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Crush Smear Cytology: A Rapid Diagnostic Technique in the Intraoperative Diagnosis of CNS Tumours

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ABSTRACT

Cytology has been shown to be of great value in intraoperative consultations of central nervous system (CNS) pathology. Intraoperative smear cytology provides a rapid and reliable intraoperative diagnosis and guidance to the neurosurgeon during surgical resection and lesion targeting. It also helps the surgeon to monitor and modify the approach at surgery. The current study was undertaken to assess the utility of intraoperative crush smear cytology and to correlate with the final histopathological diagnosis along with brief description of the cytomorphological features of commonly found brain tumours in smear preparation. It included 110 cases of CNS neoplasm which were subjected to intraoperative smear cytology. Smears were prepared from the biopsy samples sent in wet cotton pad and stained by the Haematoxylin and Eosin stain. The cytomorphological features were noted and correlated with final histopathological diagnosis. Complete correlation between intraoperative cytological and histopathological diagnosis was achieved in 89.6% of the cases. A reduction of diagnostic accuracy was seen in oligodendrogliomas and mixed gliomas (22.2%). Smear technique is a fairly accurate, relatively safe, rapid, simple, easily reproducible, and cost effective tool to diagnose brain tumours.

Keywords: *Crush smear cytology; CNS tumours; intraoperative diagnosis; HPE.*

INTRODUCTION

Central nervous system (CNS) tumours account for less than 2% of all malignancies.[1] Tumours of the nervous system are the second most common childhood tumor after leukemia, constituting approximately 35% of all childhood malignancies and remain the leading cause of cancer related deaths in children.[2,3] The intraoperative cytology preparation was first introduced by Eisenhardt and Cushing in early 1930 and by Badt in 1937.[4,5] This technique was further championed and documented by Russell et al. in 1937.[6] The intraoperative

cytology has recently gained importance because of advent of CT and MRI guided stereotactic biopsies. It provides a rapid and reliable intraoperative diagnosis and guidance to the neurosurgeon during surgical resection and lesion targeting. It also helps the surgeon to monitor and modify the approach at surgery.[7] Crush smear technique in neuropathology is now well established and continues to gain momentum. High resolution and specialized neuroimaging techniques combined with the use of stereotactic biopsies commonly require the rapid and definitive intraoperative diagnosis on minute and diminutive tissue specimens. The intraoperative smear cytology (crush smear preparation) is fairly accurate, simple and reliable tool for rapid intraoperative diagnosis of neurosurgical biopsies.[8] The aim of the study was to assess the utility of intraoperative smear cytology and to correlate with the final histopathological diagnosis along with brief description of the cytomorphological features of commonly found brain tumours in smear preparation.

Material and Method

The current study was conducted in the department of neurosurgery of our institute over a period of 24 months. It included 110 cases of CNS neoplasm which were subjected to intraoperative smear cytology. The biopsy samples obtained at the time of surgery were transported immediately to the laboratory in wet cotton pad for processing. Care was taken not to allow tissue to dry. A tiny portion (1-2 mm ^{3}) of tissue was squashed between two slides to prepare smears as described by Adams *et al*.[9] It was then fixed in 95% alcohol and stained by Hematoxylin and Eosin (H & E) stain. Further, paraffin sections were prepared by the residual tissue and stained by H & E stain. Permanent Haematoxylin and Eosin (H&E) sections were used as the "gold standard" for comparison. Relevant clinical history, age, sex and radiological data were noted. Smear cytology diagnosis was correlated with the histopathological findings.

Results

The cases where the intraoperative cytological diagnosis was same as the histological diagnosis including the grade of the tumor were considered as the complete correlation. Of the total number of cases, four cases were inconclusive i.e. they were neither diagnosed by histopathological examination (HPE) nor by crush smear cytology. 106 cases gave a definitive finding on HPE. Complete correlation between intraoperative cytological and the final histopathological diagnosis was achieved in 89.6% of the cases. Diagnostic accuracy increased when cases of partial correlation mainly due to grading deviation were included. The histological and cytological diagnosis of all the cases studied were shown in Table 1. A significant drop in diagnostic accuracy was observed in oligodendrogliomas, distinguishing between pure astrocytoma/ oligodendroglioma / mixed gliomas and the grading of gliomas. Table 2 showed the details of 11 cases misinterpreted on cytological examination. Three cases were frank errors and 8 cases showed partial correlation, comprising three cases of discrepancy in grading and five where one of the components had been missed during intraoperative consultation.

Table 1: Correlation between Histopathological and Cytological Diagnosis.

HPE DIAGNOSIS	*TOTAL NO OF CASES*	*CORRECT CYTOLOGICAL DIAGNOSIS*	*ACCURACY (%)*
Meningioma	*25*	*25*	*100*
Astrocytoma I,II	*13*	*13*	*100*
Astrocytoma III	*10*	*9*	*90*
Glioblastoma	*8*	*7*	*87.5*
Schwannoma	*15*	*15*	*100*
Oligodendroglioma & mixed glioma	*9*	*2*	*22.2*
Medulloblastoma	*6*	*6*	*100*
Ependymoma	*4*	*4*	*100*
Choroid plexus papilloma	*2*	*2*	*100*
Pituitary adenoma	*5*	*5*	*100*
Metastasis	*2*	*1*	*50*
Craniopharyngioma	*3*	*3*	*100*
Lymphoma	*1*	*1*	*100*
Central neurocytoma	*1*	*1*	*100*
Tuberculoma	*1*	*0*	*0*
Epidermoid cyst	*1*	*1*	*100*
TOTAL	*106*	*95*	*89.6*

Table 2: Cases misinterpreted in smear cytology

HPE DIAGNOSIS	*TOTAL NO OF CASES*	*SMEAR DIAGNOSIS*	*NO OF CASES MISINTERPRETED IN CYTOLOGY*
Metastatic	*2*	*Glioblastoma*	*1*
Mixed glioma	*5*	*Astrocytoma*	*4*
		Ologodendroglioma	*1*
Anaplastic astrocytoma	*10*	*Glioblastoma*	*1*
Tuberculoma	*1*	*Astrocytoma II*	*1*
Anaplastic oligodendroglioma	*2*	*Anaplastic astrocytoma*	*1*
		Oligodendroglioma	*1*
Glioblastoma	*8*	*Anaplastic astrocytoma*	*1*

The most common tumours in our series were astrocytomas (29.2%) followed by meningiomas (23.5 %) and Schwannomas (14.2%) . Astrocytomas (grade I,II,III) contributed to 21.7% of the cases. These moderately cellular neoplasms showed some degree of pleomorphism, nuclei with finely or coarsely granular chromatin, inconspicuous nucleoli, variable cytoplasmic processes and fibrillary background. Anaplastic type had in addition increased blood vessels and mitotic figures.

Glioblastoma accounted for 7.5% of the cases. The tumours spread fairly and evenly on smear and were highly cellular with discohesive cells with sparse cytoplasmic processes. Marked pleomorphism, atypia, multinucleation, mitotic figures and necrosis were noted along with the characteristic findings of marked endothelial proliferation and glomeruloid bodies. (Fig:1)

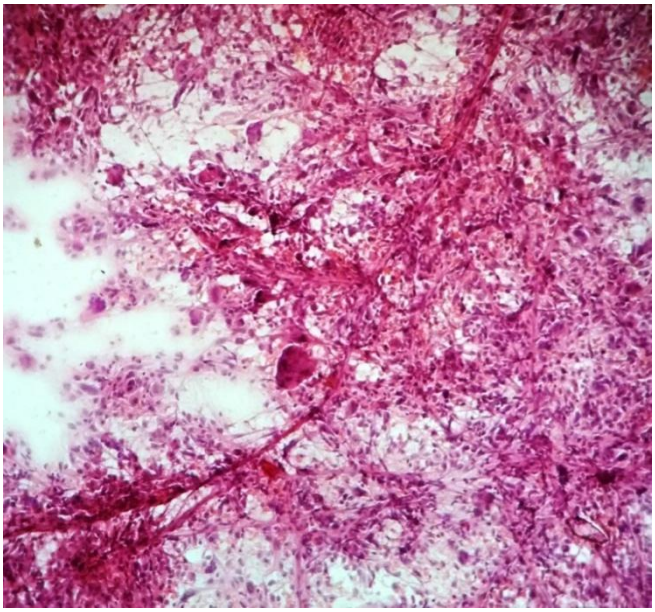

Figure 1: Smear from a glioblastoma showing hypercellularity and has abundant pleomorphic malignant cells with many bizarre and multinucleated forms. (H & E, X 100).

Meningiomas were relatively easy to smear and showed variable cellularity, high in syncytial type and low in fibrous type. The cells were fairly uniform, arranged in syncytial clusters or in whorls, with eosinophilic cytoplasm and poorly defined boundaries. The nuclei were oval with delicate chromatin and presence of intranuclear inclusions in some. Psammoma bodies were noted in some cases (Fig.2).

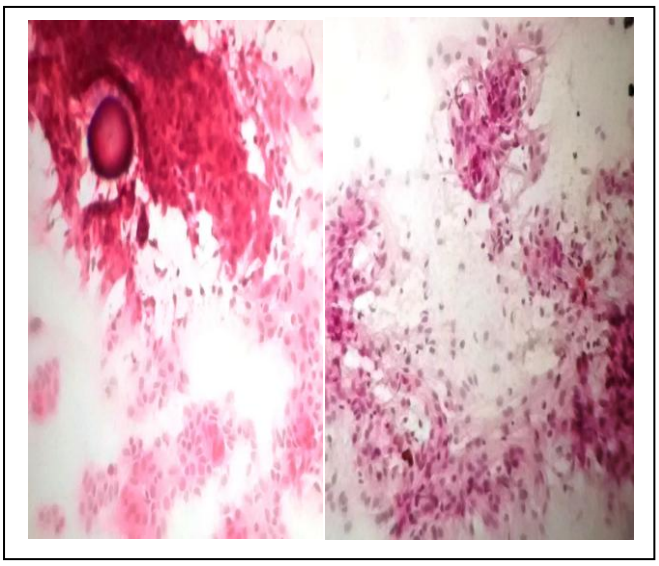

Figure 2: Smears from two meningiomas showing Psammoma body, whorls and syncytial clusters of meningothelial cells. (H & E, X 100)

Schwannomas comprised 14.2% of the cases and were difficult to smear due to cohesiveness giving rise to thick smears with three-dimensional twisted rope appearance. The tumour cells were spindle shaped with elongated, wavy nuclei. Verocay bodies and palisading were observed in some smears.

Oligodendrogliomas smear with ease appearing as moderately to highly cellular smears made up of discohesive, relatively small tumour cells with scanty, wispy cytoplasm and uniform round slightly dark nuclei with inconspicuous nucleoli. Background was finely granular and revealed no fibrillarity seen in other gliomas. Thin walled vessels were prominent and showed foci of calcification.

Medulloblastomas (5.6%) spread easily and evenly. The smears were densely cellular composed of small cells devoid of identifiable cytoplasm but with round, hyperchromatic nuclei, coarse punctuate chromatin, mitoses and individual cell necrosis.

Pituitary adenomas (4.7%) provided cellular smears of closely packed monomorphic cells with round nuclei, finely dispersed chromatin, single small nucleoli and acidophilic to faintly basophilic cytoplasm. Occasional multinucleation was observed but there were no mitoses, necrosis, pleomorphism or cellular processes.

Ependymomas represented 3.8% of cases and were cellular tumours smeared in cohesive clusters, tissue fragments and sheets of unevenly spread single cells. Perivascular pseudorosettes were seen. The cells had scanty fibrillary cytoplasm and uniform, bland, round to oval nuclei with dense, finely distributed chromatin and inconspicuous nucleoli.

Discussion

Rapid intraoperative smear cytology consultation contributes useful information in neurosurgical management. However, making an intraoperative diagnosis can be difficult due to a wide variety of CNS pathology and limitation of time and sample size during intraoperative consultation. In our study, the overall diagnostic accuracy obtained on using crush smear technique was 89.6%. Almost similar diagnostic accuracy was obtained in the study done

by Mouriquand C et al[10] and Nigam Sanjay Kumar et al[11]; while other studies have reported a diagnostic accuracy varying from 86% to 97.3%. [8, 12, 13, 14, 15, 16] Further, in our study a significant reduction in the diagnostic accuracy of oligodendroglioma was seen. The probable reason for this could be sampling error or the dense fibrillary background of astrocytic component.

No correlation with the final diagnosis was seen in four cases. Misinterpretation on cytological evaluation was seen in 11 cases. A case of reactive gliosis secondary to tuberculoma was mistaken for low grade astrocytoma on smear cytology. This was partly a sampling error as the areas diagnostic of tuberculosis had not been picked up and the reactive astrocytes were overdiagnosed as neoplastic astrocytes. Other authors[12,13,17,18] have described similar difficulties in distinguishing between reactive gliosis and low grade astrocytoma especially as both can show similar cytological features. Malignant astrocytes tend to grow along blood vessels and the subarachnoid spaces in some cases. This feature however can be seen only when the architecture is preserved as in paraffin sections. Low grade astrocytomas smear more easily compared to normal parenchyma and reactive processes. On review of the smears, reactive astrocytes had more abundant, evident cytoplasm and more prominent, numerous, long and symmetrical processes. As compared to neoplastic astrocytes, these lack hyperchromatic and lobulated nuclei, progressive atypia and mitoses. Smears of low grade astrocytoma had high cellularity, increased cell dimensions and increased pleomorphism.

An anaplastic oligodendroglioma was mistaken for an anaplastic astrocytoma on smear cytology. Roessler et al have reported similar discrepancies due to lack of uniform appearing nuclei and variable cytoplasmic processes.[12]

Mixed gliomas were also responsible for difficulties in smear interpretation due to predominance of one histological type. The smaller component may be missed especially if they are the less numerous oligodendroglial cells partly due to sampling error but also because of the dense fibrillar background of the astrocytic component.[17,19] Differentiation of astrocytic from oligodendroglial elements is of relevance during stereotactic biopsies due to prompt fixation of these biopsies, reducing the diagnostic oligodendroglial artifacts from formalin fixation, which may lead to a final histogenetic and grade misinterpretation.[18]

Improper grading of astrocytic neoplasms on cytological preparations has been documented by other authors. In fact some consider it inappropriate to attempt to grade CNS neoplasms on biopsy material whether by smear/ frozen technique as astrocytomas are known to vary significantly in grade from one area to another within a single tumour. Paraffin sections showed that in the undergraded cases there were areas of both less and more aggressive astrocytomas and the cytological sampling might have failed to show the anaplastic component. Thus small biopsies are unsuitable for grading malignancies.[13,17,19] Though grading of primary tumours may not be always possible on intraoperative smears, but their division into low or high-grade lesions is highly reliable and reproducible if due attention is paid to certain parameters like nuclear atypia, mitotic figures and necrosis. It is critical that radiological and clinical data be reviewed before intraoperative evaluation of smear cytology of brain tumours.

Conclusion

Smear cytology has been accepted as a suitable alternative to frozen section in intraoperative consultation of central nervous system tumours. Crush smear technique is a fairly accurate, relatively safe, rapid, simple, easily reproducible, and cost effective tool to diagnose brain tumours. Smear cytology is of great value in intraoperative consultation of CNS pathology. Intraoperative smears permit reliable intraoperative guidance during lesion targeting and resection.

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