Role of Squash Cytology in Intraoperative Diagnosis of Spinal Lesions

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Abstract

**Background:** Squash cytology for intra operative diagnosis of central nervous system (CNS) tumors is an immensely important modality. Though its role in brain lesions is unquestionable and has been proven in a number of studies, its utility for spinal lesions is still a grey zone. **Aims:** To assess the diagnostic accuracy of squash preparation in spinal lesions and its statistical significance (sensitivity, specificity, positive predictive value, negative predictive value) following histological confirmation. **Materials and Methods:** A total of 57 cases of spinal tumors were taken. May-Grunewald-Giemsa staining (MGG) and Hematoxylin-Eosin (H&E) were done in each one of them. Rest of the tissue was processed for histological diagnosis and results were compared. **Results:** In our study, histology was taken as the gold standard. By comparing the results, squash preparation had sensitivity of 95.75%, specificity 80.0%, positive predictive value (PPV) 95.74%, and negative predictive value (NPV) 80.80%. Schwannoma was found to be the most prevalent tumor in the spine (17/57) in our study, followed by meningioma (82.35%). Highest diagnostic accuracy was documented in intradural, extramedullary compartment. **Conclusion:** Inspite of having pitfalls and various limitations in case of spinal lesions, squash preparation is a rapid and easy method with fairly high diagnostic accuracy. So it can be reliably used as an intraoperative diagnostic tool in spinal lesions.

**Keywords:** CNS tumors, squash cytology, spinal tumors

**INTRODUCTION**

The role of squash cytology for rapid intraoperative cytological diagnosis of space-occupying lesions (SOLs) in the brain is well established and has been an important diagnostic tool over the last few decades. However, its role in spinal lesions remains unexplored. There is sparse literature available on squash cytology of spinal lesions. In the recent times, the rapid cytological diagnosis for spinal lesions is also gaining importance, which poses considerable diagnostic challenge to neuropathologists. This is useful for individualized treatment. In spite of having several pitfalls, intraoperative diagnosis is important, as it helps surgeons to decide on optimal line of management for various spinal lesions without having to wait for routine paraffin-embedded sections. The aim of this study was to evaluate the diagnostic accuracy of squash preparation of spinal lesions and its concordance with final histological diagnosis.

**MATERIALS AND METHODS**

This study was conducted over a period of 16 months in the Department of Pathology in collaboration with Department of Neurosurgery in a tertiary care hospital. A total of 57 cases of spinal tumors were included in this study. A small portion of the specimen was squashed with the help of two slides and smeared. The slides were then stained using May–Grunewald–Giemsa and hematoxylin–eosin methods. Rest of the tissue was processed for histological diagnosis and results were compared. In our study, histology was taken as the gold standard. By comparing the results, squash preparation had sensitivity of 95.75%, specificity 80.0%, positive predictive value (PPV) 95.74%, and negative predictive value (NPV) 80.80%. Schwannoma was found to be the most prevalent tumor in the spine (17/57) in our study, followed by meningioma (82.35%). Highest diagnostic accuracy was documented in intradural, extramedullary compartment.

**Conclusion:** Inspite of having pitfalls and various limitations in case of spinal lesions, squash preparation is a rapid and easy method with fairly high diagnostic accuracy. So it can be reliably used as an intraoperative diagnostic tool in spinal lesions.

**Keywords:** CNS tumors, squash cytology, spinal tumors

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Results
A total of 57 cases of spinal SOLs were studied by squash cytology and subsequent histology. The mean age of presentation was 43.35 years with no significant sex preponderance. We had 29 male patients and 28 female patients with an almost equal sex ratio. All of the 13 cases of meningioma were, however, seen in women patients. Majority of the spinal tumors were located in intradural, extramedullary (IDEM) compartment (61.4%), followed by intradural, intramedullary (IDIM) lesions (24.6%). Extradural, extramedullary (EDEM) lesions were found only in 14.03% cases. In all cases squash cytological diagnosis was compared with final histological diagnosis, which was considered to be the gold standard. The spectrum of spinal lesions in our series along with its diagnostic accuracy has been summarized in Table 1.

The commonest lesion observed in our series was Schwannoma (29.8% of the total cases) followed by meningioma (22.8%) [Figure 1a–d]. The other commonly found tumors were diffuse astrocytoma [World Health Organization (WHO) grade II], metastatic tumors, and neurofibroma. In one case we got totally necrotic material in squash preparation, which was later diagnosed to be a case of renal cell carcinoma metastasis on histology. The provisional cytological diagnosis was made and categorized as benign or malignant followed by grading. Final histological diagnosis was made according to WHO classification and grading.

In our study, diagnosis by squash preparation had a sensitivity of 95.75%, specificity 80.0%, PPV 95.74%, and NPV 80.80%. Diagnostic accuracy by squash cytology for schwannoma was fairly high (92.3%), followed by meningioma (82.35%). Gliomas also showed good concordance by squash preparation. However, we faced difficulty in diagnosing extradural lesions by squash preparation.

The major diagnostic discrepancies with regard to cell type and grading errors are tabulated in Table 2 [Figure 2a-f]. A case of spinal metastasis from renal cell carcinoma was misdiagnosed as tuberculosis of spine, as it is very common in developing countries and also shows extensive necrosis. One case of chordoma was diagnosed as chordoid meningioma [Figure 2b].

Table 1: Histological spectrum of spinal tumors along with diagnostic accuracy

| Tumor                        | Number | Percentage | Diagnostic accuracy by cytology |
|------------------------------|--------|------------|---------------------------------|
| Schwannoma                   | 17     | 29.8%      | 92.3%                           |
| Meningioma                   | 13     | 22.8%      | 82.35%                          |
| Neurofibroma                 | 3      | 5.2%       | 66.6%                           |
| Diffuse astrocytoma (WHO Gr II) | 3   | 5.2%       | 100%                            |
| Pilocytic astrocytoma        | 2      | 3.5%       | 50%                             |
| Ependymoma (WHO Gr II)       | 2      | 3.5%       | 100%                            |
| Myxopapillary ependymoma     | 2      | 3.5%       | 50%                             |
| Chordoma                     | 2      | 3.5%       | 50%                             |
| Giant cell tumor             | 1      | 1.7%       | 100%                            |
| Aneurysmal bone cyst         | 2      | 3.5%       | 50%                             |
| Metastasis                   | 3      | 5.2%       | 66.6%                           |
| Lymphoma                     | 2      | 3.5%       | 100%                            |
| Hemangiopericytoma           | 1      | 1.7%       | =                               |
| PNET                         | 1      | 1.7%       | 100%                            |
| Plasmacytoma                 | 1      | 1.7%       | 100%                            |
| Paragangioma                 | 1      | 1.7%       | =                               |
| Epidermal inclusion cyst     | 1      | 1.7%       | 100%                            |

Figure 1: (a) Smear shows cells lacking distinct cell borders and elongated nuclei swarming within bundles of fine filaments (MGG stain, ×400), suggestive of schwannoma. (b) Smear shows whorls composed of arachnoidal cap cells (MGG stain, ×400), seen in meningioma. (c) Smear shows branching blood vessels and perivascular palisading of densely packed tumor cells (MGG stain, ×400), suggestive of ependymoma. (d) Smear shows monomorphic, oval to spindle cells arranged around vascular cores and myxoid background (Papanicolaou stain, ×100), suggestive of myxopapillary ependymoma.

Figure 2: (a) Monomorphic cells with salt and pepper chromatins (MGG stain, ×100), misdiagnosed as ependymoma. (b) Tumor with myxoid matrix, vacuolated cells arranged in clumps (MGG stain, ×400), misdiagnosed as chordoid meningioma. (c) Round to ovoid cells with scanty cytoplasm resembling SRCT (Papanicolaou stain, ×400). (d) Histology of A, nested pattern of monomorphic round cells (H and E, ×400) diagnosed as paragangioma, (inset) (Reticulin stain, ×100). (e) Histology of B, vacuolated physaliphorous cells in myxoid matrix, in chordoma (H and E, ×100). (f) Histology of C, densely cellular tumor with staghorn vessels, in hemangiopericytoma (H and E, ×100).
and one case of aneurysmal bone cyst were diagnosed as giant cell tumor of spine due to presence of numerous giant cells. A case of hemangiopericytoma (HPC) was diagnosed as small round cell tumor (SRCT) [Figure 2c].

We also assessed diagnostic accuracy of squash cytology in each individual anatomical compartments, i.e., EDEM, IDEM, and IDIM. In our study the diagnostic accuracy was highest for IDEM tumors and lowest for EDEM tumors. The sensitivity, specificity, PPV, and NPV of squash cytology in each anatomical compartment have been tabulated in Table 3.

**DISCUSSION**

The use of intraoperative diagnosis by cytology was started in 1930 by Eisenhardt and Cushing, followed by Badt in 1937.[1,2] The importance of squash cytology is increasing day-by-day, especially with the introduction of computed tomography and magnetic resonance imaging-guided stereotactic biopsies.[3] The purpose of intraoperative squash cytology is mainly to provide surgeons adequate details about the lesion, so that the extent of surgery can be optimized and further therapeutic approach can be modified in an individualized manner.[4,5] The role of squash cytology in diagnosing brain lesions has been well documented in many studies, but its role in spinal tumors is still a gray zone. Hence, the aim of our study was mainly to assess its utility in spinal tumors.

**Table 2: Diagnostic discrepancies of squash cytology in spinal lesions**

| Squash cytology diagnosis | Histology diagnosis |
|---------------------------|---------------------|
| **Major diagnostic discrepancies** | |
| Total necrosis, suggestive of Tuberculosis (n=1) | Renal cell carcinoma metastasis |
| Chordoid meningioma (n=1) | Chordoma |
| Small round cell tumor (n=1) | Hemangiopericytoma |
| Giant cell tumor (n=1) | Aneurysmal bone cyst |
| **Cell type error** | |
| Schwannoma (n=1) | Fibroblastic meningioma |
| Ependymoma (n=1) | Paranglioma |
| Schwannoma (n=1) | Neurofibroma |
| Schwannoma (n=1) | Tanyctic ependymoma |
| **Grading error** | |
| Ependymoma (WHO Gr II) (n=1) | Myxopapillary ependymoma (WHO Gr I) |
| Astrocytoma (WHO Gr II) (n=1) | Pilocytic astrocytoma |
| **Partial correlation** | |
| Small round cell tumor (n=1) | PNET |
| Small round cell tumor (n=1) | Lymphoma |

In our study, the diagnostic sensitivity of squash preparation was 95.75%, specificity 80.0%, PPV 95.74%, and NPV 80.80%. We further documented diagnostic accuracy of squash cytology in individual tumors. Diagnostic accuracy for schwannoma was 92.3%, followed by meningioma (82.35%).

In a similar study, squash cytology done on central nervous system lesions (both on spinal and brain SOLs) documented sensitivity of 91.6%, specificity and PPV 100%, and 88% overall diagnostic accuracy.[6] In another series, brain and spinal lesions were taken together and documented the sensitivity, specificity, and diagnostic accuracy of squash cytology to be 94.79, 95.67, and 95.25%, respectively.[6] There is practically no data available on spinal lesions alone.

In a study done on both neoplastic and nonneoplastic brain lesions, diagnostic accuracy of squash cytology was found to be 93.3%.[7]

Although specificity and diagnostic accuracy rates are higher for brain SOLs than spinal lesions in various studies, we found that the results of our series based on spinal SOLs alone are also comparable.

In some series it has been documented that diagnosis by frozen section yields a more accurate result than squash cytology.[8,9] The soft friable nature and high water content of brain tissue impose considerable difficulty in preparing a good quality frozen section.[10] Squash is a simple, rapid method and there are less chances of contamination of instruments than in frozen section with regard to acquired immunodeficiency syndrome (AIDS), prions diseases, etc.[9,11] Although the merits of squash cytology over frozen section remain the same as in the case of brain SOLs, but presence of fibrous elements and sometimes bony spicules in spinal tissues often causes difficulty in smearing and thereby imposes difficulty in its interpretation.[12,13] Bony and gritty nature of EDEM tumors, such as aneurysmal bone cyst, giant cell tumors, etc., causes errors in proper diagnosis by squash preparation alone. In our study, we found the diagnostic accuracy of these tumors by squash to be lower than other compartments.

Squash preparation provides invaluable cytological information such as cytoplasmic and nuclear details along with important cellular architecture such as rosettes, whorls, etc.[14-16] It is devoid of freezing artifacts as seen in frozen sections. But artifacts such as crushing, overstretched, and cellular overlapping can impose problems in making an accurate diagnosis by squash. Thickness of the prepared slides is also not uniform throughout the smear. Too thick or too thin areas can also cause diagnostic discrepancies.[17]
Some of the reasons behind discordant cases were cautery artifact during surgery and presence of necrotic tissue. These factors can lead to overdiagnosis or underdiagnosis of tumors. As Pott’s spine is very common in developing countries, necrosis often leads to diagnosis of tuberculosis. In our study, a metastatic renal cell carcinoma was diagnosed as spinal tuberculosis due to presence of necrotic material only.

Interpretation of various giant cell containing lesions can also be misleading. A case of aneurysmal bone cyst was diagnosed as giant cell tumor due to presence of hemorrhage and giant cells. Due to presence of large vacuolated cells, one case was misdiagnosed as chordoid meningioma, which was later confirmed to be chordoma by histology. So, pathologists should be aware of other differential diagnosis and correlate cytological findings with clinical presentation and imaging studies.

Overlapping cellular architecture can also be dubious. Though we got a high diagnostic accuracy rate for schwannomas (92.3%) and meningiomas (82.35%), a case each of fibroblastic meningioma and neurofibroma were diagnosed as schwanna due to presence of elongated spindle cell and absence of characteristic whorls of meningioma. Similar errors in interpretation have been reported by various other studies too. HPC is a common spinal lesion of intermediate grade malignancy. Presence of round cells in HPC often causes diagnostic difficulty. In our study, a single case of HPC was diagnosed as SRCT.

Tanyctytic ependymoma is a common tumor of the spine which has relatively few perivascular rosettes, and the presence of spindle-shaped cells can be misleading. It can be mistaken for pilocytic astrocytoma or schwannoma. In our series, it was misdiagnosed as schwannoma.

Paraganglioma is very common in lower lumbar spine. The cells of paraganglioma can sometimes resemble the epithelial cells found in ependymoma. In this study one case of paraganglioma was misdiagnosed as ependymoma [Figure 2a].

Exact grading of the lesions can sometimes be erroneous. A conspicuous fibrillary background is helpful to categorize a lesion as glial tumor, but further subclassification can be a challenge due to its cellular heterogeneity. In our study, a case of pilocytic astrocytoma (WHO grade I) was misdiagnosed as diffuse astrocytoma (WHO grade II). The ependymomas can also be difficult to grade and subtype on cytology alone. A case of myxopapillary ependymoma (WHO grade I) [Figure 1d] in our series was diagnosed as ependymoma (WHO grade II).

SRCTs such as primitive neuro ectodermal tumor (PNET), lymphoma, and even hemangiopericytoma can show similar appearance on squash cytology. In our series, a case of lymphoma and one case of PNET were diagnosed as SRCT as exact differentiation could not be made on cytology.

So, we can conclude that squash cytology is an important intraoperative diagnostic tool in management of brain tumors. Its role in spinal lesion is not yet established. In our study we found that the diagnostic accuracy, sensitivity, and specificity of spinal lesion although lower but is comparable with brain SOLs, especially in case of IDEM and IDIM compartments.

It is also important in case of spinal tumors to bear in mind the radiological finding, age distribution, and anatomical location to come to an accurate cytological diagnosis.

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**Conflicts of interest**

There are no conflicts of interest.

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