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ROLE OF SQUASH SMEAR IN INTRAOPERATIVE CONSULTATION OF CENTRAL NERVOUS SYSTEM TUMORS

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INTRODUCTION:

The intraoperative cytology preparation was first introduced by Eisenhardt and Cushing in early 1930 and by Badt in 1937.[1] Squash smear technique has recently gained importance because of image-guided stereotactic biopsies.A rapid pathological diagnosis of the space occupying lesions of the nervous system helps the neurosurgeon to plan the extent of surgery.[2] This technique is simple, rapid, inexpensive, fairly accurate, and dependable intraoperative diagnostic tool. The soft consistency of the central nervous system (CNS) tissue is best suited for squash cytology, which is a hindrance for frozen section. Moreover, ice crystal artefacts may make morphological interpretation of frozen sectioned tissue difficult.[3,4] The present study was done to assess the accuracy and utility of intraoperative squash cytology in the evaluation of CNS tumors.

MATERIALS AND METHODS:

This prospective study was done in our Medical College Hospital from January 2016 to June 2018. All patients with clinical diagnosis of CNS tumors were enrolled inthis study. Detailed clinical history of the patients such as presenting symptoms, location of lesion, and radiological findings were noted. During the study, a total of 82 neurosurgical specimens were received. Of these, seven cases were excluded due to nonrepresentative sampling in five cases and inadequate tissue for squash smear preparation in two cases.

The specimens were sent in normal saline. Squash smears were prepared with small bits of tissue measuring 1–2 mm size without exerting undue pressure. Minimum of five to eight smears were made depending on the amount and sample of tissue received. Smears were fixed in 99.9% isopropyl alcohol and stained with rapid hematoxylin and eosin (H and E) stain. The remaining tissue was processed routinely for paraffin sections. Clinical and radiological findings were taken into consideration while interpreting the smears and the diagnosis was informed to the neurosurgeon preoperatively. Remaining tissue was subjected to routine tissue processing and stained with H and E. Immunohistochemistry (IHC) was performed in paraffin blocks in difficult cases.

Cytology results were classified into the following categories: true negative (absence of tumor correctly diagnosed); true positive (presence of tumor correctly diagnosed); false negative (the cytological specimen failed to diagnose as tumor); and false positive (the cytological specimen was incorrectly diagnosed as tumor). The tumors were classified and graded according to the World Health Organization (WHO) classification of CNS neoplasms 2007. Data analysis was based on Galen and Gambino method which calculated sensitivity, specificity, and positive and negative predictive value.

RESULTS:

We received 82 samples from neurosurgery department during the study and after exclusion 75 cases were taken up for study. Clinically, the patients presented with headache, vomiting, altered sensorium, seizures, and neurological deficits. Cerebellopontine (CP) angle tumors had giddiness, auditory, and visual disturbances. Some pediatric patients presented with hydrocephalus. Most common location of tumor was cerebrum comprising about 64% (48 cases), followed by 28% (21 cases) in CP angle/posterior fossa and 4%(3 cases) in both sellar/suprasellar and spinal regions. Glial tumors were most common comprising about 33.3% (25 cases), followed by 24% (18 cases) of meningioma, 18.6% (14 cases) of schwannoma.

A maximum number of cases was seen in 31–40 years age group (20 cases) and a slight female preponderance 52% (39 Cases) was noted. Squash smear findings were noted and the diagnosis was informed to the neurosurgeon intraoperatively.

The distribution of tumors according to final histopathological diagnosis, and accuracy of squash smear diagnosis is illustrated in Table 1. Out of 75 cases, 68 cases showed complete correlation with histopathological diagnosis. The details of misdiagnosed cases in squash smears are given in Table 2. Thus, the overall diagnostic accuracy of squash cytology in the evaluation of CNS tumors was 90.67%. Sensitivity, specificity, positive, and negative predictive value were 98.7%, 93.2%, 97.4%, and 99.2%, respectively.

Table 1: Correlation of histopathological versus cytological diagnosis

Histopathological diagnosis	Number of cases	Number of cases correctly diagnosed by squash cytology	Discordant cases	Accuracy (%)
Low grade (Grade II) astrocytoma	9	9	Nil	100
High grade (Grade III) astrocytoma	5	4	1	80
Glioblastoma multiforme	6	6	Nil	100
Oligoastrocytoma	2	0	2	0
Oligodendroglioma	1	1	Nil	100
Anaplastic ependymoma	1	1	Nil	100
Myxopapillary ependymoma	1	1	Nil	100
Medulloblastoma	2	2	Nil	100
Anaplastic medulloblastoma	1	0	1	0
Meningioma	18	18	Nil	100
Schwannoma	14	14	Nil	100
Craniopharyngioma	1	1	Nil	100
Pituitary adenoma	1	1	Nil	100
Metastatic adenocarcinomatous deposits	4	2	2	50
Hemangioma	1	1	Nil	100
Hemangioendothelioma	1	0	1	0
Olfactory neuroblastoma	1	1	Nil	100
Epidermoid cyst	1	1	Nil	100
Colloid cyst	1	1	Nil	100
Dermoid cyst	1	1	Nil	100
Tuberculoma	2	2	Nil	100
Abscess	1	1	Nil	100
Total	75	68	7	90.67

Table 2: Discordant cases on cytology

Histodiagnosis	Cytological diagnosis
Metastatic carcinomatous deposits	Ependymoma
Metastatic carcinomatous deposits	Gemistocytic astrocytoma
Anaplastic astrocytoma	Metastatic carcinomatous deposits
Anaplastic medulloblastoma	Anaplastic ependymoma
Hemangioendothelioma	Low grade astrocytoma
Oligoastrocytoma	Oligodendroglioma
Oligoastrocytoma	Grade II astrocytoma

DISCUSSION:

Intraoperative diagnosis in CNS tumors can be done by squash smear cytology or frozen sections or in combination depending on the type of neurosurgical procedure, availability of technology, and amount of tissue sampled. With the advent of stereotactic or endoscopic approach to inaccessible lesions, a tiny tissue is provided which might necessitate squash cytology. Frozen section provides good cytomorphological details and finer histological typing if there is no limitation of tissue availability. However, it requires costly equipment, and may cause ice crystal formation particularly in astrocytomas, and the freezing artifacts cause distortion of architecture. The advantages of squash smears are that it is easy to smear CNS tumors with good cellularity, can be done even when the sample is limited, and intraoperative diagnosis can be rendered within 10 min.[3,5]

Out of these 82 samples received during the study, 7 cases were

excluded due to nonrepresentative sampling and inadequate material for smear preparation. Cases with insufficient and nonrepresentative material were mentioned in many studies.[4,6] The youngest patient in our study was 3 years female child and the oldest being an 80 years male. Peak incidence of brain tumors was observed in 31–40 years age group comprising about 19 cases (25.3%), followed by 41–50 years and 21–30 years age groups comprising about 13 cases (17.3%) and 12 cases (16%), respectively. A similar finding was found in studies done by Deshpande et al.,[5] Jha et al.[7] and Shukla et al.[8] In contrary to most studies, we encountered a slight female preponderance of 52% (39 cases) with Male to Female ratio of 1:1.1 which was consistent with the report by Sharma et al[9] and Krishnaprasad et al[10]

Astrocytomas formed the largest category accounting for 26.7% (20 cases) of the total cases reported in our study. All astrocytomas except one case showed concordance with the final histopathological diagnosis. Low-grade astrocytoma had low to moderate cellularity, the cells tend to aggregate around blood vessels, and nuclei were oval to elongated with fibrillary cytoplasm [Figure 1] as reported by Deshpande et al.[5] High-grade astrocytomas had moderate to high cellularity, marked nuclear pleomorphism, tumor giant cells, and mitotic figures. Bhagyalakshmi et al. stated that the presence of single mitotic figure in squash smear indicates that the tumor is high grade.[2] Glioblastomas showed anaplastic cells, endothelial proliferation [Figure 2], necrosis with a mixed inflammatory infiltrate, and the neoplastic cells were closely related to blood vessels.[7]

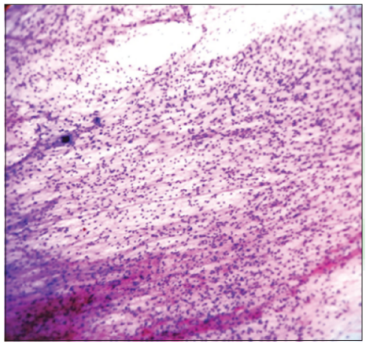

Figure 1: Squash smear of low grade astrocytoma shows neoplastic astrocytes with fibrillary cytoplasm (H and E, ×100)

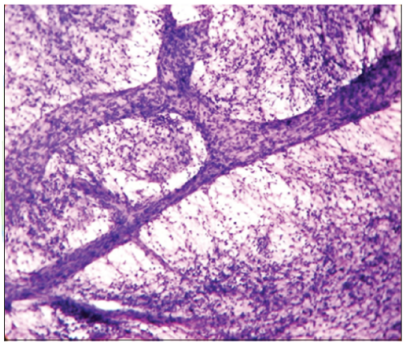

Figure 2: Squash smear of glioblastoma with prominent endothelial proliferation (arrow) (H and E, ×100)

A case of high-grade astrocytoma was misinterpreted as metastatic carcinomatous deposits, in which gemistocytes were mistaken for mucinophages. Radiologically, it was reported as multiple frontal lobe abscesses. The same difficulty was encountered in various studies.[11-13]

We encountered two cases of oligoastrocytoma; both were misinterpreted on squash smear cytology as oligodendroglioma and Grade II astrocytoma. In one case, it was a recurrent mass with an initial histodiagnosis of oligodendroglioma, which turned out to be oligoastrocytoma in the recurrent mass. This was misinterpreted as oligodendroglioma in squash smear. Other case was a cerebellar lesion reported as Grade II astrocytoma in smear cytology. In both cases, the reported component in squash smear cytology was the predominant one, and the smaller component was missed which may be due to sampling error or either due to less number of oligodendroglial cells or dense fibrillary background of astrocytic component. Such misdiagnosis has been reported in literature and this can reduce the diagnostic accuracy of squash smears.[4,5,14]

Sampling errors affect the grading of glial tumors, and the tumor can be overdiagnosed or misdiagnosed because the grading may vary from one area to another area in the same tumor.[5,14-17] However, the WHO classification does not specify about the quantitative criteria for the proportion of glial components, interobserver variability is more likely to occur while diagnosing mixed gliomas.[18,19]

Smears from oligodendroglioma had neoplastic cells with uniform round nuclei and speckled nuclear chromatin which lacks the dense fibrillary background as that of astrocytoma, foci of calcification was noted [Figure 3]. Perinuclear halo was not seen in contrast to paraffin sections as reported in many studies. [4,5,12,15]

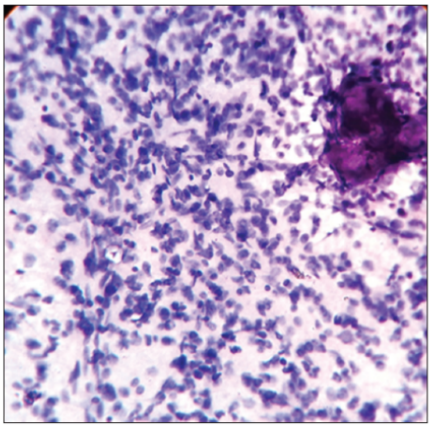

Figure 3: Squash cytology of oligodendroglioma showing cells uniform round nuclei and focal calcification (arrow) (H and E, ×400)

Cytology of ependymoma showed neoplastic cells in papillary architecture around blood vessels having a prominent fibrillary matrix with pseudorosettes as shown in Figure 4 and these findings correlated well with literature.[4-6,12,17] A case of myxopapillary ependymoma in filum terminale had high cellularity with papillary pattern [Figure 5] in a prominent myxoid matrix as reported in other studies.[15,20] Nanarng et al. state that caution must be taken while diagnosing myxopapillary ependymoma as it mimics chordoma and metastatic papillary adenocarcinomatous deposits.[15] The absence of physalliferous cells and marked nuclear pleomorphism ruled out both possibilities, respectively.

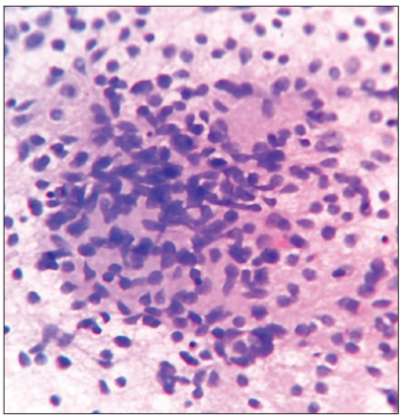

Figure 4: Smear from ependymoma exhibiting rosettes in a fibrillary background (H and E, ×400)

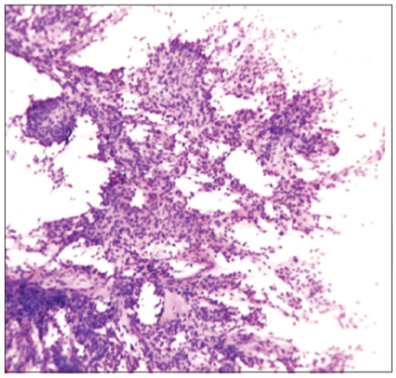

Figure 5: Squash smear of myxopapillary ependymoma exhibiting papillary fronds in a myxoid background (H and E, ×100)

Of the two cases of cerebellar medulloblastoma reported one patient developed drop metastases in spinal cord during follow-up. Smears had high cellularity with cells exhibiting carrot shaped nuclei, nuclear molding, scant cytoplasm with Homer-Wright pseudorosettes [Figure 6] as described in other studies.[5,6] Folkerth [21] states that medulloblastoma can mimic lymphoma on cytology, as both will produce highly cellular smear with discohesive cells and increased nuclear-cytoplasmic ratio.

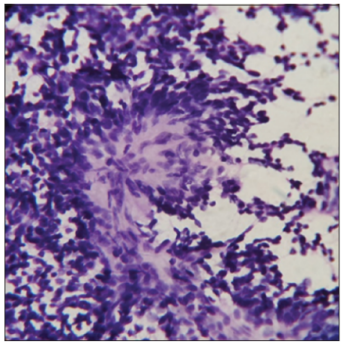

Figure 6: Smear from medulloblastoma shows rosettes and cells with carrot shaped nuclei (H and E, ×400)

A case of anaplastic medulloblastoma was misinterpreted as anaplastic ependymoma on smear cytology in a 4 years child. Computerized tomography image revealed a posterior fossa lesion with calcification obstructing the fourth ventricle. Smears showed increased cellularity, cells with pleomorphic hyperchromatic nuclei, scant cytoplasm with mitosis and occasional pseudorosettes. Based on these findings, a cytodiagnosis of anaplastic ependymoma was made. However, a final histodiagnosis of anaplastic medullobl astoma was made based on strong immunoreactivity for synaptophysin by IHC as shown in Figure 7 and ki-67 showed moderate proliferating index. The probability of misdiagnosing medulloblastoma as ependymoma and vice versa is 11.7% as stated by Roessler et al.[22] The cells of the granular layer of cerebellum are uniformly smaller and round and had to be distinguished from tumor cells.[20]

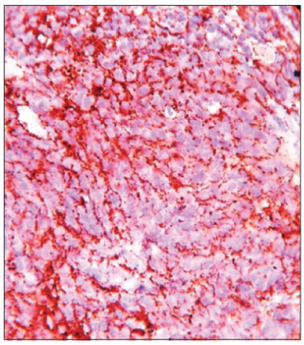

Figure 7: Section from anaplastic medulloblastoma exhibiting strong immunoreactivity for synaptophysin (IHC, ×400)

Meningioma constituted 24% of total cases with complete concordance with histopathological diagnosis similar to other reports.[10,14] The different subtypes of meningioma reported were meningothelial meningioma (ten cases); psammomatous, transitional, angiomatous, and metaplastic meningioma. Characteristic type of each meningioma was finally confirmed in histopathological diagnosis mainly for transitional, metaplastic, and angiomatous meningioma. Jha et al. noted that it was not possible to provide clear-cut differentiation between types of meningioma on cytology as in our study.[7] Most cases showed plump to ovoid meningothelial cells arranged in syncytial and whorling patterns [Figure 8] as reported in literature.[10,14]

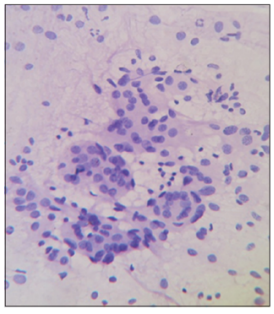

Figure 8: Squash smear of meningioma with cells in whorls and syncytium (H and E, ×400)

]Krishna Prasad et al.[10] stated that psammomatous meningiomas invariably occurs in spinal canal, but in our study, all three cases were located in the supratentorial region. All meningiomas except psammomatous and transitional type with high fibrous component were easy to spread.[10,14] Both schwannoma and meningioma especially fibrous type can pose diagnostic difficulty as both are tough and resistant to smear. Features such as whorling, psammoma bodies, and plump to ovoid cells with syncytial cytoplasm favors meningioma.[13,15]

We got 100% accuracy in diagnosing schwannoma on smear cytology. It was difficult to smear due to cohesiveness of cells which leads to twisted rope appearance in cytology [Figure 9] as reported in literature.[5,14,20] Characteristic hypercellular and hypocellular areas were noted in most of the cases. We found vague nuclear palisading and verocay bodies were difficult to appreciate in smears as stated by Sharma and Deb[12] Nuclear atypia with vacuolation (so called kern-loche) was seen in two cases and was diagnosed as ancient schwannoma in tissue sections. These findings were documented in literature.[6]

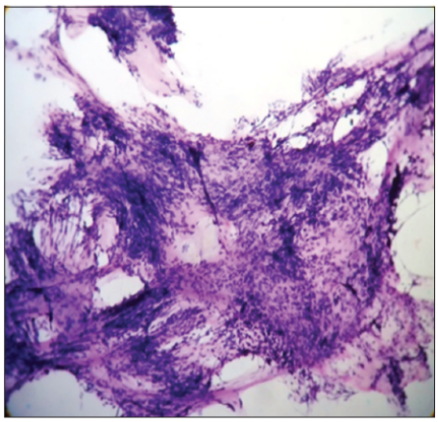

Figure 9: Squash smear showing cohesive spindle cells with twisted rope appearance in schwannoma (H and E, ×100)

Smears from craniopharyngioma revealed cohesive sheets of squamous cells with vague peripheral palisading and calcification [Figure 10] as found in literature.[14,20] Craniopharyngioma will produce florid reactive gliosis as seen in our case and it may be mistaken for low-grade astrocytoma and hence care must be taken while diagnosing suprasellar lesions.[13]

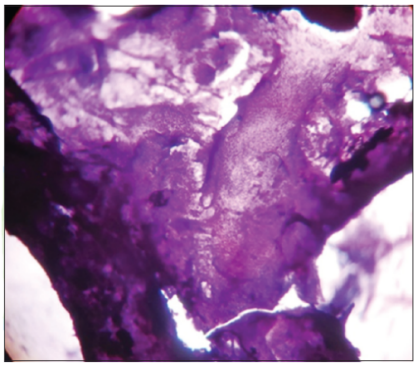

Figure 10: Squash cytology of craniopharyngioma exhibiting sheets of neoplastic cells with peripheral palisading and calcification (H and E, ×100)

Of the four cases of metastatic deposits, two showed concordance with histopathological diagnosis. Smears had neoplastic cells arranged in discohesive sheets and papillary architecture as reported in literature.[5,14] These neoplastic cells were seen embedded in the apparently normal brain parenchyma which showed reactive gliosis containing numerous macrophages (Gitter cells). Sharma and Deb[12] found that tumor differentiation in metastatic carcinomatous deposits was very difficult to interpret on smear cytology. In this study, one case showed extracellular mucin and diagnosed as metastatic carcinomatous deposits in squash smear. On subsequent evaluation, the patient had colorectal carcinoma. Sampling needs to be extensive as surrounding reactive gliosis may be florid, and the tumor tissue may be easily missed. In this case, we found tumor cells only in two out of eight smears.

A case of metastatic carcinomatous deposits was misinterpreted as gemistocytic astrocytoma as the mucinophages were mistaken for gemistocytes [Figure 11]. Such misinterpretation of high-grade glioma as metastatic carcinomatous deposits and vice versa in squash smears has been reported in literature.[11,13] In another case, D7-D8 level spinal lesion, the smear had pleomorphic neoplastic cells with attempted pseudorosettes and was diagnosed as ependymoma. However, histodiagnosis turned out to be metastatic adenocarcinomatous deposits and the glandular pattern in smears was mistaken for rosettes, and the same difficulty was encountered by Roessler et al.[22]

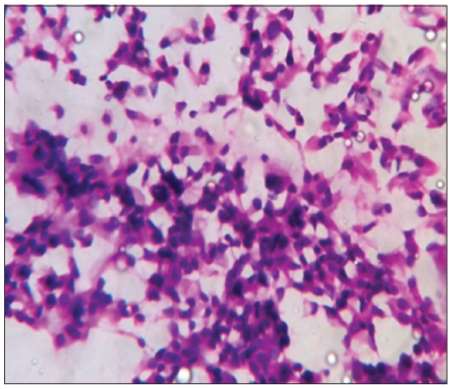

Figure 11: Squash smear of metastatic adenocarcinomatous deposit showing mucinophages mistaken for gemistocytes (H and E, ×400)

Normal pituitary parenchyma was firm and difficult to spread and the cells showed greater variation in size due to the presence of different population of cells.[15] Whereas in adenomas, smears were easy to spread and had uniform round cells, often arranged in papillary pattern and nuclei showed fine powdery chromatin in as seen in our case.[14]

A case of cerebellar hemangioblastoma with cystic component was misinterpreted as low-grade astrocytoma on smear cytology. Tumor tissue was difficult to make smear as stated by Mitra et al.[16] Smear showed few round to oval cells admixed with elongated spindle-shaped cells in a fibrillary background leading to make a diagnosis of low-grade astrocytoma. Similar misinterpretation due to reactive gliosis around tumor tissue and cystic component was noted in many studies.[2,13,16]

Tissues from epidermoid cyst were easy to smear and revealed anucleate squamous cells. It may be confused with craniopharyngioma but characteristic cytological features, clinicoradiological information can help in the differentiation.[15] Overall diagnostic accuracy in our study was 90.67% which correlates well with reports by other authors [Table 3]. The sensitivity and specificity of squash preparations correlated well with the reports by Bhardwaj et al.(97.2% and 100%)[6] and Sanjeev et al.(94.79% and 95.67%)[23] respectively.

Table 3: Diagnostic accuracy of smear cytology in various studies

Study	Number of cases	Accuracy (%)
Jaiswal *et al.*[20]	326	83.7
Goel *et al.*[13]	3057	84.9
Kini *et al.*[14]	100	86
Pawar *et al.*[4]	50	88
Mitra *et al.*[16]	96	88.5
Shrestha *et al.*[11]	60	88
Nanarng *et al.*[15]	75	89.2
Roessler *et al.*[22]	4172	89.8
Deshpande *et al.*[5]	238	91
Sharma and Deb[12]	89	93.3
Sanjeev *et al.*[23]	358	95.25
Present study	75	90.67

CONCLUSION:

This study shows a very high degree of cytohistological correlation. With better and precise radio imaging and stereotactic biopsies, the percentage of cytohistological correlation can improve and increase further. Some cases will always require histopathological study and/or immunohistochemical markers for definitive diagnosis, but for most of the lesions, cytology of the CNS tumors performed intraoperatively fulfills all the determinants of an excellent diagnostic modality.

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