A study of squash smear cytology in intraoperative diagnosis of CNS tumors and its histopathological correlation

P Tejaswi1,*, K Shirisha1, Ashok Kumar D1

1 Dept. of Pathology, Kamineni Academy of Medical Sciences and Research Centre, Hyderabad, Telangana, India

ABSTRACT

Background: Intra operative cytology is an important diagnostic modality especially central nervous system tumors. Squash smears most widely used cytological technique is rapid, accurate and relatively uses little tissue. It also helps the surgeon to plan the extent of surgery.

Aim: To assess the accuracy of squash smear by comparing with the histopathological diagnosis.

Materials and Methods: A study of 52 cases at our institute. Complete clinical, demographic and radiological findings were recorded. The smears were stained by rapid haematoxylin and eosin and toluidine blue stain. The cytomorphological features were studied and were compared with the tissue sections.

Results: Glial tumors formed the largest category of tumors constitutes 40.3%. A total 47 cases showed complete concordance with histodiagnosis with a diagnostic accuracy of 95.9%. Conclusion: Squash smear cytology proved to be a simple, inexpensive, rapid intraoperative neuropathological technique particularly useful in astrocytomas.

© 2020 Published by Innovative Publication. This is an open access article under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/)

1. Introduction

Squash smear is the most widely used cytological technique in intraoperative neuropathology. Although frozen section is superior to smear cytology for assessment of architectural features smear cytology allows better demonstration of nuclear morphology particularly in distinguishing astrocytic and oligodendroglial tumors. Squash smear cytology was first introduced by Eisenhardt and Cushing in early 1930.3 This technique is simple, rapid, inexpensive, accurate intraoperative diagnostic modality2 which provides a rapid pathological diagnosis of the space occupying lesions of the nervous system. It provides adequate material has been obtained and helps the neurosurgeon to plan the extent of surgery. The soft consistency of the central nervous system (CNS) tissue is best suited for squash cytology which in fact is a hindrance for frozen section.3 The objective of this study is

1. To study the CNS tumors on squash smear cytology.
2. To correlate squash cytology with histopathology examination.

2. Materials and Methods

The study was done at our institute over a period of 2 yrs. A complete clinical and radiological findings were noted.4 A total of 52 neurosurgical specimens were received intraoperatively for squash smear cytology.

Preparation of crush smear: Tissue was first grossly examined for necrosis and hemorrhage. Two or more tissue fragments measuring no more than 2 mm diameter were taken.5 Tissue was placed on the Centre of a labeled glass slide. A second labeled slide was placed over the first slide and smeared with the second glass slide without exerting too much pressure.6–8 Smears were fixed in 99.9% isopropyl alcohol and stained with rapid hematoxylin and eosin (H and E) stain and toluidine blue. Minimum of five to eight smears were made. Remaining tissue was submitted for
medulloblastomas were common in children.

Brain and other nervous system cancers account for 1.4% of all new cancer cases in the US. The intraoperative neuropathological techniques used includes frozen section histology and smears or imprint cytology. Frozen section is superior to cytology for the assessment of architectural features, however squash smear cytology allows better nuclear morphology and provides rapid diagnosis and do not need any sophisticated instrumentation. The squash technique was introduced in as early as the 30’s. However with the advent of stereotactic neurological techniques that produce very small specimens that are difficult to section on the cryostat has resulted in increased popularity of the squash preparation in rapid diagnosis.13 The advantages of squash smears are that it is easy to smear with good cellularity, can be done even when the sample is limited, and intraoperative diagnosis can be rendered within 15-20 min.14

The cytologic details well-illustrated on smears include astrocytic processes in astrocytomas, neuropil background in neurocytomas, oligodendroglial/neurocytic cells with round and regular (if not anaplastic) nuclei that are smaller than an astrocyte with dense chromatin, ganglion cell type neurons with large nuclei with prominent nucleoli, and meningothelial cells plump nuclei with pseudo-inclusions and pituitary lesions with rounded nuclei with punctate ‘salt and pepper’ chromatin. However, interpretation of each of these entities is benefited by synchronous evaluation of histologic patterns which is better achieved on frozen section. A pit fall of using frozen sections is that, subsequent formalin-fixed paraffin wax embedded sections, show freezing artifact. Cytological technique are more rapid and require little tissue.15

Our study includes 52 cases the youngest patient being 1yr old and oldest being 68yr old. With slight male preponderance. Peak incidence of brain tumors was observed in 41-60 yrs of age.

Astrocystomas constituted largest category accounting for 40.38%. Of which high grade glioma were 11 cases and low grade gliomas were 8 cases. On cytology, the low grade glioma showed low to moderate cellularity with the tumor cells round, oval to slightly elongated normochroic nuclei rimmed by pale staining cytoplasmic processes. (Figure 1). Squash smears of high grade gliomas (55.5% cases) were highly cellular and showed tumor cells against a fibrillary background with necrotic debris and marked endothelial proliferation (Figure 2 a, b). Subsequent histopathology sections showed high cellularity with bizarre tumor cells, tumor giant cells, large areas of necrosis, mitosis, pseudo palisading around tumor necrosis and endothelial proliferation (Figure 2 c, d). Similar findings were reported by other authors.16

In our study discordance was seen mostly in case of gliomas. 3 cases of high-grade gliomas were reported as low grade gliomas on squash. Possibility of sampling errors or lack of representative sample and often necrosis doesn’t stick to the slide. Similar difficulty was observed in other studies.17,18

A case of reactive gliosis on smear cytology reported as low-grade glioma. Reactive gliosis is often interpreted as low grade glioma because during intraoperative biopsy
Table 1: Distribution of cases

<table>
<thead>
<tr>
<th>Histopathology diagnosis</th>
<th>No of cases</th>
<th>No of cases correctly diagnosed by squash</th>
<th>Discordant cases</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade astrocytoma</td>
<td>08</td>
<td>07</td>
<td>01</td>
<td>87.5%</td>
</tr>
<tr>
<td>High grade astrocytoma/ GBM</td>
<td>11</td>
<td>08</td>
<td>03</td>
<td>72%</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>02</td>
<td>01</td>
<td>01</td>
<td>50%</td>
</tr>
<tr>
<td>Anaplastic ependymoma</td>
<td>03</td>
<td>03</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>Embryonal tumor</td>
<td>01</td>
<td>01</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>Meningioma</td>
<td>08</td>
<td>07</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>Schwannoma</td>
<td>06</td>
<td>06</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>Metastasis</td>
<td>04</td>
<td>04</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>03</td>
<td>03</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>DNET</td>
<td>01</td>
<td>01</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>02</td>
<td>01</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>01</td>
<td>01</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>Inflammation</td>
<td>02</td>
<td>02</td>
<td>00</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>47</td>
<td>05</td>
<td>90.38%</td>
</tr>
</tbody>
</table>

Table 2: Comparison with other studies

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of cases</th>
<th>Correlated with HPE</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malhotra et al</td>
<td>18</td>
<td>18</td>
<td>100%</td>
</tr>
<tr>
<td>Shukla et al</td>
<td>140</td>
<td>135</td>
<td>96.42%</td>
</tr>
<tr>
<td>Padma et al</td>
<td>11</td>
<td>9</td>
<td>81.8%*</td>
</tr>
<tr>
<td>Brommeland et al</td>
<td>100</td>
<td>95</td>
<td>95%</td>
</tr>
<tr>
<td>Present study</td>
<td>53</td>
<td>47</td>
<td>95.9%</td>
</tr>
</tbody>
</table>

Fig. 1: Glioma; a: Glial cells with rounded nuclei with relatively open chromatin in a find fibrillary squash (Toluidine blue; 10X); b: Histopathology (H&E 10X)
Fig. 2: Glioblastoma; a: Markedly cellular pleomorphic malignant astrocytic cells in well-formed fibrillary mesh work with hyperplastic blood vessel, bizarre astrocytic cells against afibrillary background, tumor giant cells; b: Palisading necrosis inset shows glomeruloid proliferation of blood vessel

Fig. 3: a: Pictomicrograph shows meningotheial cell with oval nucleus and some showing intranuclear vacuolations, squash (toluidine blue; 40X) b; Histopathology (H&E; 40X)
Fig. 4: a: Smear showed spindle shaped cells with wavy nuclei on a fibrillary background, squash (toluidine blue; 10X); b: Verocay bodies (H&E 10X)

Fig. 5: a: Metastatic adenocarcinoma: cohesive cluster of malignant epithelial cells; b: Histopathology (H&E 10X)

it is very important to identify the reactive gliosis with glioma and then biopsy taken. Typically, in gliosis, reactive astrocytes tend to be evenly distributed, with slightly enlarged and eccentric nuclei, abundant, eosinophilic cytoplasm with stellate longer, tapering processes and low nucleus/cytoplasmic (N/C) ratio. In contrast, low-grade fibrillary astrocytomas have uneven distribution of neoplastic cells with increased N/C ratios, irregular hyperchromatic nuclei with coarse chromatin, shorter, thinner and more variably intersecting processes, mitotic figures especially atypical ones, and at times microcystic change.

The next most common tumors were meningioma includes 8 cases Radiologically, meningiomas are isointense to cerebral cortex in MR images and show homogenous contrast enhancing tissue in MR and CT with “dura tail” smears. On squash they revealed firm rubbery soft tissue showing psammoma bodies, fibrillary matrix, nuclear inclusion, and a characteristic whorling pattern was seen along with a few spindle cells in some areas. our study showed 100% concordance.

The next group of tumors encountered was schwannomas which constituted 6 (11.5%) showed 100% concordance in accuracy. They were difficult to smear and cytosmears showed cohesive fragments of spindle cells and subsequent histopathology revealed a distinctive biphasic pattern composed of Antoni A and Antoni B areas with interlacing bundles of uniform cells in parallel array of palisades (verocay bodies).

Next most common tumors in present study are metastasis which constituted about 4/52 cases. Metastatic carcinomas displayed cohesive nests of atypical cells with high-grade with nuclear anaplasia, prominent nucleoli, frequent mitoses and a necrotic background.

Our study also included two cases of medulloblastoma and two cases of lymphoma which showed similar findings on histopathological diagnosis.

One case of clinical diagnosis of dysembryoblastic neuroepithelial tumor was made which on squash smear showed small uniform cells with delicate capillary vasculature and microcystic pattern admixed were large ganglion cells and on histopathology typically seen as
floating neurons.

One case of small blue round cell tumor was diagnosed on squash which correlated with histopathology and the final diagnosis was lymphoma on immunohistochemistry.

This study showed diagnostic accuracy of 95.9% which correlated with other studies.

5. Conclusion

Our study shows a high degree of cyto-histological correlation (95.92%). With better and precise radio imaging the percentage of cyto-histological correlation can improve and increase further. Some cases will always require histopathological study and/or immunohistochemical markers for definitive diagnosis, but for most of the lesions cytology of the CNS tumors performed intraoperatively fulfills all the determinants of an excellent diagnostic modality. Squash also replaces frozen section in diagnosis for CNS tumors. Frozen section needs cryostat, technical experience and ice crystal artifacts makes squash a better diagnostic modality for CNS tumor intraoperatively.

6. Source of Funding

None.

7. Conflict of Interest

None.

References


Author biography

P Tejaswi Postgraduate
K Shirisha Assistant Professor
Ashok Kumar D Professor

Cite this article: Tejaswi P, Shirisha K, Kumar D A. A study of squash smear cytology in intraoperative diagnosis of CNS tumors and its histopathological correlation. Indian J Pathol Oncol 2020;7(3):441-446.