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Original Research Article

Diagnostic utility of intraoperative cytology in CNS lesions

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Abstract

Background: Squash smears of CNS lesions are easy to perform, inexpensive and permit high diagnostic accuracy.
Aims: 1) Study the cytology of various CNS lesions by squash technique.2) Assess the utility of squash preparation, as an aid to frozen section study.3) Assess the accuracy of squash preparation, by comparing it with histopathological section.
Setting and Design: This study was conducted in the Department of Pathology over a period of 3½ years.
Materials and Methods: A total of 59 neurological specimens received in the department of pathology for frozen section were studied. Squash preparation was done on all the cases and stained with H & E staining. An intraoperative diagnosis was made on squash preparation and frozen section slides. The diagnosis on squash smears were compared with the final diagnosis.
Results: Cytological correlation was seen in 54 out of 59 cases. Thus overall accuracy of squash cytology in intraoperative diagnosis of CNS lesions in our study was 91.52%. Erroneous intraoperative diagnosis was given in one case each of meningioma, anaplastic medulloblastoma, oligodendroglioma, arachnoid cyst and a case of gemistocytic astrocytoma.
Conclusion: Intraoperative cytology is fairly accurate in the diagnosis of low grade astrocytoma, medulloblastoma, ependymoma, subependymoma, lymphoma, pituitary adenoma, metastases and certain inflammatory lesions. Diagnosis of high grade gliomas, certain cystic lesions and spindle cell lesions posed difficulties.
Keywords: Squash smears, intraoperative diagnoses, neurosurgery.

1.Introduction

Intraoperative cytology preparation was first introduced by Eisenhardt and Cushing in early 1930's and by Badt in 1937 [1]. Intraoperative consultation for CNS lesions is frequently sought to ensure proper sampling of the lesion tissue. It is of value to the neurosurgeon if an unexpected lesion is encountered during surgery or when the appearance of lesion seen during surgery is different from that visualised preoperatively [2,3].

The knowledge of this technique is beneficial in centres where facility for frozen section is unavailable or in case of power breakdown or lack of trained technical personnel [4]. Another advantage of squash smear technique is that minimal tissue is required for diagnosis and the remaining tissue is available for further processing and ancillary techniques to aid in the final diagnosis [5]. Squash preparation is also of importance in processing samples from patients of AIDS and slow virus diseases, considering the contamination of instruments used by fresh unfixed tissues [6,7]. Thus squash preparation serves as a valuable diagnostic tool in experienced hands.

Squash preparation is an adjuvant in diagnosing CNS lesions on tissue sent for frozen section. Crush smear cytology better suits CNS lesions than any other tumours because of scanty connective tissue in the CNS. Most of the lesions are solid and creamy. Therefore a comparative analysis is being done between squash preparation and regular histopathological examination, with an aim to identify the advantages of using squash studies along with frozen sections for intraoperative diagnosis.

2. Materials and methods

A prospective study of squash smears of CNS lesions was carried out over a period of $3^{1/2}$ years. Neurological specimens received in the department of pathology for frozen section were studied. Ethical

clearance for conducting this study was obtained from the institutional ethical clearance committee. Specimens received in fresh state only were considered for the study and those sent in formalin fixative were excluded.

Preoperatively, clinical details of the patient like age, sex, symptoms and signs on presentation, clinical diagnosis along with other relevant clinical and radiological findings were noted. Tissue sent for intraoperative consultation was used for squash preparation that is, one to two millimetres of tissue was crushed between two slides in order to spread the tissue into a thin film, fixed in 95% alcohol and stained by hematoxylin and eosin (H & E). In a few cases, one of the smears was also air dried and stained with Romanowsky stain. Cytomorphological findings were observed on squash preparation. Frozen section slides were prepared by cutting the tissue on a cryostat apparatus and staining with haematoxylin-eosin stain. Intraoperative diagnosis was given based on the findings observed in the squash smears as well as the frozen section slides. Remaining tissue was processed routinely to produce paraffin embedded sections. These were observed and reported by another pathologist, who was blinded to the intraoperative diagnosis. Later on, the findings observed on cytology smears were compared with those present in the paraffin embedded sections. Comparing the initial intraoperative cytology and final histopathology diagnoses, established the diagnostic utility of squash cytology as an adjunct to frozen section in the diagnosis of CNS lesions.

3. Results

Squash smears from 59 cases were analyzed. Of these 59 cases, 32 were females and the other 27 cases were males. Maximum number of cases that is 17 cases was seen in the age group of 41-50 years, followed by 15 cases in the age group of 51-60 years. Eight were children <20 years of age. Most of the lesions, that is 51 were located intracranially, the remaining 8 were spinal lesions. Figure 1 shows the various lesions seen in this study. Schwannomas formed the majority of cases that is 11 cases (18.64%) followed by meningiomas, 10 cases (16.94%).

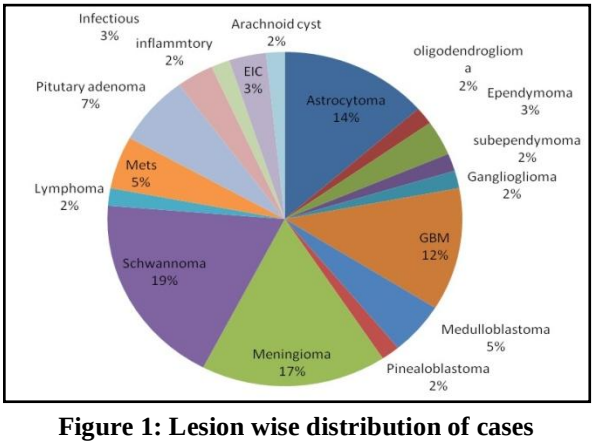

Figure 1: Lesion wise distribution of cases

Glial neoplasms: Amongst glial neoplasms maximum number of cases (8 cases, 13.55%) was astrocytoma grade I-III. One case of pilocytic astrocytoma was reported in an 8 year old male patient, which showed elongated piloid processes and Rosenthal fibres in the squash smears. Five cases of diffuse fibrillary astrocytomas were reported which showed highly cellular smears with pleomorphic astrocytes on smears.

Second most common glial neoplasm in the present study (7 cases, 11.86%) was glioblastoma multiformae. Squash smears showed increased cellularity, nuclear pleomorphism, and numerous atypical mitotic figures in a necrotic background. Paraffin embedded sections also showed similar features along with vascular endothelial proliferation.

One case (1.69%) of subependymoma was reported. The squash smears showed dense glial tissue having either patches of high cellularity and paucicellular fields with few cells. (Figure 2) Since scant tissue was sent for intraoperative consultation no representative tissue could be seen in the frozen section. Diagnosis was give based on clinical and cytology findings. Further the diagnosis was confirmed by features observed on histopathology sections.

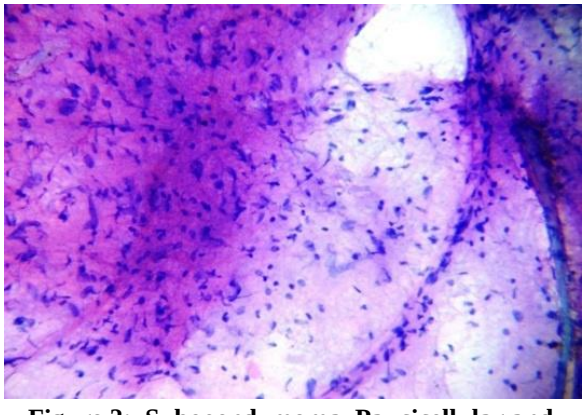

Figure 2: Subependymoma. Paucicellular and hypercellular areas. Squash smear H&E 10X

One case (1.69%) of ganglioglioma was reported which on squash smears showed large ganglion cells with round nucleus, prominent large nucleoli and moderate to abundant granular cytoplasm, along with neoplastic astrocytes.

Two cases (3.38%) of ependymomas were reported in the present study. Squash smears showed monomorphic cells forming perivascular pseudorosettes along with occasional true rosettes. Permanent sections showed monomorphic cells forming ependymal canals and perivascular pseudorosettes.

Non glial neoplasms: Maximum number of non glial neoplasms in the present study was schwannoma (11 cases, 18.64%). Majority of them presented as cerebellopontine (CP) angle masses on MRI. Squash smears showed spindle cells with elongated nuclei

arranged in fascicles. Paraffin sections showed hypercellular (Antoni A) and hypocellular (Antoni B) areas with occasional Verrocay bodies.

Ten cases (16.94%) of meningiomas were seen in the present study. Two cases of psammomatous meningiomas were reported which showed spindle cells in whorls with psammoma bodies on squash smears.

Three cases (5.08%) of medulloblastoma were reported in the present study. 2 cases on squash smears showed small round cells forming occasional neuroblastic rosettes.

Three metastatic lesions (5.08%) were seen in the present study. These being one case of metastatic squamous cell carcinoma, one case of metastatic adenocarcinoma and a case of metastases from a malignant phyllodes tumour breast.

One case (1.69%) of pinealoblastoma in a female aged 15 years was seen, which showed cells with round to angulated nuclei forming occasional perivascular pseudorosettes. (Figure 3)

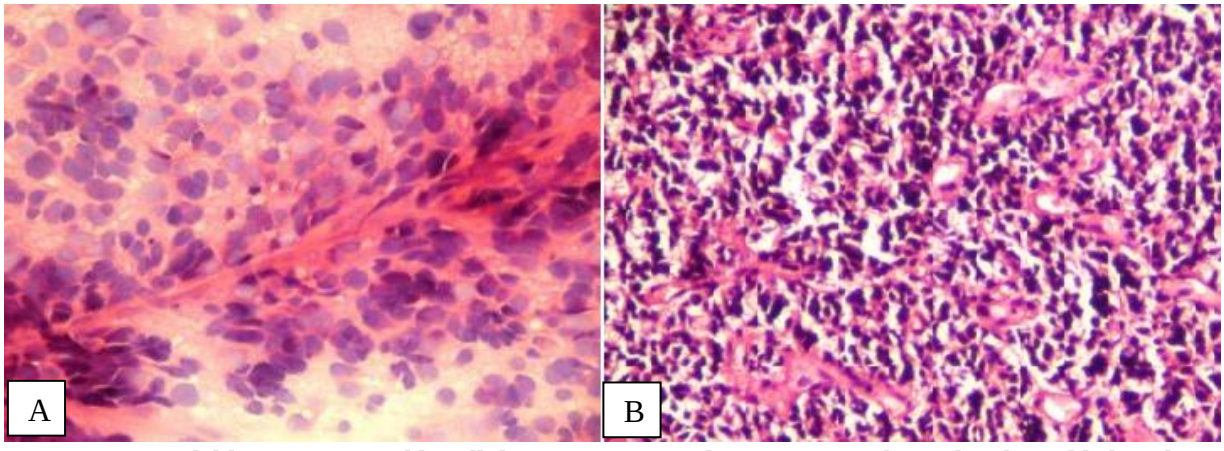

Figure 3: Pinealoblastoma. A, Highly cellular smear. Hyperchromatic, round/angulated moulded nuclei, perivascular pseudorosette. Squash smear H&E 10X. B, Highly cellular tumour showing undifferentiated small cell histology with rosettes. Paraffin section, H&E 10X

One case of primary CNS lymphoma (PCNSL) (1.69%) was reported in the present study in a 38 year old male patient, which showed uniform small round cells arranged in singles, background showed few lymphoglandular bodies on squash smears and angiotropism was seen on permanent sections.

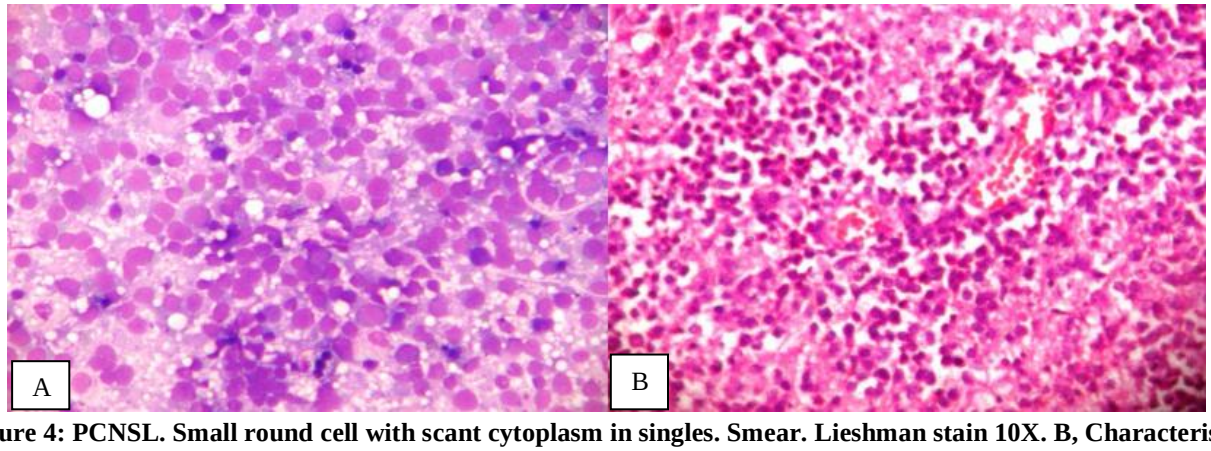

Figure 4: PCNSL. Small round cell with scant cytoplasm in singles. Smear. Lieshman stain 10X. B, Characteristic perivascular spread of tumour cells. Paraffin section H&E 10X

We reported 3 cases (5.08%) as inflammatory/infectious lesions. 2 cases were of tuberculosis and 1 case of chronic inflammatory lesion secondary to prolapsed disc.

There were 2 cases (3.38%) of epidermal inclusion cyst which on smears showed sheets of anucleate squames, both in squash smears and permanent sections.

Cytological correlation was seen in 54 out of 59 cases. Thus overall accuracy of squash cytology in intraoperative diagnosis of CNS lesions in the present study was 91.52%. Figure 5 shows the diagnostic accuracy of squash cytology in the diagnosis of individual lesions.

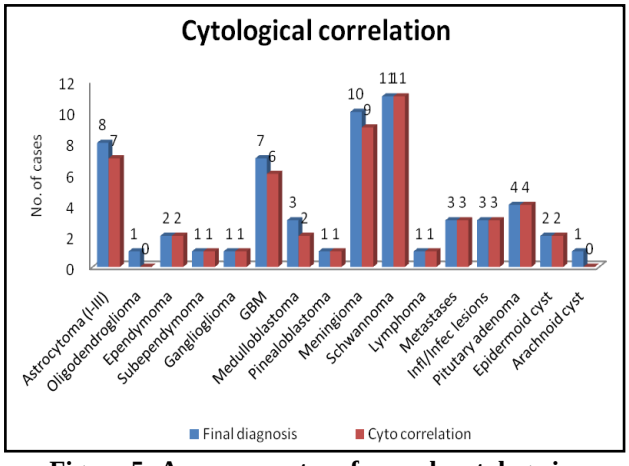

Figure 5: Accuracy rates of squash cytology in diagnosis of individual CNS lesions

Discrepancies between the intraoperative cytological diagnosis and the final diagnosis were observed in 5 cases, as summarized in Table No I.

Table I: Cases in which there were discrepancies between the intra-operative cytological diagnosis and the final diagnosis

Sl No	Final diagnosis	Intraoperative diagnosis	No. of cases
1	Meningioma	Schwannoma	01
2	Anaplastic medulloblastoma	Glioblastoma multiformae	01
3	Oligodendroglioma	Grade II astrocytoma	01
4	Arachnoid cyst	Infective lesion	01
5	Gemistocytic astrocytoma	Grade IV astrocytoma	01

4. Discussion

Following the introduction of intraoperative cytology technique by Eisenhardt and Cushing in early 1930's, this technique was championed and documented by Russel *et al*, in 1937. The present technique was introduced by her along with Sir Hugh Cairns in 1930's [1]. Various intraoperative cytology techniques include touch preparation, crush and squash preparations. Touch preparations are useful in dyscohesive lesions like abscesses, lymphomas and some metastatic lesions, however it is not of much use in extremely soft small biopsies and also fibrous lesions as the lesional cells are not shed easily. Crush smears leave a big glob of tissue in centre of slide which is too thick to be useful under microscope. Squash smears are easy to prepare and convenient to study the cytological features of the lesional cells. Hence squash preparations are more useful for intraoperative diagnosis [8].

Intraoperative consultation is asked for by the neurosurgeon mostly in cases of dilemma regarding the diagnosis being neoplastic or infectious. If there is an infectious process, an additional sample has to be sent for microbiological examination and also post-operative intensive antibiotic treatment needs to be started. In case of a neoplastic lesion, the type of neoplasm, as to that amenable to thorough surgical resection only or those requiring additional chemotherapy and radiotherapy as well, can be determined besides proving if the tumour is a primary or metastases from a distant site [9].

In our study, hundred percent cytological correlations was seen in diagnosis of low grade astrocytoma, ependymoma, subependymoma, ganglioglioma, pinealoblastoma, schwannoma, lymphoma, metastatic lesions, pituitary adenoma, epidermal inclusion cyst and infectious/inflammatory lesions. Similar results were seen in other studies conducted by Kini *et al* [10], Roessler *et al* [11], Shukla *et al* [12], Nigam *et al* [13] and Khamechian *et al* [14]. Lesions like subependymoma and ganglioglioma were not seen in a few of these studies.

One case of meningioma was misdiagnosed as schwannoma due to similarity in cytomorphology of both the lesions that is spindle cells in fascicles (Figure 6). However meningiomas show broadened intercellular bridges and cells are arranged in whorls. Occasional psammoma bodies were seen in paraffin embedded sections which were not seen in the squash smears in our case. This might have occurred due sampling of certain bits of the biopsy only, preserving majority of the specimen for regular histopathology processing. Such pitfall has been observed by other authors like Goel *et al*. & Din *et al* [15,16].

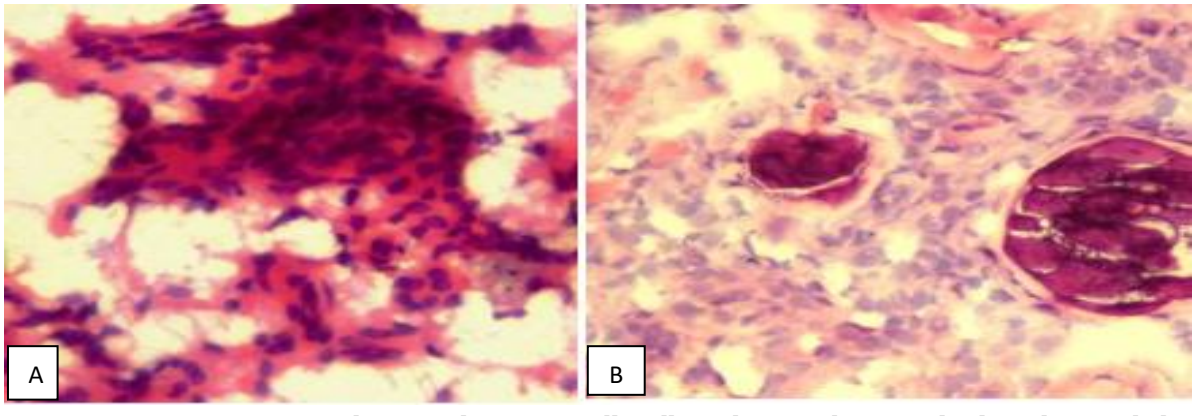

Figure 6: Meningioma. A, squash smear showing spindle cells in sheets and vague whorls. B, histopathology section showing spindle cells in sheets, whorls and few psammoma bodies. H&E 10X

One case of anaplastic medulloblastoma was misdiagnosed as giant cell glioblastoma due presence of necrotic background, highly pleomorphic cells, giant cells and increased mitotic activity in the smears. However the presence of nuclear moulding, apoptotic bodies and wrapping of one tumour cell around another on histology sections lead to the diagnosis of anaplastic medulloblastoma [2]. (Figure 7)

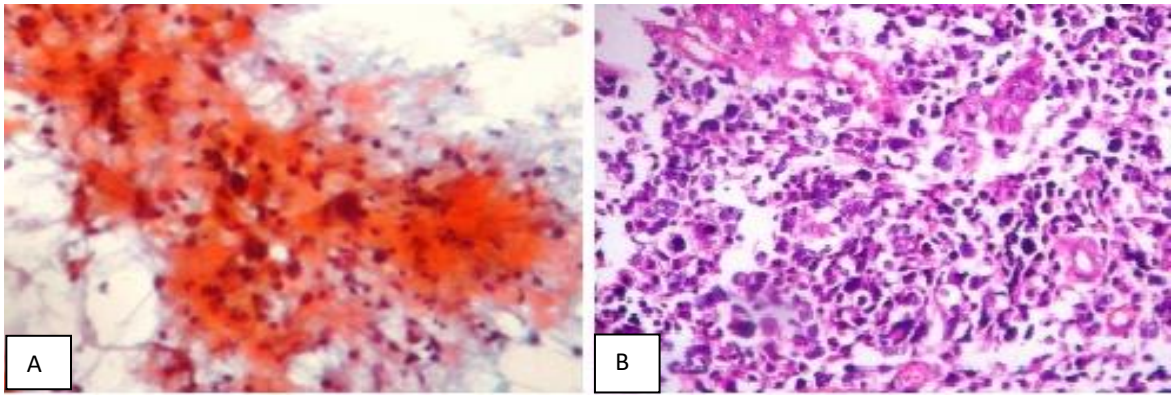

Figure 7: Anaplastic medulloblastoma. A, highly pleomorphic cells in a necrotic and fibrillary background, diagnosed as glioblastoma. Pap stain 10X. B, pleomorphic tumour cells, few giant cells and cell wrapping in paraffin sections. H & E 10X

One case of oligodendroglioma was misdiagnosed as low grade astrocytoma on cytology. This is observed in many other studies as well. Smears from oligodendrogliomas are highly cellular, show discohesive relatively small tumour cells, scant wispy cytoplasm, uniform round dark nuclei, finely granular chromatin with no identifiable cytoplasmic borders [10]. Calcification and microgemistocytes may be present [17]. Definitive diagnosis of oligodendroglioma is possible mostly on histology sections, due to presence of perinuclear halo which is a processing artefact and the "chicken wire" pattern of blood vessels. (Figure 8) These characteristic features are not seen in cytology and frozen section preparations, thus making intraoperative diagnosis of low grade oligodendrogliomas difficult.

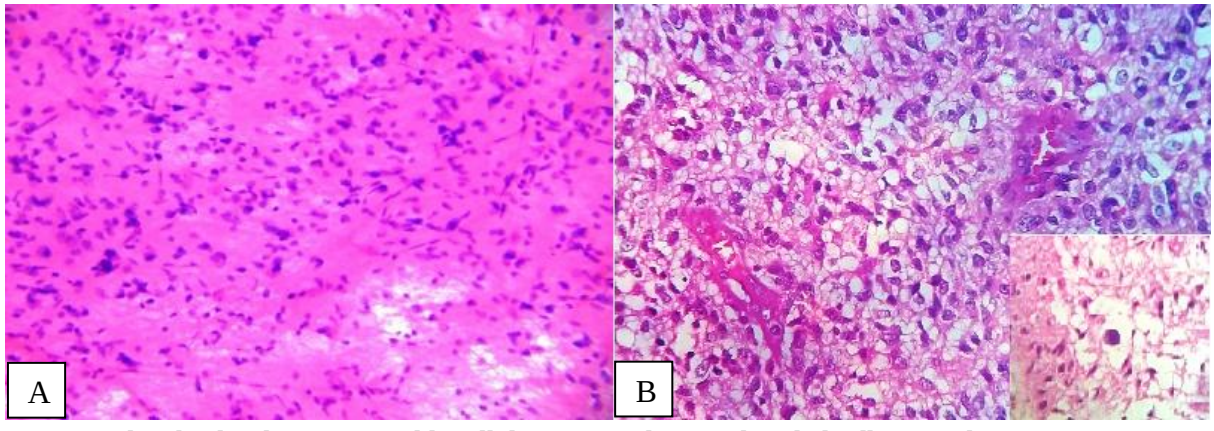

Figure 8: Oligodendroglioma. A, Highly cellular smear, pleomorphic glial cells. Squash smear H&E 10X. B, neoplastic oligodendroglial cells surrounded by a clear halo, thin walled blood vessels (chicken wire pattern), Inset - Calcification. Paraffin section H&E 40X.

One case of arachnoid cyst was seen in the present study. Arachnoid cysts are accumulations of CSF in the leptomeninges, occurring mostly in the posterior fossa or temporal lobe areas. These cysts are lined by an attenuated layer of meningothelial cells. Cytology smears might show flattened sheets of polygonal cells [18,19]. However in our case, squash smears showed only mixed inflammatory cells and giant cells in a necrotic background. Therefore a diagnosis of inflammatory lesion was given intraoperatively. Permanent sections showed cystic structures lines by flattened cells with focal giant cell reaction. (Figure 9) Thus establishing a final histopathological diagnosis of arachnoid cyst.

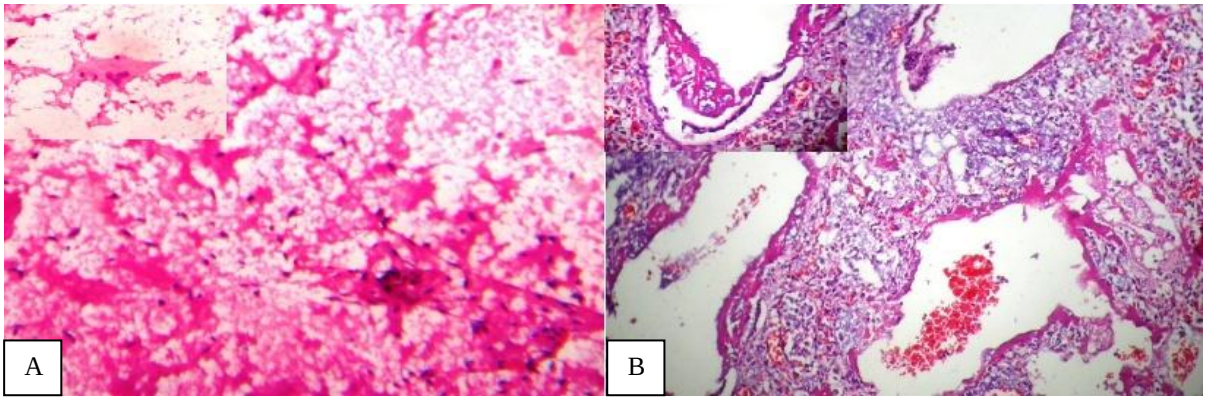

Figure 9: Arachnoid cyst. A, Smear showing chronic inflammatory cells in a background of necrosis. Inset – giant cell. B, Arachnoid cyst. Cyst wall lined by arachnoid cap cells, inset - giant cell reaction. Paraffin section H&E 10X

Improper grading of CNS tumours on cytological preparations has been documented in many studies. As in the present study a case of low grade astrocytoma was over diagnosed as glioblastoma on cytology. Many authors consider it inappropriate to grade tumours on cytology, as tumours vary in grade from one area to another within a single tumour. In undergraded cases with both less and more aggressive astrocytomas, cytological sampling might fail to show the anaplastic component. Thus small biopsies are unsuitable for grading malignancies [10].

Over all diagnostic accuracy of intraoperative squash cytology in the present study was 91.52%. This is similar to the diagnostic accuracy obtained in the studies done by Kini *et al* (86%) [10], Shukla *et al* (87.76%) [12], Khamechian *et al* (84%) [14] and Nigam *et al* (89.3%) [13]. (Table II)

Table II.Comparision of overall accuracy rates of squash cytology in diagnosis of CNS lesions with other studies

Sl No	Studies	Accuracy rates (%)	Number of cases
01	Roessler *et al*[11]	95	4,172
02	Kini *et al*[10]	86	100
03	Iqbal *et al*[20]	95.36	151
04	Ghoshal *et al*[17]	93	306
05	Shukla *et al*[12]	87.76	278
06	Kamechian *et al*[14]	84	139
07	Nigam *et al*[13]	89.3	75
08	Mitra *et al*[21]	88.5	114
09	Shah *et al*[22]	89.7	183
10	Verma *et al*[23]	88.9	63
11	Torres *et al*[24]	97.3	650
12	**Present study**	**91.52**	**59**

The diagnostic accuracy in the present study was slightly lesser than that obtained in the studies carried on by Roessler *et al* (95%) [11], Iqbal *et al* (95.36%) [20] and Ghoshal *et al* (93%) [17]. This difference is probably due to larger sample size and longer duration of study in these studies compared to the present study.

Recent advances in detection and management of CNS lesions have placed great onus on pathologists for accurate diagnosis of these lesions. Therefore it is important that proper radiological and clinical data be reviewed before intraoperative evaluation of CNS lesions.

5. Conclusion

The aims of present study were to study the cytomorphology of various CNS lesions and to assess the utility of intraoperative cytology as an adjunct to frozen section in the diagnosis of these lesions. We understand that the number of cases in certain categories was insufficient to draw definitive conclusions; however an overall accuracy rate of 91.52% was obtained which is in comparison with other studies. Thus cytology has emerged as a useful and preferred method for intraoperative diagnosis, as cellular morphology is sharply defined; ice artefacts and other distortions introduced by frozen section technique are avoided. In case of scanty tissue sent for frozen section, squash smears can be reliably used to give an impression intraoperatively with expert opinion and optimum clinical details. Therefore cytology aids in reliable intraoperative diagnosis and guidance during targeting and resecting lesions in neurosurgery. In conclusion, squash smear technique is fairly accurate, relatively safe, rapid and simple tool for intraoperative diagnosis of CNS lesions.

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