Intraoperative Squash Smear Cytology in CNS Lesions: A Study of 150 Pediatric Cases

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Abstract

Background: Tumors of the central nervous system in the pediatric age group occur relatively frequently during the early years of life. Brain tumors are the most common solid malignancies of childhood and only second to acute childhood leukemia. Squash cytology is an indispensable diagnostic aid to central nervous system (CNS) lesions. The definitive diagnosis of brain lesions is confirmed by histological examination.

Aim: To study the cytology of CNS lesions in pediatric population and correlate it with histopathology.

Materials and Methods: One hundred and fifty cases of CNS lesions in pediatric patients were studied over a period of 2 years. Intraoperative squash smears were prepared, stained with hematoxylin and eosin, and examined. Remaining sample was subjected to histopathological examination. Results: Medulloblastoma (24.0%) was the most frequently encountered tumor followed by pilocytic astrocytoma (21.33%) and ependymoma (13.33%). Diagnostic accuracy of squash smear technique was 94.67% when compared with histological diagnosis.

Conclusion: Smear cytology is a fairly accurate tool for intraoperative CNS consultations.

Keywords: Central nervous system tumors, child, intraoperative diagnosis, squash preparation

Introduction

Brain and other nervous system cancers account for 1.4% of all new cancer cases in the US.[1] Tumors of the central nervous system (CNS) account for nearly 20% of all childhood tumors.[2] Although gross and microscopic features of brain tumors in adult and pediatric age group are the same, the incidence and type of pathology varies. The most common tumors in adults include anaplastic astrocytoma, glioblastoma, meningioma, metastatic tumors, pituitary tumors, and acoustic tumors.[3] However, in children, astrocytomas tend to be histologically and biologically benign, and meningiomas and metastatic tumors are rare. Certain tumors are specific to the pediatric age group such as juvenile pilocytic astrocytoma, brain stem gliomas, embryonal neuroepithelial tumors, mixed glioneuronal tumors, choroid plexus tumors, germ cell tumors, and atypical teratoid rhabdoid tumors. Another fundamental difference between adult and pediatric CNS tumors is the site of origin. Seventy percent of childhood CNS tumors arise in the posterior fossa; a comparable number of tumors in adults arise within cerebral hemispheres above the tentorium.[2]

Smear cytology has emerged as an important indispensable intraoperative diagnostic aid guiding the neurosurgeon and helping in better management of patients. However, definitive diagnosis is still confirmed by histopathological examination. The current study was undertaken to study CNS tumors in the pediatric population by squash smear cytology and correlating them with histological diagnosis.

Materials and Methods

This study included 150 pediatric patients with intracranial space occupying lesions admitted in the Department of Neurosurgery during a period of 2 years (January 2011 to December 2012). The tissue samples were received intraoperatively to prepare the cytosmear. A small piece of tissue received was dissected with a scalpel. Necrotic tissue-like material and blood clots were avoided. The tissue was crushed with just enough pressure to...
prepare a smear. Slides were immediately fixed in 95% ethanol and stained with rapid hematoxylin and eosin staining method. Relevant clinical and radiological data were noted in each case. Remaining tissue was fixed in 10% neutral buffered formalin for histopathological analysis. Squash cytology diagnosis were compared with histopathological findings. The tumors were classified according to the World Health Organization classification of CNS neoplasms 2007.\[^4\]

**Results**

A total of 150 squash smears comprising 90 males and 60 females (M:F = 3:2) were included in the study. The age ranged from 3 months to 18 years. Of the 150 cases, 63 cases were supratentorial (42%) and 87 were infratentorial (58%). Incidence of CNS tumors seen in this study showed medulloblastoma (24.0%) was the most common tumor in this age group followed by pilocytic astrocytoma (21.33%) and ependymoma (13.33%).

Complete correlation was obtained in 94.67% cases. The cases with the same diagnosis and grade on cytology and histopathology were considered as complete correlation [Tables 1 and 2]. Deviations of grade of tumor with lesser grade on squash smear cytology were included in partially correlated cases [Table 3]. The cases where intraoperative cytological diagnosis did not correlate with the histological examinations were categorized as discrepant cases [Table 3].

Tumors were graded according to the WHO grading system.\[^4\] We encountered 55 cases of astrocytomas, of which 40 were low grade and 15 were high grade. Low grade included pilocytic astrocytomas ($n = 32$), diffuse infiltrating low-grade astrocytomas grade II ($n = 7$), and subependymal giant cell astrocytoma ($n = 1$). Pilocytic astrocytomas show stellate/spindle cells with bland nuclear chromatin, mild increase in cellularity, and mild nuclear atypia and pleomorphism. Rosenthal fibres and eosinophilic granular bodies were noted in some cases [Figure 1a]. Occasionally, thick-walled vessels were noted. Mitosis was sparse. Necrosis was absent in all the cases.

One case of Subependymal giant cell astrocytoma (SEGA) was encountered in our study in a 12-year-old male patient with a history of tuberous sclerosis. Smears showed large cells having large vesicular nucleus with prominent nucleoli and abundant eosinophilic cytoplasm [Figure 1b].

Low-grade astrocytomas (diffuse infiltrating astrocytomas) had increased cellularity with mild nuclear atypia and pleomorphism. No mitosis, necrosis, and vascular proliferation was noted [Figure 2a].

High-grade astrocytomas included anaplastic astrocytomas WHO grade III ($n = 2$) [Figure 1c] which showed increased cellularity with mitosis and glioblastoma WHO grade IV ($n = 13$) [Figure 1d] which also showed vascular endothelium proliferation and necrosis [Figure 1c]. The appearance varied widely with some lesions composed of astrocytes with abundant cytoplasmic processes to poorly differentiated tumor cells with presence of giant cells.

### Table 1: Cytohistological correlation of CNS lesions

| Histopathological diagnosis                  | Total cases | Correct cytopathological diagnosis |
|----------------------------------------------|------------|-----------------------------------|
| Pilocytic astrocytoma                        | 32         | 31                                |
| Subependymal giant cell astrocytoma          | 01         | 01                                |
| Astrocytoma grade II                         | 08         | 08                                |
| Astrocytoma grade III                        | 02         | 01                                |
| Glioblastoma                                 | 13         | 10                                |
| Oligodendroglioma                            | 02         | 02                                |
| Ependymomas                                  | 20         | 19                                |
| Medulloblastoma                              | 36         | 36                                |
| Meningioma                                   | 07         | 07                                |
| Tuberculosis                                 | 05         | 05                                |
| Hemangioblastoma                             | 05         | 05                                |
| Schwannoma                                   | 03         | 03                                |
| Craniopharyngioma                            | 08         | 08                                |
| Pituitary adenoma                            | 06         | 06                                |
| Epidermoid cyst                              | 02         | 02                                |

### Table 2: Overall accuracy

| Distribution of cases | Number |
|-----------------------|--------|
| Total number of cases | 150    |
| Complete correlation in cases | 142    |
| Partial correlation in cases | 5      |
| Discrepant cases | 3      |
| Overall accuracy | 94.67%  |

**Figure 1:** (a) Pilocytic astrocytoma with eosinophilic granular bodies and Rosenthal fibres (H and E stain ×400). (b) Subependymal giant cell astrocytoma showing small astrocytic tumor cells with fibrillar matrix along with large cells and pale round nuclei (H and E stain ×400). (c) Anaplastic astrocytoma with increased cellularity, nuclear atypia, and mitotic figures (H and E stain ×400). (d) Glioblastoma with marked nuclear atypia and increased cellularity (H and E stain ×100).
Oligodendrogliomas \((n = 2)\) (one case each of grade II and grade III) were not common in children. Smears were moderately to highly cellular with monotonous small cells with uniform dark staining nuclei and scanty cytoplasm. Fine branching capillary network was noted in these tumors [Figure 2b]. Calcification was noted in both the cases.

Ependymomas \((n = 20)\) represented 13.33% cases of our study. These tumors were cellular with sheets of unevenly spread cells with prominent perivascular pseudorosettes [Figure 2c].

Medulloblastomas \((n = 36)\) represented 24% cases being the most common tumors in our study. The smears were highly cellular, comprising sheets of uniform, round to oval cells with hyperchromatic nuclei, irregular contour, mitosis, and apoptosis. Rosettes were identified in some cases [Figure 2d]. Few cases showed normal cerebellar tissue also where the cells of granular layer had to be differentiated from tumor cells.

Meningiomas \((n = 7)\) were relatively difficult to smear and showed high cellularity. The cells were arranged in clusters, in whorls, with fairly uniform nuclei with delicate chromatin, and with occasional presence of intranuclear inclusions. The cytoplasm of these cells was eosinophilic, wispy with ill-defined cell borders [Figure 3a].

Schwannomas \((n = 3)\) were easily identified by their twisted rope appearance at low power [Figure 3c]. The tumor cells had spindle-shaped nuclei.

Craniopharyngioma \((n = 8)\) were difficult to smear, showed isolated sheet of cohesive uniform epithelial cells along with fibrous tissue, along with isolated squamous cells and nonnucleated squames. In 4 cases, only keratin material was observed with fibrous tissue; hence, correlating with clinical and radiological findings, a diagnosis of craniopharyngioma was suggested while epidermoid cyst only showed anucleated squames [Figure 3b].

Pituitary adenoma \((n = 6)\) had a soft texture and were easily smeared. Characteristically, there were monolayers of individual cells with little cohesiveness. Some of them showed papillary formation with vascular core. The cells had eosinophilic granular cytoplasm, at places showing rosettes/glandular pattern.

There were 5 cases of tuberculosis. Squash smear showed epithelioid cells along with lymphocytes, plasma cells, and neutrophils. One case showed caseous necrosis.

**DISCUSSION**

The technique of intraoperative cytological examination was first introduced by Eisenhardt and Cushing in 1930.[5] Smear cytology is an indispensable intraoperative diagnostic aid for CNS lesions. It guides the neurosurgeon in monitoring and modifying the surgery, which may affect the extent of resection. The current study was undertaken to assess the utility of intraoperative consultations for cytomorphological diagnosis by smear technique and to correlate it with the final histopathological diagnosis.

**Table 3: Partially correlated cases and discrepant cases**

| Histopathological diagnosis | Cytopathological diagnosis | No. of cases |
|-----------------------------|-----------------------------|--------------|
| Glioblastoma                | Astrocytoma grade III       | 3            |
| Astrocytoma grade III       | Astrocytoma grade II        | 1            |
| Anaplastic ependymoma       | Ependymoma                  | 1            |
| Pilocytic astrocytoma       | Ependymoma                  | 1            |
| Tubercular granulation tissue | Astrocytoma grade II | 2            |

**Figure 2:** (a) Low-grade astrocytoma with increased cellularity and mild nuclear atypia \((H \ and \ E \ stain \times 100)\). (b) Oligodendroglioma showing finely branching capillary network \((H \ and \ E \ stain \times 100)\). (c) Ependymoma showing isolated tumor cells in between fronds of more cellular perivascular tissue and nuclear palisading \((H \ and \ E \ stain \times 400)\). (d) Medulloblastoma showing small round cells with nuclear molding and rosette-like structure \((H \ and \ E \ stain \times 400)\)

**Figure 3:** (a) Meningioma showing whorls with concentric arrangement of tumor cells. The arachnoidal morphology of nuclei is apparent \((H \ and \ E \ stain \times 400)\). (b) Epidermoid cyst showing keratinous flakes \((H \ and \ E \ stain \times 400)\). (c) Schwannoma showing fascicle of tumor cells from dense Antoni A areas intertwined about each other by smearing process \((H \ and \ E \ stain \times 400)\). (d) Inflammatory lesion \((H \ and \ E \ stain \times 400)\)
In our study, the most common tumor in the pediatric age group was medulloblastoma followed by pilocytic astrocytoma. However, most of the western literature reported the cystic cerebellar astrocytoma to be the most common tumor in the posterior fossa. In our study, 24% of tumors were constituted by medulloblastoma. This finding was also noted in a study conducted by Mehta et al. [3]

The overall diagnostic accuracy in our study was 94.67%. Other studies have also reported diagnostic accuracy ranging 76–96%. [6-18]

In our study, three cases were misinterpreted on smear cytology. One case of pilocytic astrocytoma was misdiagnosed as ependymoma on squash smear. There was presence of perivascular radiating processes. No Rosenthal fibres or eosinophilic granular bodies were noted in the smear. It may be noted that, at times, pilocytic astrocytoma may show areas of ependymal differentiation.

In two cases, reactive gliosis secondary to tuberculosis gave a mistaken diagnosis of low-grade astrocytoma on squash smear. This was partly a sampling error as the areas diagnostic of tuberculosis had not been picked and reactive astrocytes were overdiagnosed as neoplastic astrocytes. Other authors [6,11,19,20] have also described similar difficulties in distinguishing between reactive gliosis and low-grade astrocytoma, especially as both can show similar cytological features.

The astrocytomas vary histologically within a single tumor as areas of both low grade and high grade are present. Hence, if the tissue is not sampled well in a large tumor, the chances of error in grading the neoplasm may occur. Undergrading of tumor was done in five cases in our study. Other authors [11,12,20] have also encountered similar difficulties. Hence, it is advisable not to grade the astrocytomas on small tissue biopsies.

**CONCLUSION**

Smear cytology is of great importance in the intraoperative diagnosis of CNS pathology. However, it is important to know the age, clinical details of patient, duration and type of symptoms, whether onset was sudden or insidious, radiological findings, and site of tumor before evaluating the smear intraoperatively.

Squash smear cytology is fairly accurate, relatively safe, rapid, and simple tool to diagnose brain tumors and guiding the neurosurgeon.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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