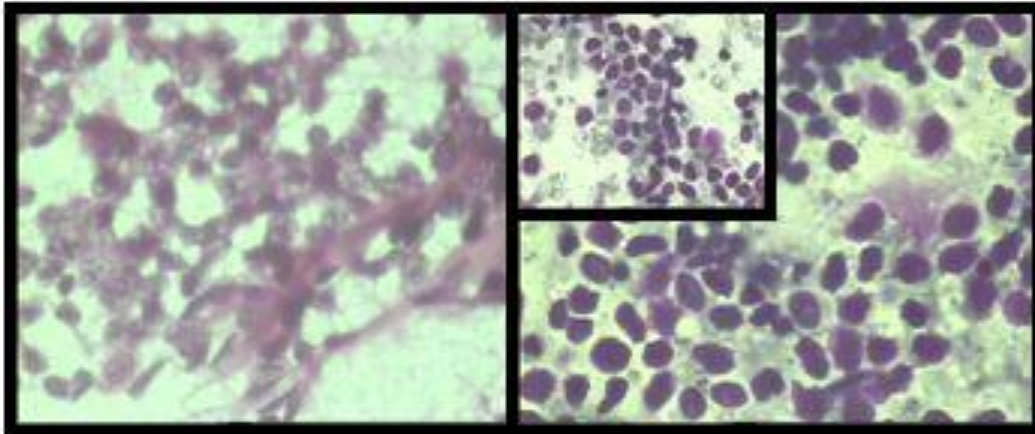


Figure 8 (a)

Figure 8 (b)

Fig.8a Smear of Medulloblastoma showing dense cellularity, round to oval hyperchromatic cell(H:E,40X). Fig.8b Smear of malignant round cell tumour(M.G.G,40X). Inset shows rosette formation (M.G.G, 40X)

cellular and revealed uneven cell distribution finely granular background. The cells were oval, wavy and elongated with an indistinct cytoplasm and finely dispersed nuclear chromatin. Foamy macrophages (Fig.9b), Verocay bodies and palisading were noted in some smears (Fig.9a).

The tumors of cranial and paraspinal nerves included 12 cases of grade I tumor Schwannoma, were moderately

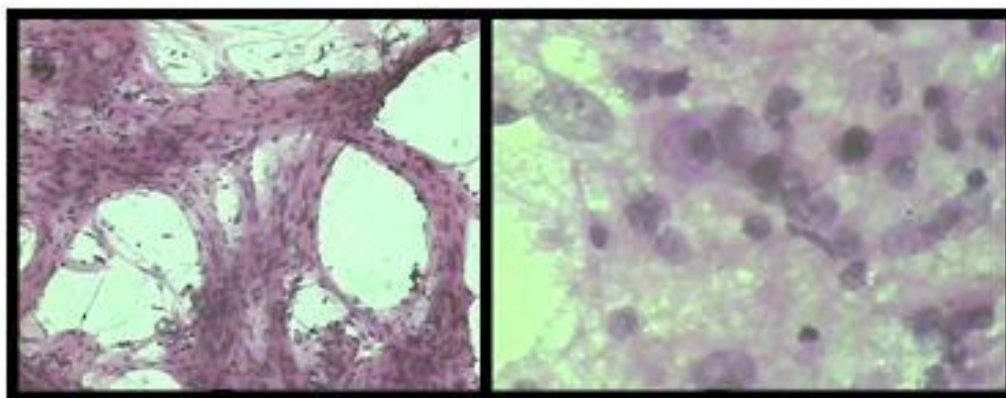


Figure 9 (a)

Figure 9 (b)

Fig 9a Smear of Schwannoma showing elongated cells with oval to wavy nuclei in palisades(H:E,10X).9b) shows foamy macrophages(H:E,40X)

Meningothelial meningioma and low in fibroblastic type. The cytoplasm was eosinophilic with round to oval nuclei with pale, margined chromatin and inconspicuous nucleoli and intranuclear pseudoinclusions. In addition, whorl like pattern was evident in Fibroblastic meningioma(Fig.10b). Psammoma bodies were noted in some cases (Fig.10d). Single case of Chordoid meningioma was characterized by

Twenty six cases of grade I tumors Meningiomas included meningothelial meningioma, fibroblastic meningioma, transitional meningioma, psammomatous meningioma and chordoid meningioma. The cellularity was variably high in

cohesive clusters of eosinophilic vacuolated cells in a myxoid background (Fig.10e).

The Grade III meningioma included three cases of Anaplastic Meningioma that showed pleomorphic

meningothelial cells with scant cytoplasm and round nucleus with vesicular chromatin and prominent nucleoli. Multinucleate giant cells and mitotic figures were also seen (Fig.10f).

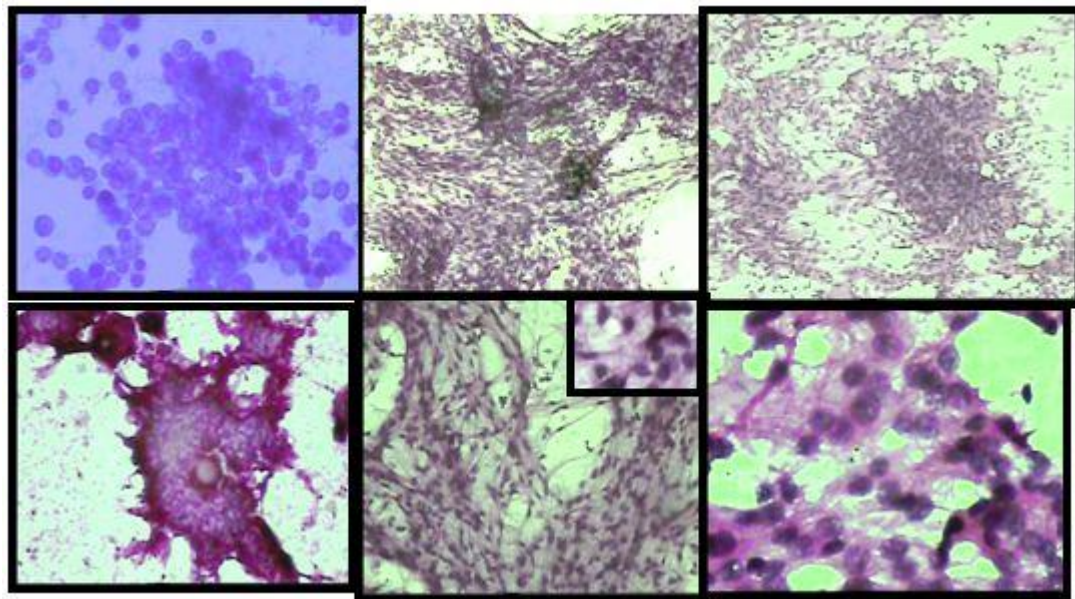


Figure 10: (a) Smear of meningothelial meningioma (Methylene blue,40X) Fig. 10b Smear of fibroblastic meningioma showing spindle shaped cells (M.G.G,10X) Fig. 10c Smear of transitional meningioma (H:E,10X) Fig. 10d Smear of Psammomatous meningioma.(H:E,20X) Fig. 10e Smear of Chordoid meningioma showing chains of eosinophilic cells, occasionally vacuolated in a myxoid background(H:E,10X). Inset shows eosinophilic vacuolated cells(H:E,40X) Fig. 10f Smear of anaplastic meningioma showing pleomorphic, hyperchromatic cells in necrotic background(H:E,40X).

The mesenchymal tumor included single case of Grade I Hemangioblastoma the smears of which were difficult to spread and revealed anastomosing trabeculae of blood vessels and cells with vacuolated cytoplasm with moderate pleomorphism (Fig.11). Vascular proliferation and Hemosiderin laden macrophages were also seen.

Seven cases of pituitary adenoma smeared out evenly and showed monomorphic population of dispersed cells in a clean background (fig.12a). The cytoplasm was acidophilic to faintly basophilic with oval to round nucleus and finely dispersed chromatin and inconspicuous nucleoli (Fig.12b).

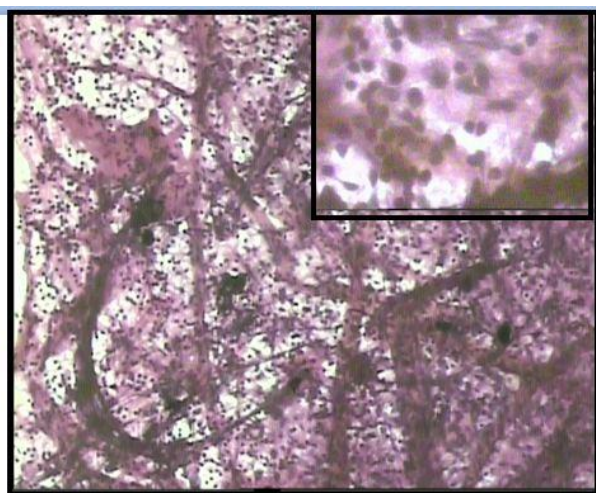


Figure 11: Smear of Haemangioblastoma containing anastomosing trabeculae of blood vessels and stromal cells with vacuolated appearance (H: E,10X). Inset shows cells with foamy cytoplasm (H; E, 40X).

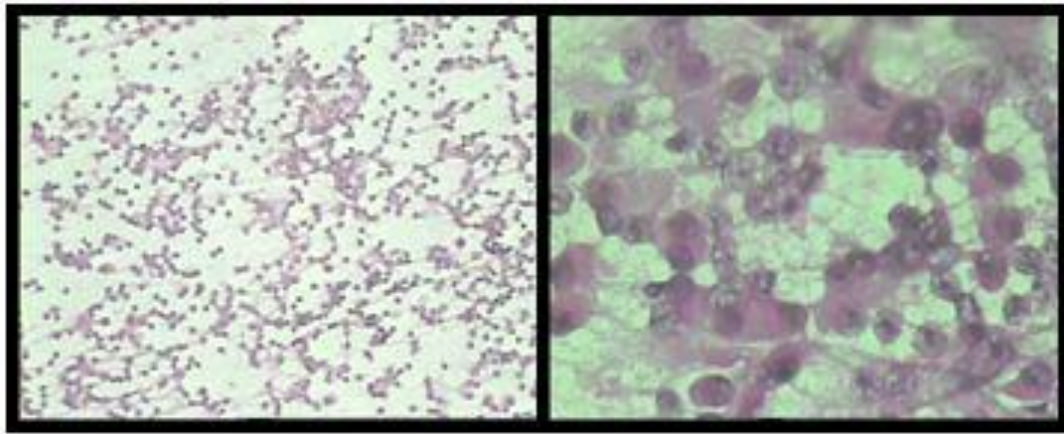


Figure 12a

Figure 12b

Fig. 12a Smears of Pituitary adenoma showing disorganized monomorphic population of cells(H:E, 10X) 12b Smears of Pituitary adenoma showing round to oval nuclei with finely dispersed chromatin and conspicuous nucleoli, mild nuclear hyperchromasia, binucleate forms(H:E,40X).

neoplastic cells with pleomorphism and high nucleocytoplasmic ratio and prominent nucleoli. Mitotic figures and necrosis were also seen. Perivascular arrangement of tumor cells was also seen in one case. A single case of Metastatic malignant melanoma showed disorganized clusters of tumour cells which contained obvious melanin pigment granules (Fig13b,c)

Ten cases of metastatic tumors were encountered in our study and on cytological analysis revealed easily smeared

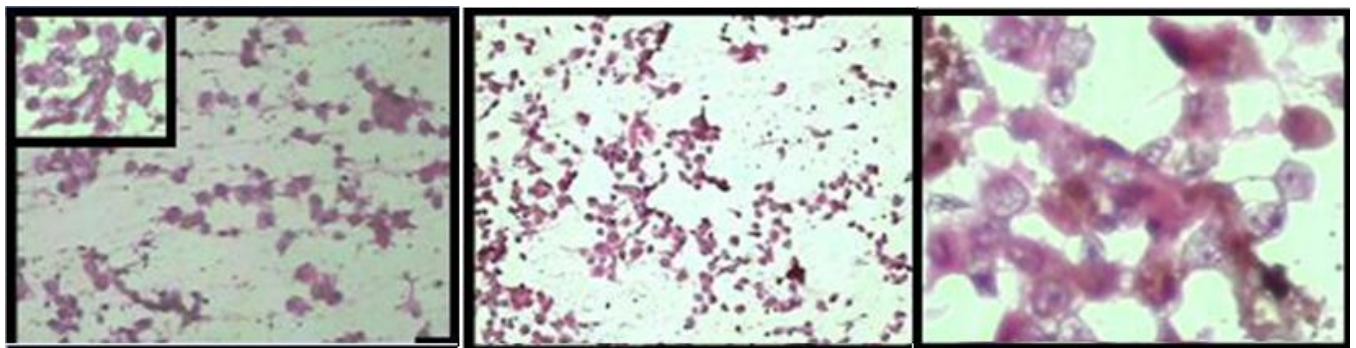


Figure 12 (a)

Figure 12 (b)

Figure 13 (c)

Fig.13a Smear of Metastatic adenocarcinoma shows cluster of small, disorganized cell groups (H: E, 10X).Inset shows pleomorphic, high N/C ratio, vesicular chromatin, prominent nucleoli (H: E,40X). Fig. 13b Smear of Metastatic malignant

melanoma showing disorganized tumor cells (H: E, 10X).Fig.13c shows pleomorphic, hyperchromatic cells, and prominent nucleoli. Melanin pigment granules seen. (H: E,40X).

Table 2: Cyto-Histopathological Correlation Of Neoplasms (n=102)

HPE Diagnosis	Total No.Of Cases	Cytology	Incorrect Cytology	Accuracy %
Pilocytic astrocytomas	04	04	-	100
Diffuse astrocytoma	01	0	01(Reactive gliosis)	0
Pleomorphic Xanthoastrocytoma	01	01	-	100
Glioblastomas multiforme	17	14	03(necrotic debris)	82.35
Gliosarcoma	04	04	-	100
Anaplastic oligodendroglioma	01	01	-	100
Ependymoma	05	04	01(necrotic debris)	80
Anaplastic ependymoma	02	02	-	100
Choroid plexus papilloma	01	01	-	100
Central neurocytoma	01	01	-	100
Embryonal tumors	06	06	-	100
Schwannomas	12	11	01(Fibroblastic meningioma)	91.67
Meningiomas	29	28	01(Inadequate)	96.67
Haemangioblastoma	01	01	-	100
Pituitary adenoma	07	07	-	100
Metastatic	10	10	-	100
Total	102	95	07	93.13

Thus, complete correlation between cytological and the final histopathological diagnosis in the central nervous system tumors was achieved in 93.1 % of cases.

4. Discussion

Cytologic examination by squash smear preparation of central nervous system lesions was used in intraoperative neurosurgical diagnosis as early as 30s. Dudgeon and Patrick in (1927)⁸ were the pioneers in utilizing 'wet-film' technique for rapid diagnosis of tumors. Supravital staining was performed by Cushing and Eisenhardt (1930)⁷ by using neutral red dye and later on by other workers. Daery (1936)⁹ used a mixture of Azure A and Erie garnet B, Russel *et al.* (1937)¹⁰ fixed the smear by Schaudinn's fluid and subsequently stained by toulidene blue or Mann's methyl blue stain. Morris (1947)¹¹ used eosin-methylene blue, Mcmenemy (1960)¹² used 0.25% Toulidene blue for staining of the wet fixed smears. Marshall *et al.* (1973) advocated fixation of smears in 95% alcohol and then staining by 1% toulidene blue. Goel *et al.* (2005)²⁷ used 1% alcoholic toulidene blue. The basic shortcoming of supravital technique experienced in our study and previous workers^{7,9,11} was that permanent preparation of the material was not possible as the stain faded away quickly and since the cells were not in a fixed state, autolytic changes soon set in to vitiate the cytomorphology. After supravital staining of smears, we performed the routine Hematoxylin and eosin method of staining of squash smears. Similar technique and staining method was performed by previous workers.^{6, 15, 21, 26, 29,30,32,35}

In this study, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, gliosarcoma, anaplastic oligodendroglioma, anaplastic ependymoma, choroid plexus papilloma, central neurocytoma, embryonal tumours, hemangioblastoma, pituitary adenoma and metastatic tumours showed 100% diagnostic accuracy cytohistological correlation. Whereas, the difficulty was found in few cases of glioblastoma multiforme and ependymoma as smear was prepared from necrotic tissue and a single case of diffuse astrocytoma which on smears was diagnosed as reactive gliosis. Single case of schwannoma was diagnosed as fibroblastic meningioma. Single case of meningioma could not be interpreted due to scanty number of cells possibly due to non-squashable nature and firm consistency of meningiomas.

Thus, the overall diagnostic accuracy among the central nervous system tumors in our study was 93.1% (95/102).

There are few limitations of the squash smear technique that it relies on tissue soft enough to smear, histological architecture is not apparent, it relies on accurate localization by the surgeon and the minute quantity of specimen may not represent all the characteristics of the regional variation expected in the tumor. The specimen might be taken from the necrotic or hemorrhagic part of the specimen.

5. Conclusions

To conclude, squash smear technique should be incorporated for rapid diagnosis of neurosurgical lesions as it is simpler,

accurate and cost-effective in comparison to frozen sections. The immediate knowledge of the cytological characteristics of the lesion gives an indication of the prognosis so that appropriate measures can be taken accordingly. For example, if the lesion is benefited by radiotherapy, it may be instituted without delay. In case of necrotic or hemorrhagic specimen, immediate further sampling is required, and the accuracy of diagnosis and grading can be increased by assessing more cellular regions predominated by neoplastic cells. To ease the limitation, radiological correlation becomes important. The diagnosis can be made on a very small sample. The small amounts of tissue received from neurosurgical biopsies and the soft consistency of many brain tumors of the glioma group frequently render it impossible to obtain good frozen sections, and hence smear preparations have to be employed. Under good conditions, smear preparations may present a beauty of cell details and the characteristic features which are closely comparable with histopathology sections. Besides diagnosis and grading, smear cytology may provide resection guidance in cases of well-delineated tumors.

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