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Research Article

Squash Smear Technique in Rapid Intraoperative Diagnosis of Non-neoplastic and Cystic Lesions of CNS

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Abstract

In neurosurgical practice, a rapid intraoperative diagnosis helps the neurosurgeon to monitor and modify the approach at surgery. Smears and frozen sections are the two rapid tissue preparations that may be used by neuropathologists for giving opinion on intraoperative biopsy specimens of suspected lesions. The smear technique, which could be either squash/crush smears or impression smears, has been applied in neurosurgical units worldwide. This method plays a very important role in the analysis of sample from craniotomies and the small specimens obtained from stereotactic biopsies.

Squash smear technique is a very rapid technique and the study was designed to assess the accuracy of squash smear in the rapid intraoperative diagnosis of non-neoplastic and cystic lesions of CNS and to document the cytomorphology of these lesions. A total of 23 cases were studied. The tissue was removed at craniotomy or by burr hole biopsy.

In all the cases clinicoradiological correlation was done with smear diagnosis. Smears were stained with 1% Toluidine blue and H&E. In all cases, results were compared with the paraffin section prepared from the tissue remaining after the squash smears. Immunohistochemistry was done in one case. Special stains were used as required.

Amongst non-neoplastic lesions, tuberculous lesions comprised the maximum number of cases (n=9), two cases of PML, one case of fungal lesion, one case of non-specific abscess, one case of infarct, only reactive changes in one and normal cortex and white matter in one. In cystic lesions of the CNS, there was one case of Rathke's Cleft cyst, four cases of Epidermoid cyst and two cases of arachnoid cyst. Cases of PML and Rathke's cleft cyst could not be identified on smears. In all the cases, smear diagnosis was compared with histopathological diagnosis.

Keywords: Squash smear, Intraoperative diagnosis, Infective lesion, Cystic lesion, Non neoplastic lesion

Introduction

Lesions of central nervous system are diverse comprising of variety of neoplastic as well as non-neoplastic lesions. Diagnosis of these lesions is one of the most challenging domains of neuropathologists. A broad spectrum of space occupying non-neoplastic lesions may mimic tumors, both clinically and radiologically. Non-neoplastic lesions belong to different etiologies like infections, demyelination, infarcts, reactive changes, etc.

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In this study, we have included a series of cases that presented both clinically and radiologically as intracranial or intraspinal mass lesions with a possibility of non-neoplastic lesion, and cases of cystic lesions of CNS. There can be significant overlap in radiological findings between neoplastic and non-neoplastic lesions. Both neoplastic as well as non-neoplastic lesions can produce abnormal contrast enhancement, mass effect and perilesional edema on both CT and MRI. Clinically also, non-neoplastic lesions may mimic symptoms of tumor.

The role of rapid intraoperative diagnosis is crucial to neurosurgeon to not only define surgical approach, but also to ensure that minimum injury is caused to normal structures surrounding the lesion. But with the advent of stereotaxic biopsies as a neurosurgical procedure to establish preoperative diagnosis of intracranial space occupying lesion, has resulted in renewed appreciation of the value of the smear preparation, because very small specimens are obtained with this procedure. Squash smear technique is a very rapid technique and enables a neuropathological diagnosis within five minutes of receiving the tissue, and thus the lesion is diagnosed and neoplasia is confirmed or excluded. This is most important as further surgical management depends on the diagnosis, e.g., maximum resection of some primary brain tumors as meningioma and medulloblastoma, brachytherapy for high grade gliomas, and immediate postoperative medical treatment for some non-neoplastic lesions, e.g., tuberculosis, brain abscess and infarct.

The smear technology is suitable for almost the entire spectrum of CNS lesions. Brain tissue is usually very soft and edematous, making it easy to smear whereas with cryostat sections, the edema in the tissue makes it prone to freezing artefacts, which can mar the histologic details.

Another advantage is that even infected specimens may also be processed (e.g., AIDS, viral encephalitis, syphilis, etc.) without the risk of contamination of the cryostat.

This study was designed to assess the accuracy of intraoperative squash smear in the rapid intraoperative diagnosis of non-neoplastic and cystic lesions of CNS.

Materials and Methods

The study was conducted in the departments of Pathology and Neurosurgery, GB Pant Hospital, New Delhi. All patients in which either clinical diagnosis or differential diagnosis of non-neoplastic lesion/cystic lesion was considered, were enrolled in the study. 23 consecutive biopsy specimens of suspected non-neoplastic CNS lesions were studied and rapid intraoperative diagnosis was rendered to the neurosurgeon. Patients were of varied age groups with various clinicoradiological diagnoses.

The clinical details of the patient, including history of present illness, any significant past history, neurological examination, and radiological findings (CT/MRI/X-Ray) were obtained prior to the surgery. The space occupying lesion varied from single to multiple. The specimen for study was obtained immediately after it was removed intraoperatively from the affected area/areas by the neurosurgeon, in a covered container, without any fixative. Small 1-mm sized fragments were selected from multiple sites in the sample to ensure adequate sampling. For making the smear, sample was gently crushed by a second slide held at right angles. The slide was gently rotated to spread the specimen evenly across the base before it was drawn along the length of the labelled slide.

Smears were stained both with toluidine blue and rapid H&E method. Toluidine blue method that we have used in our study was introduced by Russel et al.[1] Goel et al.[2] had also used toluidine blue in their study. In this method, smears prepared were fixed with absolute methanol for 1 min, stained with toluidine blue for 1 min, rinsed in tap water and then dried on the hot plate, cleared in xylene, and mounted in DPX.

In rapid H&E method, smears were fixed in absolute methanol for 1 min, rinsed in tap water, stained with Harris hematoxylin for 2–3 min, rinsed in tap water followed by two quick dips in acid alcohol, then dipped in ammonia water till the smears were bright blue. Slides were then rinsed in tap water and stained with eosin solution for 30 to 45 sec. Special stains like Z-N stain and luxol fast blue were done, wherever necessary, and immunocytochemistry was done in one case.

A diagnosis on the smear appearances was communicated to the neurosurgeon and the remaining tissue left after smear preparation was processed for paraffin sections for subsequent correlation.

Results

We studied 23 cases of CNS lesions in which the lesions were non neoplastic or cystic, and assessed the accuracy of squash smears in the diagnosis of non neoplastic and cystic lesions of CNS.

For interpreting the smears, preliminary information regarding age and sex of the patient, relevant clinical details and site of biopsy were all taken into account.

The appearances described are on toluidine blue and rapid H&E stained smears. In toluidine blue stained smears, nuclei appeared blue and cytoplasm and processes, various shades of grey blue. Metachromatic material, if present, stained reddish violet. In rapid H&E method, nuclei appeared blue and cytoplasm appeared pink.

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Various non-neoplastic lesions diagnosed in our study are:

Normal cortex and white matter was reported in a 54-year-old patient with multifocal white matter hypodense lesions which were non contrast enhancing. Clinicoradiological impression was ?Metastatic ?Tubercular. But, however, the first biopsy specimen showed only normal white matter and cortex. Smear showed pyramidal-shaped large neurons with nuclei showing prominent nucleolus, glial cells with smaller nuclei, distinctively blue staining neuropil in background. Other portions of smear showed astrocytes and oligodendroglial cells. On smears, therefore, it was reported as normal cortex and white matter. Because of strong clinical suspicion, a second biopsy from suspected area was taken, which was finally reported as progressive multifocal leucoencephalopathy.

In one case, only reactive changes were seen in the smear. This was from a 53-year-old patient, presented with multifocal white matter lesions. Smears showed much paler neuropil in the background, edema, and slightly increased cellularity. Reactive astrocytes were large with more eosinophilic cytoplasm, well-defined cell margins, and processes in H&E stained smears. In toluidine blue smears, a slightly larger nucleus with coarse, evenly distributed chromatin, and occasional prominent nucleolus was appreciable. Lipid phagocytes, reactive micro glial cells, and capillary proliferation with prominent endothelial cells were also seen (Fig. 1).

A diagnosis of infarct was made in one case in a 60-year-old male patient who had presented with paraparesis, and MRI showed subcortical zones of decreased density in the left post frontal and partial area with irregular contrast enhancement. Possibility of ?infarct ?low-grade glioma was considered. Smears showed tissue from white matter as well as cortex. Background showed edema with much paler neuropil. Cellularity was increased with presence of reactive astrocytes with variation in nuclear sizes and numerous foamy macrophages. Capillary network was more prominent. Diagnosis of infarct was made in keeping with MRI findings also. Subsequent paraffin section was consistent with infarct.

Two patients 53- and 54-year-old were finally diagnosed as progressive multifocal encephalopathy, but could not be diagnosed on smears. They had presented with subacutely evolving motor deficits, memory impairment and headache. MRI revealed multiple, discrete white matter lesions. These were hypodense, non-contrast enhancing and not associated with mass effect. Clinically suspected diagnosis was ?metastatic ?tubercular. In the first case, first specimen revealed only normal cortex and white matter. Second specimen was obtained which showed numerous reactive astrocytes with bizarre nuclei, giant cells and numerous foamy macrophages and necrosis in the background (Fig. 2). A diagnosis of granulomatous lesion was made on the smears ?tubercular.

The corresponding paraffin section showed much enlarged oligodendroglial nuclei (about 4 times the normal size), which were hyperchromatic with smudged, homogenously, dense chromatin and with basophilic inclusions. Foamy macrophages and perivascular lymphocytic cuffing was prominent. Reactive astrocytes with bizarre nuclear abnormalities and giant cell forms were seen. GFAP done on sections confirmed astrocytic origin of the giant cells (Fig.3). Luxol fast blue stain confirmed presence of extensive demyelination. A diagnosis of progressive multifocal leucoencephalopathy (PML) was thus made.

In the second case, smears and corresponding paraffin section of the biopsy showed only reactive change. Diagnosis of PML was made on remaining tissue removed at surgery.

In this study, there were nine cases which were diagnosed as tuberculous. Six cases were diagnosed as tuberculomas on squash smears. One case was from cerebellar space occupying lesion and five cases with thoracodorsal vertebral body destruction, disc prolapse and soft tissue epidural mass. Possibility of tuberculosis was considered in these cases. The smears showed inflammatory background with presence of lymphocytes, plasma cells along with epithelioid cell granulomas and langhans giant cells in few and caseous necrosis in the background. Stain for AFB was negative. Final diagnosis was spinal tuberculomas and cerebellar tuberculoma (Fig. 4).

Four patients aged 7, 13, 21, and 23 had presented with headache, vomiting and ataxia. MRI showed a well-defined, rounded, space occupying lesion with an enhancing rim in the cerebellum. Clinicoradiological impression was of a granulomatous lesion ? Tubercular ? Fungal. The smears in three cases showed inflammatory background with presence of lymphocytes, plasma cells along with epithelioid cell granulomas and langhans giant cells in few and caseous necrosis in the background. In two cases from cerebellum and one from spinal lesion, Z-N staining showed a large number of bacilli. Diagnosis of cerebellar abscess was made in cerebellar SOL in keeping with the MRI findings. Spinal lesion was diagnosed as tuberculous lesion, which was finally diagnosed as TB abscess on paraffin section. Smears from one of the cases of cerebellar space occupying lesion showed granulomatous inflammation and presence of fungal hyphae and spores. Diagnosis of fungal infection was made.

A diagnosis of non-specific abscess was made in a 32-year-old patient presenting with seizures and neurological deficits. MRI revealed irregular areas of decreased density in the parietal lobe with associated edema. There was a ring of contrast enhancement around this. Clinicoradiological

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impression was granulomatous disease ?Tubercular ? Fungal.

Smears showed necrotic background with mixed inflammatory infiltrate, foreign body-type giant cells, and foamy macrophages. There was absence of epithelioid cells or any fungus. Z-N staining was negative. Diagnosis of a non-specific abscess was made on smear.

Corresponding paraffin section showed findings of non-specific abscess.

A case of Rathke's cleft cyst was diagnosed in a 12-year-old child, who had presented with headache and visual disturbance. MRI showed an intrasellar cystic lesion. Diagnosis of arachnoid cyst or a cystic pituitary adenoma was kept in mind. The tissue was firm and did not spread well. However, smear showed a cystic lining by a single layer of monomorphic cells with little cytoplasm and vesicular nucleus and isolated, and groups of similar cells were seen. Diagnosis of arachnoid cyst was made on smears, but paraffin section was consistent with Rathke's cleft cyst. The subepithelial cells at places showed pituitary cells. Thus there was failure to type this cystic lesion correctly.

Four cases of epidermoid cyst were diagnosed in our study in patients aged 20–40 years, presenting with headache, dizziness and seizures. MRI revealed a predominantly cystic tumor at CP angle/prepontine region, which was focally solid and there was minimal contrast enhancement. Clinicoradiological impression was ?epidermoid cyst ? meningioma ?hemangioblastoma. The contents of the cyst were easy to spread whereas cyst wall was firm to spread. Smears showed keratinous debris and flakes, groups of nuclear squames, foreign body-type of giant cells, and foamy macrophages diagnosis of epidermoid cyst was made on smears, confirmed on paraffin section too.

In two cases, diagnosis of arachnoid cyst was correctly made on smears. CT revealed a low-density area in the left temporal region with expansion of bony middle fossa. The other case showed well circumscribed cystic lesion in CP angle. Clinicoradiological impression was ?arachnoid cyst ? meningioma in first case and ? arachnoid cyst ? epidermoid cyst in the second case.

The biopsy tissue was firm to smear. Smears showed meningothelial cells in irregularly placed groups and at places lying scattered with occasional whorl formation (Fig. 5). Diagnosis of arachnoid cyst was made on the smear in keeping with the characteristic findings on MRI and gross findings too. Paraffin section was also consistent with arachnoid cyst.

Table 1

S. No.	Smear Diagnosis	No. of Cases	Paraffin Diagnosis/Final Diagnosis	Accuracy	Overall Accuracy
1	Normal cortex and white matter	1	Normal cortex and white matter	100%	87.5%
2	Reactive changes	1	Reactive changes	100%	
3	Reactive Changes and granulomatous	2	PML	0%	
4	Infarct	1	Infarct	100%	
5	Tuberculous lesion	1	Tuberculoma cerebellum	100%	
6	Tuberculous lesion AFB positive	3	Tuberculous abscess	100%	
7	Tuberculous lesion	5	Spinal tuberculoma	100%	
8	Non-specific abscess	1	Non-specific abscess	100%	
9	Fungal infection	1	Fungal infection	100%	

Table 2

S. No.	Smear Diagnosis	No. of Cases	Paraffin Diagnosis/Final Diagnosis	Accuracy	Overall Accuracy
1	Arachnoid cyst	1	Rathke's cleft cyst	0%	85.7%
2	Epidermoid cyst	4	Epidermoid cyst	100%	
3	Arachnoid cyst	2	Arachnoid cyst	100%	

Thus, amongst non-neoplastic lesions, tuberculous lesions comprised maximum number of cases (n=9), two cases of PML, one case of fungal lesion, one case of non-specific abscess, one case of infarct, only reactive changes in one and normal cortex and white matter in one case. In cystic lesions of the CNS, there was one case of Rathke's cleft cyst, four cases of epidermoid cyst and two cases of arachnoid cyst. Cases of PML and Rathke's cleft cyst could not be identified on smears.

Accuracy in diagnosis of tuberculous lesions, fungal infection, infarct, and reactive changes was 100% on smears. Overall accuracy in diagnosis of non-neoplastic lesions was 87.5% (Table 1) and diagnostic accuracy for cystic lesions of CNS was 85.7% (Table 2) in our study after cytogical and histopathological correlation.

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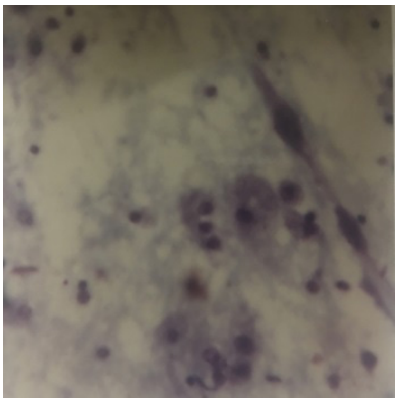

Figure 1.(1) Smear: Reactive changes showing edema and prominent endothelial cells . Toluidine bluex200.

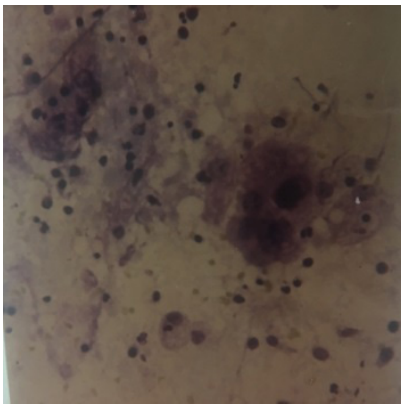

Figure 2.(2) Smear: Case of PML showing Giant cells and foamy macrophages besides other cells. Toluidine bluex200.

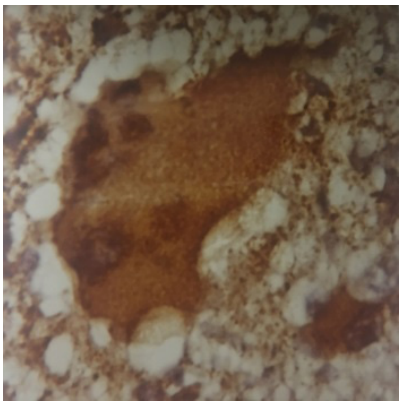

Figure 3.(3) Section: Case of PML showing GFAP positive Giant cell. GFAPx400.

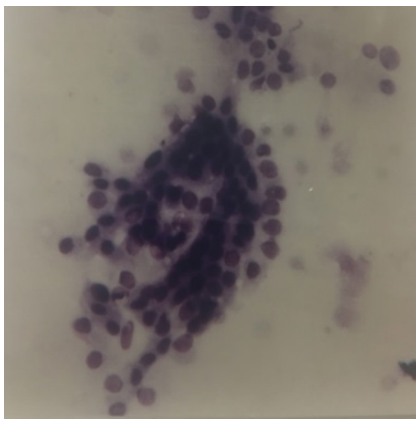

Figure 4.(4) Smear: A case of Tuberculoma. Toluidine bluex200.

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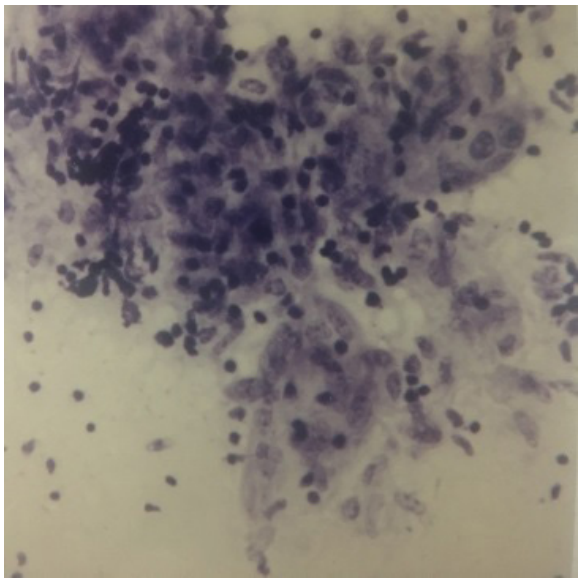

Figure 5.(5) Smear: A case of arachnoid cyst showing meningothelial cells with focal whorling.Toluidine bluex200.

Discussion

The intraoperative cytology preparations were first introduced by Eisenhardt and Cushing[3] in early 1930s.

The squash smear technique is a very rapid technique and very advantageous for offering rapid intraoperative diagnosis to the operating neurosurgeons. The technique is best suited for neurosurgical specimens because they are soft, gelatinous and easy to smear; however some difficulty was encountered in some of the lesions.

The first biopsy specimen of a case of progressive multifocal leucoencephalopathy revealed only normal cortex and white matter. The error in sampling was from the neurosurgeons side, who had not sampled the representative lesion. Diagnosis of normal cortex and white matter could be made on smears, and rapid communication of report to operating neurosurgeon facilitated the neurosurgeon to take another sample. So, in this study, normal cortex and white matter could be identified with 100% accuracy.

Reactive changes were identified in 1+(2) cases. The first case, which was finally diagnosed as PML, showed only reactive changes. This was again a sampling error from the neurosurgeon's side. Reactive changes were also identified in cases of infarct and non-specific abscess. In these cases, thick fibrillary gliosis was seen as presence of numerous large, fiber-forming astrocytes along with reactive astrocytes. Capillary formation was conspicuous along with prominent endothelial cells (Fig. 1). So, accuracy of diagnosing reactive changes in this study was 100%.

Results of previous studies show that reactive gliosis was misdiagnosed as low-grade astrocytoma. Jindal et al.[4] reported misdiagnosis of two cases of reactive gliosis secondary to tuberculomas as low-grade astrocytoma. It is a big challenge as there is marked difference in prognosis and therapy after pathological diagnosis. Zengotita[5] mentions some common diagnostic pitfalls as gliosis associated with brain tumor mimics, including demyelinating diseases and infections, and gliosis associated with non-glial tumors such as craniopharyngoma. Bushati et al.[6] excluded the possibility of glioma on smears and reported reactive gliosis. However, on histopathology, case was finally reported as corticaldysplasia.

Reactive gliosis can be seen in association with non-neoplastic, neoplastic or cystic lesions. Das et al.[7] found florid reactive gliosis in a case of craniopharyngoma and states that it may be mistaken for low-grade astrocytoma and hence care must be taken while diagnosing supra seller lesions. Govindaraman[8] also shared a similar experience.

Diagnosis of infarct was made in one case. Clinicoradiologically, possibility of low-grade glioma was considered. On smears, possibility of tumor was not considered because of the polymorphous nature of the cell content. Possibility of infarct was thus considered after correlating smear findings with the MRI findings.

In this study, there were two cases which were finally diagnosed as PML. In one case, the first biopsy specimen had shown only cortex and white matter, and diagnosis of PML was established on second specimen after immunocytochemistry on smears. In the second case, the biopsy showed only reactive changes, and diagnosis of PML was established on rest of the tissue sent later for histopathological examination. In the first case, smears from second specimen showed numerous reactive astrocytes with bizarre nuclei, oligodendroglial cells, giant cells (Fig. 2) with multiple nuclei, numerous foamy macrophages along with lymphocytes and plasma cells and presence of necrosis in the background. A diagnosis of granulomatous lesion

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was made on the smears? tubercular. The corresponding paraffin sections showed enlarged oligodendroglial nuclei with basophilic inclusions. Another supporting evidence was the demonstration of extensive demyelination revealed by luxol fast blue stain done on smears and GFAP staining done on smears which showed astrocytic nature of the giant cells (Fig. 3). On review of the smears, enlarged hyperchromatic oligodendroglial nuclei with amphophilic to basophilic inclusions could be appreciated too.

In this study, it was possible to diagnose tubercular pathology with 100% accuracy (n=9 cases) and it was possible due to extensive sampling of the tissue so as to pick up characteristic epithelioid cell granulomas, and positive staining for AFB clinched the diagnosis in three cases.

Sundaram[10] found tuberculosis as the commonest infective lesion of CNS (70.83% cases).

Jindal et al.[4] reported misdiagnosis of tuberculomas in two cases as low-grade astrocytoma. The error was due to misinterpretation of reactive gliosis as tumor.

In one case, smears showed granulomatous inflammation with presence of fungal hyphae and spores.Diagnosis of fungal infection was made on smears. In two cases from cerebellum and one from spinal lesion, Z-N staining showed large no of bacilli which clinched the diagnosis of tuberculous abscess. Desai et al.[11] reported intracranial fungal granulomas can be misdiagnosed clinically and radiologically as neoplastic lesions. Deshpande et al.[9] reported accurate diagnosis of fungal infection in two cases in their study.

In this study, there was one case which was diagnosed as non-specific abscess. Smears showed necrosis, polymorphous cell infiltrate and foreign body giant cells. No epithelioid cell granulomas were seen. Z-N staining for AFB was negative and no fungus was also seen. A diagnosis of non-specific abscess was therefore made.

In this study, there was one case which could be diagnosed only on paraffin section as Rathke's cleft cyst. It showed cystic spaces lined by single layer of cuboidal epithelium and at places ciliated epithelium. The sub-epithelial tissue at places showed pituitary cells. There was failure to diagnose it on smears. The tissue was firm and did not spread well, and also, sampling error, whereby the pituitary component was missed.

Four cases of epidermoid cyst could be correctly diagnosed on smears. Characteristic smear findings of keratinous debris, groups of squamae, foreign body giant cells and foamy macrophages, and coupled with MRI finding of a cystic lesion at CP angle enabled the correct diagnosis. Deshpande et al.[9] reports correct diagnosis of all 13 cases of epidermoid cyst correctly on squash smears in their study. Das et al.[7] also diagnosed a case of epidermoid cyst on smears correctly.

Two cases of arachnoid cyst could also be correctly diagnosed on smears. Smears showed scattered and groups of meningothelial cells with occasional whorl formation. Diagnosis was made in keeping with the characteristic CT and gross findings. Deshpande et al.[9] reports correct diagnosis of two cases of arachnoid cyst on squash smear cytology. Nanarng et al.[12] reports missing a case of arachnoid cyst intraoperatively due to sampling error on cytology.

Other cystic lesions reported in other studies are hydatid cyst by Aggarwal et al.[13] with findings of eosinophilic laminated layers, and mucocele in sellar region by Jha et al.[14]

Thus we found a fairly good overall diagnostic accuracy of squash smears in non-neoplastic lesions and cystic lesions of CNS also. Many studies have shown comparable diagnostic accuracy of squash smears in neoplastic as well as non-neoplastic and cystic lesions.

Conclusion

Squash smear is a simple, rapid and fairly accurate diagnostic tool for rapid intraoperative diagnosis and consultation in all kinds of CNS lesions. Smears provide good cytomorphology and with good clinicoradiological correlation, squash smear technique could be the preferred method for intraoperative diagnosis of CNS lesions.

Conflict of Interest: None

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