Correlation of Intraoperative Cytological and Final Histological Diagnoses

A Retrospective 10-Year Study of Neurosurgical Cases From Ibadan, Nigeria

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Objective: *Intraoperative cytology is a cost-effective, rapid, and easy technique, and studies have shown good correlation between intraoperative cytology and histology. We undertook this study to compare the intraoperative cytology diagnoses of brain lesions made in our unit over a 10-year period with the definitive histological diagnoses. The aim was to determine the degree of accuracy of this procedure.*

Study design: *This is a retrospective study of intraoperative neuropathology consultation cytology smears or imprints and histology of 69 cases obtained over a 10-year period. Cytology smears were stained using both Papanicolaou and Giemsa. Histology sections were prepared from routine formalin-fixed paraffin-embedded tissue and stained using H and E method. Each of the smears and histology samples were assessed by at least two pathologists. Cytological diagnosis was correlated with final histological diagnosis. The sensitivity and specificity of cytological diagnosis was evaluated using final histological diagnosis as gold standard.*

Results: *Correlation was strongest with inflammatory lesions followed by low-grade neoplasms. High-grade neoplasms also showed good concordance, but the degree of correlation was lower than in the other categories. Misdiagnosis was commonest with benign tumors.*

Conclusion: *Intraoperative cytology is a relatively simple, reliable, and accurate diagnostic technique and should be more commonly used, particularly in low-resource settings.* Diagn. Cytopathol. 2015;43:195–201.

Key Words: cytology; intraoperative; correlation; histology

One of the central roles of the histopathologist is the provision of timely diagnoses that can impact significantly on surgical management. This is particularly pertinent in the intraoperative management of neurosurgical cases. Close collaboration and unfettered two-way communication between the neurosurgeon and the histopathologist is a *sine qua non* for the optimal management of these cases.

In developing countries, intraoperative consultation usually presents considerable and peculiar challenges. The gold standard for intraoperative consultation is the provision of frozen section services. This is, however, often not possible because of lack of functional equipment, constant interruptions in electricity supply, and unavailability of carbon dioxide or liquid nitrogen.[1,2] In such circumstances, an acceptable substitute for frozen section is intraoperative brain smear cytology.

Intraoperative brain smear cytology is cheap, rapid, simple, and inexpensive.[2] Several studies have shown a good level of comparison between frozen section and intraoperative cytological diagnoses.[1,3] One of the main advantages of intraoperative cytology is the ability to maximally use small samples to achieve more than can be done with frozen section.[4,5] Typically, the cytology specimen may not usually show the three-dimensional architecture of the tissue, but it often displays to good effect the cytological details of the individual cells,

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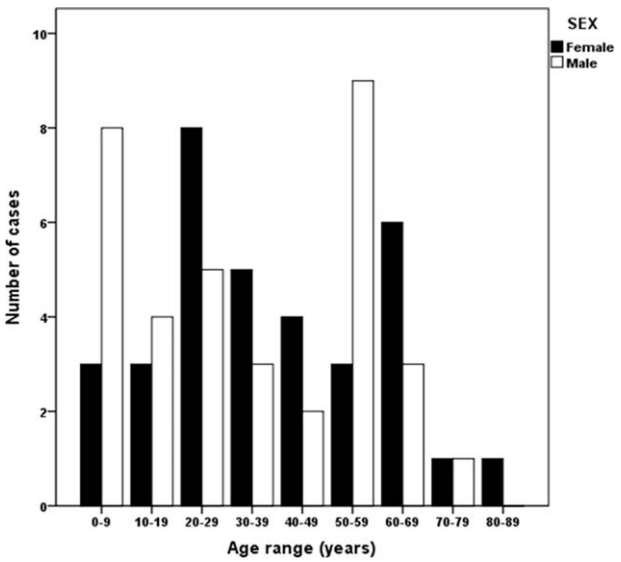

Fig. 1. Bar chart showing the age and sex distribution of patients in the study.

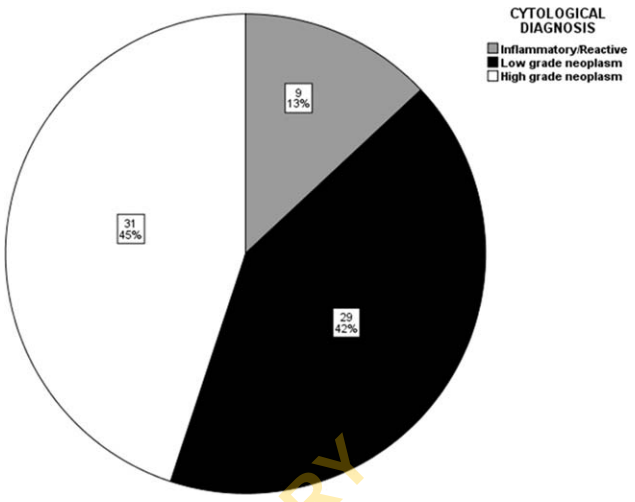

Fig. 2. Pie chart showing the distribution of the three categories of lesions in the study.

particularly the nucleus. [5] In some cases, the diagnostic features often seen in histological specimens still appear visible.[4,5] These features are often lost in frozen section specimens because of the alterations made by ice crystal artefacts on the tumor cells.[4]

A study by Marshall et al.[6] in 1973, the first to compare intraoperative smears with final histological diagnosis of specimens from brain lesions, showed good concordance between the two techniques. Several other subsequent studies have also shown a relatively high level of concordance between diagnosis made from cytological smears and those from corresponding histological specimens.[1–3,7] This level of accuracy is further enhanced by the advantage of being able to produce multiple slides from the small tissue sample received. This allows better sampling of different areas from which diagnostic features may be seen. This is not often the case with frozen sections from which a single slide may have to be produced from the tiny section.[8]

We undertook this study to compare the intraoperative cytology diagnoses of brain lesions made in our unit over a 10-year period with the definitive histological diagnoses, so as to determine the degree of accuracy of this procedure.

Materials and Methods

The study is a retrospective study of intraoperative neuropathology consultation cytology smears or imprints and histology of corresponding specimen obtained over a 10-year period. Overall, 69 cases were suitable for the study comprising of imprints and smears. The cytology smears were stained using both Papanicolaou and Giemsa, whereas the histology was processed using the standard H and E method. Each of the smears and histology samples were assessed by at least two pathologists. The results were assigned a scale of 1, 2, and 3 for inflammatory, benign, and malignant, respectively, and analysis was made for degree of agreement, correlation, and statistical significance using SPSS statistical software version 20. The accuracy (total number of true positives and true negatives/number of tests), sensitivity (true positives/true positives + false negatives), and specificity (true negatives/true negatives + false positives) were also calculated.

Results

The age range of the patients varied from 5 months to 87 years, and samples were from both males and females with a male-to-female ratio of 1:1. The sex distribution for various age categories is as shown in Figure 1.

The main categories of cases seen include benign and malignant neoplasms and inflammatory lesions within the brain and spinal cord. There was a strong positive correlation between the cytology and histology with a Pearson's r of 0.509 and good agreement with kappa value of 0.603.

Malignant neoplasms constitute the highest percentage of the cases (45%) seen, followed by benign neoplasms, which accounted for 42%. Inflammatory cases were the least frequent lesions, constituting 13% of all cases seen (Fig. 2).

Meningiomas were the commonest cases seen in the benign category, whereas high-grade gliomas were more common in the malignant series. Inflammatory lesions had the highest accuracy of 88% with sensitivity and specificity of 87.5 and 94.9%, respectively (Table I).

Low-grade neoplasms showed a high specificity of 91.2% but a relatively lower sensitivity of 75.8% with an

Table I. Correlation of Cytology and Histologic Diagnosis Seen in the Study

Age	*Sex*	*Location*	*Cytological diagnosis*	*Histological diagnosis*
5 months	Male	Cerebral	Epidermoid cyst	Meningocele
1	Female	Left orbital	Meningioma	Meningioma
1	Male	Skull base	Grade II or III meningioma	Grade I meningioma
14 months	Female	Cerebral	Acute necrotizing inflammation	Acute suppurative inflammation
3	Male	Right hemispheric	Medulloblastoma	Medulloblastoma
6	Male	Frontoparietal	Malignant (PNET and grade III ependymoma)	Anaplastic ependymoma
7	Male	Posterior fossa	Malignant astrocytoma	Pilocytic astrocytoma
8	Male	Posterior fossa	Medulloblastoma	Pilocytic astrocytoma
9	Male	Suprasellar	Pilocytic astrocytoma	Pilocytic astrocytoma
9	Male	Cerebellum	Medulloblastoma	Medulloblastoma
9	Male	Cerebellum	Medulloblastoma	Medulloblastoma
12	Male	Cerebellar	Medulloblastoma	Medulloblastoma
14	Male	Paraspinal	Suspicious (not specified)	Malignant mesenchymal neoplasm
15	Male	Posterior fossa	Chronic necrotizing inflammation	Granulomatous inflammation
15	Female	Spinal	Reactive inflammatory lesion	Grade II astrocytoma
15	Female	Intraorbital	Meningioma	Meningioma
18	Female	Cerebral	Benign, reactive	Epidermoid cyst
18	Male	Parietotemporal	Low grade glioma or meningioma	Meningioma
20	Female	Posterior fossa	Inflammatory	Arachnoid cyst
21	Male	Temporoparietoccipital	Oligodendroglioma	Meningioma
21	Male	Pineal region	Malignant (atypical meningioma or dysgerminoma)	Pinealoblastoma
22	Male	Spinal	Acute on chronic inflammation	Acute on chronic inflammation
23	Female	Skull base	Chronic inflammation	Phycomycosis
24	Female	Left cerebral hemisphere	Infarct or low grade glioma	Oligodendroglioma
24	Female	Sellar	Meningioma	Meningioma
26	Female	Pineal region	Germinoma	Pinealoblastoma
26	Female	Pineal region	Germinoma	Pinealoblastoma
28	Female	Sellar	Craniopharyngioma	Pituitary adenoma
28	Male	Posterior fossa	Ependymoma, anaplastic astrocytoma, medulloblastoma	Medulloblastoma
29	Female	Intraspinal	Meningioma	Ependymoma
29	Female	Spinal	Acute on chronic inflammation	Grade III astrocytoma
30	Female	Anterior cranial fossa	Meningioma/craniopharyngioma	Ossifying fibroma
30	Female	Cerebellum	Medulloblastoma	Medulloblastoma
32	Male	Occipital	Meningioma	Meningioma
32	Female	Sellar	Pituitary adenoma	Pituitary adenoma
35	Male	Right frontal	High grade glioma (glioblastoma)	Glioblastoma
35	Female	Right parietal	Oligodendroglioma	Glioblastoma
39	Female	Petroclival	Meningioma	Meningioma
40	Male	Conus medullaris	Chordoma	Myxopapillary ependymoma
40	Female	Sellar	Meningioma	Meningioma
40	Male	Left hemispheric	Metastatic carcinoma	Metastatic adenocarcinoma
40	Male	Left hemispheric	Metastatic carcinoma	Metastatic adenocarcinoma
41	Female	Sellar	Craniopharyngioma	Craniopharyngioma
44	Female	Frontoparietal	Atypical meningioma	Meningioma
48	Female	Middle cranial fossa	Meningioma	Meningioma
50	Male		Inflammatory smear	Glomus tumor
50	Female	Frontotemporal	Meningioma	Meningioma
50	Male	Brain	Malignant (high grade glioma)	Glioblastoma
51	Female	Left frontal	Meningioma	Meningioma
52	Female	Sellar	Pituitary adenoma	Pituitary adenoma
52	Male	Extra-axial	Malignant astrocytoma (glioblastoma)	Meningioma
53	Male	Left parietal	Meningioma	Meningioma
53	Male	Parasagittal	Anaplastic astrocytoma or glioblastoma	Glioblastoma
55	Male	Spinal	Pleomorphic sarcoma	Granulomatous inflammation
55	Male	Right frontal	Low grade astrocytoma	Meningioma
55	Male	Right cerebellar	Metastatic carcinoma (possible thyroid or prostate)	Metastatic adenocarcinoma
56	Male	Parasagittal	Meningioma	Meningioma
60	Female	Parietal	Malignant astrocytoma (glioblastoma)	Malignant glioma
60	Female	Left parietal	Malignant (glioblastoma)	Glioblastoma
60	Female	Parietal	Malignant astrocytoma (glioblastoma)	Malignant glioma
61	Male	Anterior cranial fossa	Meningioma	Meningioma
61	Male	Left parietal	Meningioma	Meningioma
65	Female	Right cerebral	Atypical meningioma	Grade II Meningioma
65	Female	Frontoparietal	Malignant (glioblastoma)	Gliosarcoma
67	Female	Right parietal	Malignant meningioma	Rhabdoid meningioma
68	Female	Posterior fossa	Low grade astrocytoma	Cavernous hemangioma
68	Male	Right hemispheric	Low grade astrocytoma	Glioblastoma
70	Female	Paraspinal	Anaplastic carcinoma, lymphoma, sarcoma	Pleomorphic sarcoma
77	Male	Right frontal	Malignant glioma (glioblastoma)	Glioblastoma
87	Female	Posterior fossa	Glioblastoma	Glioblastoma

Table II. Cytological Features Causing Errors in Diagnosis With Clues to Accurate Diagnosis

Diagnosis	*Confusing features*	*Diagnostic clues*
Meningioma	May show fibrillary background causing a misdiagnosis of a glioma	Whorling Syncytial appearance Clustering and cohesiveness Intracytoplasmic inclusions
Pilocytic astrocytoma	May show foci of dysplastic cells simulating a high grade glioma Focal areas of increased cellularity	Presence of Rosenthal fibers and bipolar cells Bland nuclear features
High-grade gliomas	May show only inflammatory cells from the edge of the tumor Highly differentiated areas may be sampled giving an impression of benign cells	Sampling from multiple areas of the tissue needed to show more representative areas. Nuclear features of malignancy such as clumped chromatin and macronucleoli in a Papanicolaou stain will aid diagnosis
Cavernous hemangioma	Stromal cells gave a fibrillary appearance "Dirty background" due to hemorrhagic necrosis simulate a high grade lesion	Stromal cells show variation in shape compared with monomorphic tumor cells in glioma
Myxopapillary ependymoma	Degenerating cells may mimic physaliphorous cells. Uncommon location in the thoracic spine	Presence of papillary features may help in the diagnosis of myxopapillary ependymoma Bland nuclei in tumor cells can help in separating from a malignant neoplasm
Small round blue cell tumors	Sampling in focal areas of marked cellularity may cause a misdiagnosis of malignancy in a benign tumor	High cellularity of monomorphic cells with scanty cytoplasm Abnormal mitotic figures may be seen. Rosettes may be seen in some tumors
Chronic inflammation	Cells may show bizarre shapes in areas of chronic inflammation Areas of necrosis may also mimic high-grade gliomas	Multiple inflammatory cell types Multiple smears from tumor Nuclear features particularly with Papanicolaou stain

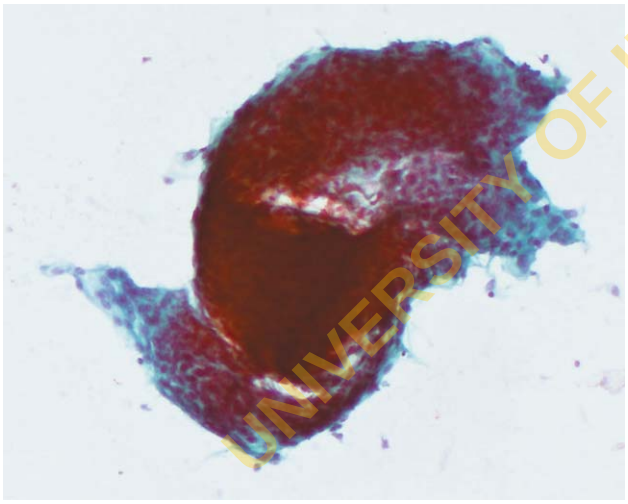

Fig. 3. Cytology smear showing syncytial-like clusters of epithelial cells arranged in whorls. Both the intraoperative and histological diagnoses were meningioma (original magnification ×250). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

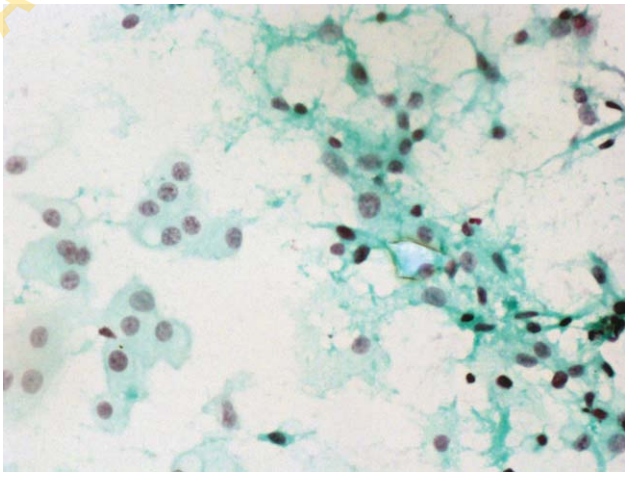

Fig. 4. Cytology smear showing elongated cells with moderate cytoplasm and bipolar nuclei. Both the intraoperative and histological diagnoses were pilocytic astrocytoma (original magnification ×400). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

accuracy of 84%. High-grade neoplasms also show a relatively high sensitivity and specificity of 84.6 and 89.5%, respectively, and an accuracy of 83%. Meningiomas were the commonest tumors to be misdiagnosed (Table I).

Glioblastomas and pinealoblastomas also showed a high number of false negatives (Table I). Pilocytic astrocytomas were seemingly often misdiagnosed as high-grade neoplasms.

Figures 3 and 4 show representative cases in which there was agreement between the intraoperative cytological diagnosis and final histological diagnosis.

Figures 5–9 show representative cases in which there was discordance between intraoperative cytological diagnosis and final diagnosis.

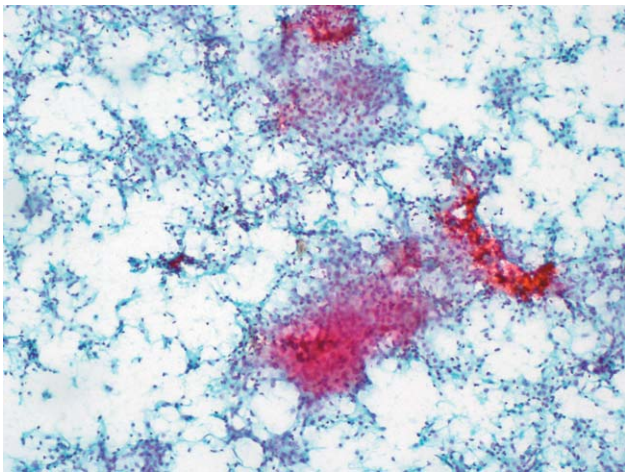

Fig. 5. Cytology from a case misdiagnosed as astrocytoma because of the "fibrillary" background (original magnification ×250). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

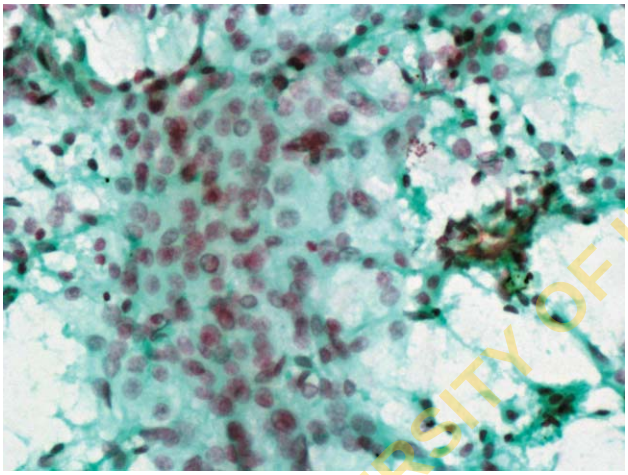

Fig. 6. Higher magnification as same case as in Figure 7 showing prominent cytoplasmic inclusions. The histological diagnosis was meningioma (original magnification ×250). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

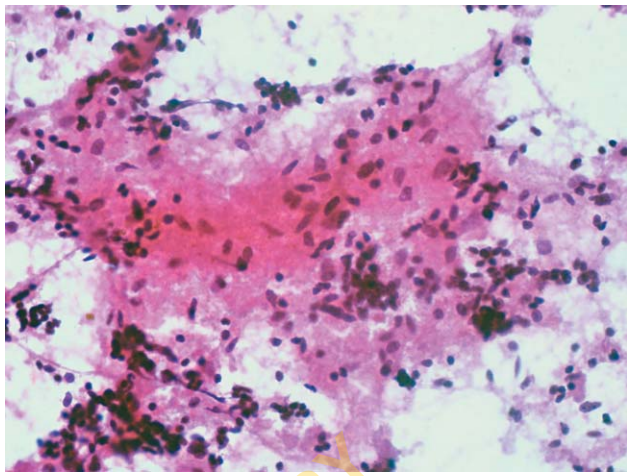

Fig. 7. Cytology of a case with erroneous intraoperative diagnosis of astrocytoma. The histological diagnosis was cavernous hemangioma. Note admixture of reactive stromal cells (original magnification ×250). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

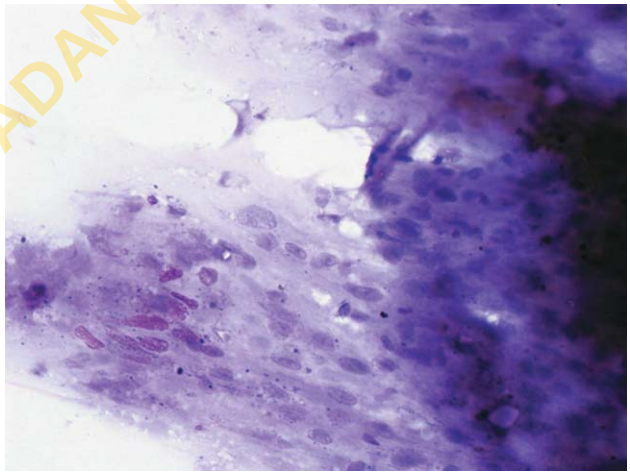

Fig. 8. Cytology of a case of myxopapillary ependymoma with intraoperative diagnoses of chordoma. Note the cells with intracytoplasmic bubbles mimicking physaliphorous cells (original magnification ×400). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Discussion

Making a diagnosis from an intraoperative smear can present a daunting challenge. The concordance rate obtained in this study is in keeping with results obtained by other investigators.[3,7] Although frozen sections are still considered to be a better method in intraoperative diagnosis because the tissue architecture and cellular outlines can be appreciated, cytology has been shown to have its place in making a fast evaluation for the surgeon.[9,10] The inherent softness of brain tissue, which may allow the formation of ice crystal artefacts and the fact that often small tissue samples are available for analysis, can make diagnosis from frozen sections to be difficult.

The pitfalls often seen in cytology may also cause a misdiagnosis to be made as can be seen in some of the cases in this study. Meningiomas are notorious for being too cohesive and giving poor yield as a result of clustering by the cells. Some of the cells in a cluster may show syncytial like appearance which may be the clue in making a diagnosis of the neoplasm. In some cases, however, the cells may lack the typical cohesiveness and have fibrillary background, which may simulate a glial tumor as was seen in this study.[9] Pilocytic astrocytomas are classified by the World Health Organization as a grade I neoplasm, which usually has an indolent course and is of

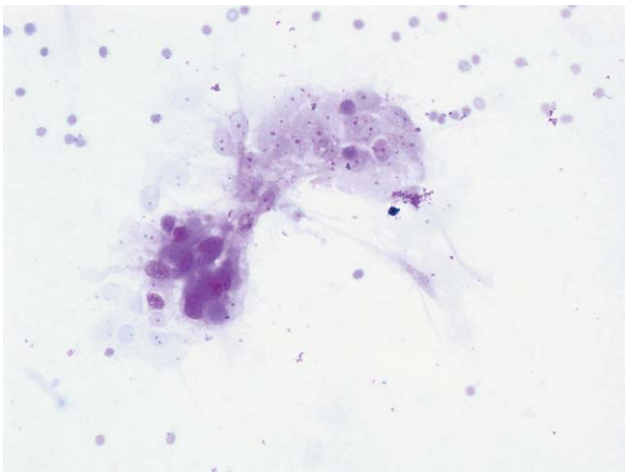

Fig. 9. Cytology from a case misdiagnosed intraoperatively as malignant astrocytoma. The histology was syncytial meningioma. Note the multiple nucleoli highlighted by the Giemsa stain (original magnification ×400). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

good prognosis. It is usually characterized by the presence of bipolar astrocytes with admixed Rosenthal fibres and granular eosinophilic bodies.[11] The occasional foci of dysplastic cells observed in some cases does not imply malignancy but can cause a misdiagnosis of a high-grade glioma as was seen in this study.[9,11,12] Bizarre cells are often seen in many benign lesions and cause confusion with malignant neoplasms. Sometimes, focal cellularity in a pilocytic astrocytoma may also create room for a misdiagnosis. In such cases, the absence of nuclear features such as clumped chromatin, irregular nuclear membrane, and prominent or multiple nucleoli are often helpful in arriving at the right diagnosis. Papanicolaou stain is particularly helpful in the diagnosis of meningioma by showing the cohesiveness of the meningothelial cells. This is in contrast to Giemsa staining, which, although good for highlighting the intracytoplasmic inclusions that may be seen in meningiomas, can sometimes overemphasize nuclear features such as the appearance of multiple nucleoli.[13] This can lead to a wrong diagnosis of a malignant tumor.

Small round blue cell tumors are a heterogeneous group, which usually show marked cellularity on smears. The cells are usually monomorphic and have scanty cytoplasm but the nuclear features of malignancy will also help in the diagnosis of these groups of tumors, particularly in cases that are more differentiated. The nuclei will usually show clumped chromatin and nuclear membrane irregularities that are best highlighted with the Papanicolaou stain. The presence of abnormal mitotic figures, if seen, can help in pointing to the diagnosis. Occasionally a benign tumor may show cellularity that is characteristic of these grade IV tumors due to the smear being made from an area of active proliferation, which may cause a misdiagnosis of a malignant lesion[9]. In some instances, smears may sometimes be made from more differentiated areas within the tumor, which may give an appearance of normal brain tissue or a benign lesion[6].

A significant proportion of high-grade gliomas may have inflammatory edges, a feature that can affect evaluation of a smear if the sample is taken from the inflammatory area.[6] Necrosis may be seen with many different lesions, including inflammatory, benign, and malignant lesions. Although in our environment there are many infections that may cause a necrotizing inflammation, especially in a setting of HIV/AIDS, it must also be borne in mind that neoplastic lesions can mimic inflammatory processes; therefore, necrosis in a smear requires cautious interpretation. The presence of chronic inflammatory cells may be helpful in some cases, but they are also seen in reactive tissues, thus limiting their usefulness.

One of the misdiagnosed cases recorded in our study was a cavernous haemangioma that was initially called an astrocytoma. The cytology of this lesion was misleading because of the abundance of stromal cells present in the lesion. The misleading fibrillary background created by reactive stromal cells mimicked a glial tumor. Some vascular neoplasms may demonstrate a dirty background arising from hemorrhagic necrosis within the lesion, simulating high-grade astrocytic tumors.[8] True astrocytic tumors generally have round to oval nuclei, which may be monomorphic except in the high-grade lesions which can show pleomorphism. In retrospect, the stromal cells in the lesion we misdiagnosed showed variation in shape, a useful clue to a reactive lesion.

Some tumors can be difficult to diagnose accurately, particularly when they arise in an unusual primary site such as a myxopapillary ependymomas arising in the thoracic spine. Myxopapillary ependymoma and chordoma share some features with some malignant tumors such as chondrosarcoma. The characteristic physaliphorous cells, usually seen in chordoma, are fragile and easily destroyed by the process of smearing, but degenerating cells in myxopapillary ependymoma can simulate such cells.[8] Misdiagnosis in the case of myxopapillary ependymomas may arise due to lack of papillary or radial features which can help in separating the two, because both lesions tend to have a myxoid background. However, both chordomas and myxopapillary ependymomas usually have nuclei with benign features that distinguish them from a chondrosarcoma. Nonetheless, some cases may require the permanent section before a diagnosis can be made.[11]

Radiological images and clinical data are highly important in reducing errors in neurocytological diagnosis.[1,8,12] The images help to localize the tumor and allow elimination of some of the differential diagnoses based on the regional location and age of occurrence. It may however

be difficult to offer a single diagnosis in many cases, and in such situations, a list of differentials can be offered as was the experience in some of the cases in this study.[11]

Conclusion

Intraoperative cytology has high concordance rate with permanent histology sections. In view of the ease of preparation and easier access to facilities, particularly in low-resource settings, it should be a more widely used tool. However, the pathologist should be aware of the possible pitfalls and guard against them to reduce misdiagnosis.

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