RESEARCH COMMUNICATION

Comparative Efficacy of Imprint and Squash Cytology in Diagnosing Lesions of the Central Nervous System

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Abstract

Introduction: Central nervous system lesions can be localized precisely with the help of computerized tomography (CT). However, rapid cytological diagnosis of lesions by imprint and/or squash smear technique is useful to assist the operating surgeon to avoid unnecessary craniotomy. The present study was conducted with the aim to study the sensitivity, specificity and diagnostic accuracy of the two types of smears in central nervous system lesions while taking histology as gold standard. Materials and Methods: In the present study 149 lesions of the central nervous system were studied by squash and imprint smear techniques and results were compared with histology. Cytology smears were stained with May-Grunwald-Giemsa and Pap stain. Histopathology smears were stained with haematoxylin and eosin stain. Results: Out of 149 cases, 85 were malignant and 64 cases were either benign or non-neoplastic lesions. The sensitivity, specificity and diagnostic accuracy by squash smear were 90.6%, 87.5% and 89.3% respectively and that of the imprint smears were found to be 92.9%, 90.6% and 92.0%. The combined sensitivity, specificity and diagnostic accuracy of both techniques were even greater (95.3%, 93.8% and 94.6%). Conclusions: The present study showed that imprint smear examination is superior to the squash smear for diagnosing lesions of the central nervous system.

Keywords: Central nervous system lesions - squash smear - imprint smear - intraoperative diagnosis

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Introduction

The central nervous system (CNS) is one of the most challenging domains for the neurosurgeon. Making a diagnosis of CNS lesions is difficult on the basis of clinical and radiological findings only. Cytological and/or histological diagnosis is required for confirmation and proper management. In this regard, imprint cytology or squash smear prepared from small biopsy during surgery seems to be very helpful for the operating surgeon to make a decision on further management.

In contrast to the usual method of tissue processing, the application of smear techniques as a means of obtaining rapid diagnosis for neurosurgical biopsies was first advocated by Eisenhardt and Cushingin in the USA in early 1930. Since then, although the technique has been modified by various individuals by changing the fixative or stain, the basic principle has remained unchanged. Though this technique receded to the background with the advent of computerised tomography (CT) guided stereotactic biopsy, it has regained importance due to its technical simplicity.

In the United Kingdom the smear technique has been extensively used for the examination of neurosurgical biopsies for at least 40 years as an adjunct to and sometimes replacement for frozen section, particularly in centers performing many needle or stereotactic biopsies (Russel, 1937). Different studies by the groups of Asha (1989), Pogady (1993), Ili (1995), Imtiaz (2006) and Iqbal (2007) have proven the utility of squash smear technique as an intraoperative diagnostic tool in comparison to frozen section and have shown a variable accuracy rate of 87% - 95.35%.

Imprint smear inspite of its advantages has not received enough attention for rapid diagnosis of central nervous system lesions. Martinez et al (1988) studied 100 CNS lesions by touch preparation and frozen section and compared both these techniques with paraffin sections. They observed 76% accuracy in imprint smear and 88% in frozen section as compared to paraffin section. They also observed that touch preparations were superior to frozen section particularly for evaluating soft or highly cellular tumour and for preliminary diagnosis from a minute surgical specimen (eg. stereotactic biopsy).

To the best of our knowledge there is no study comparing intraoperative squash and imprint techniques in the same specimen. Therefore the present study was undertaken to compare the efficacy of squash and imprint cytology for intraoperative diagnosis of central nervous system lesions.

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Materials and Methods

One hundred and thirteen patients with intracranial space occupying lesions and spinal cord lesions were studied. Detailed clinical history, neurologic manifestations and duration of the symptoms were recorded. Particular attention was paid to the findings obtained from computerized tomography (CT) scan.

All the biopsy specimens were obtained per-operatively except in one case, where burr-hole technique was used. Cytology smears(imprint and squash) were prepared immediately before fixing the biopsy material in formalin. Imprint smears were prepared by touching the cut surface of the tissue over the slide. For squash smear a small bit of tissue was placed between two clean, grease free glass slides and gently pressed (Takahashi, 1981). Smears thus prepared were immediately fixed in 95% ethyl alcohol and methanol for 30 seconds and stained with haematoxylin-eosin and Giemsa stains. The formalin fixed biopsy material was then processed for histopathological examination and 3 micron thick sections were stained with haematoxylin- eosin.

The cytological details of the squash and imprint smears were studied (see Figure 1) and a provisional diagnosis was made. The findings were compared with the final histological observation. All the observations were made by two pathologists, independently to avoid any bias. Finally the percentage of misdiagnosed cases by smear techniques and sensitivity, specificity and diagnostic accuracy of each individual technique as well as for combined technique (imprint and squash) were calculated.

Results

The study included 149 patients. Their mean age was 29 years and M:F ratio was 1:1.13. Out of 149 lesions 85 (57.7%) were malignant neoplasms and 64 (42.3%) were either benign neoplasms or non-neoplastic lesions clinically and radiologically masquerading as neoplasms. Of the benign or non-neoplastic lesions meningioma was found to be the most frequent(17.5%) followed by abscess and schwannoma (7.4% each). Among the malignant lesions astrocytoma was the most frequent lesion (43.0%) followed by metastatic tumours (4.7%).

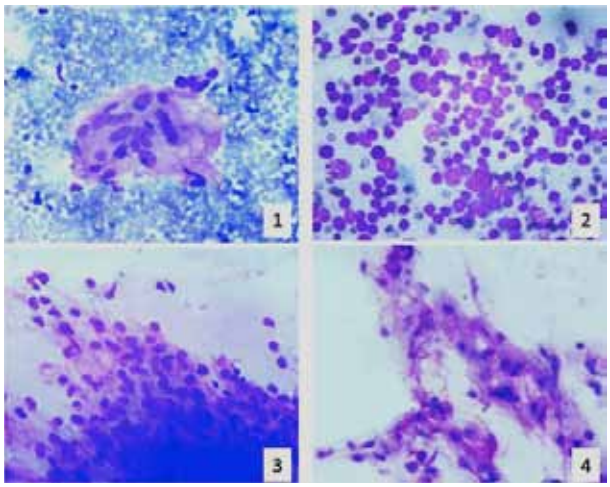

Figure 1. Illustration of Smears. 1) Meningioma, Imprint; 2) Ependymoma, imprint; 3) Astrocytoma, squash; 4) Dysembyroplastic neuroepithelial tumour, squash

Table 1. Sensitivity, Specificity and Diagnostic Accuracy of Squash and Imprint Smears

Smear	Cytological diagnosis	Final diagnosis B	M	Sens (%)	Spec (%)	Accuracy (%)
Squash						
M	85	8	77	90.6	87.5	89.3
B	49	56	8			
Imprint						
M	85	6	79	92.9	90.6	92.0
B	49	58	6			
Combined						
M	85	4	81	95.3	93.8	94.6
B	49	60	4			

Sens, sensitivity; Spec, specificity; B, benign & non- neoplastic; M, malignant

Considering the histology as gold standard, total 16 (10.7%) cases were misdiagnosed on squash smears. Of the 64 cases of astrocytoma 43 were low grade and 21 were high grade. Out of these, four cases of low grade astrocytoma and two cases of high grade astrocytoma were false positive by squash technique and finally confirmed as meningioma and ependymoma respectively on histology. We had only one case of mixed glioma which was misinterpretated as reactive gliosis on squash smear, while the cases of reactive gliosis and dysembryoplastic neuroepithelial tumour were misjudged as low grade astrocytoma. A single case of oligodendroglioma was correctly diagnosed on cytology.

Two cases of lymphoma were seen - one from a male aged 14 years and the other from a female of 50 years. Correct diagnosis of non-Hodgkin's lymphoma was made in one case by the squash smear technique but the other case was misdiagnosed as inflammatory lesion. Both the cases were confirmed to be non- Hodgkin's lymphoma, (lymphoblastic variety) on histology. A case of temporal swelling that was diagnosed as anaplastic spindle cell tumour on cytology was confirmed as mesenchymal chondrosarcoma on histology.

Out of 6 metastatic tumours three were in the brain and three in the spinal cord. Squash smears were studied in all the 6 cases; of which 2 cases were misdiagnosed; one as haemangioblastoma and the other as inflammatory lesion. Histology helped in diagnosing the exact morphological nature of all 6 metastatic lesions. They comprised of adenocarcinoma, melanoma, squamous cell carcinoma and adenoid cystic carcinoma. Out of 5 cases of ependymoma, one was reported falsely positive, one falsely negative and one was under graded.

Of the total 21 cases of meningioma (confirmed on histology), one case was false positive and four were false negative by squash technique. Both the cases of haemangioblastoma were misdiagnosed on squash smear and labelled as vascular tumours. A correct diagnosis could be made on histology only.

Considering the histology as agold standard, a total of 9 (8.0%) cases were misdiagnosed on imprint smears. Of the total 21 cases of meningioma (confirmed on histology) all the cases but one were diagnosed by imprint smear. In addition one case of meningioma was false positive

by imprint which was finally confirmed as low grade astrocytoma. Of the five cases of ependymoma, three were misinterpretated as astrocytoma or medulloblastoma. The case of mixed glioma (100%) and mesenchymal chondrosarcoma were mistaken for reactive gliosis and spindle cell anaplastic tumour respectively. Out of two cases diagnosed as inflammatory lesion by imprint, one case was confirmed as non- Hodgkins lymphoma (lymphoblastic variety) and the other as metastatic tumour on histology. Out of 5 cases of ependymoma, one was reported falsely positive, one falsely negative and one was was undergraded. Both the cases of haemangioblastoma (100%) were labelled as vascular tumour by the imprint smear technique.

The sensitivity, specificity and diagnostic accuracy of imprint smear were found to be higher than that of the squash smear (92.9%, 90.6% and 92.0% respectively) (see Table 1). When both the techniques were combined they were much higher (95.3%, 93.3% and 94.6% respectively).

Discussion

The smear technique can provide a rapid, reasonably accurate and reliable diagnosis to the operating surgeon. Using even a very small piece of tissue that is insufficient for frozen section, it is possible to detect cellular details including vessels and to make a definite pathologic diagnosis by the smear techniques. Besides this, the use of haematoxylin-eosin and Giemsa stains in contrast to the usual supravital stains has facilitated a permanent preparation and easy comparison of the smears with the paraffin sections for accurate interpretation.

In the present study, results of the smear techniques (squash and imprint) were compared to the final histological diagnosis. Study included a total of 149 central nervous lesions with 85 (57.05%) malignant and 49 (42.95%) benign lesions. Smear techniques diagnosed 85 malignant and 49 benign lesions. For malignant cases 8 were false positive and 8 were false negative by squash technique; whereas 6 were false positive and 6 were false negative by imprint technique. When both techniques were combined 4 were false positive for malignancy, 4 were false negative. The sensitivity, specificity and diagnostic accuracy of smear technique in the present study are consistent with that of the previous studies. Asha et al (1989) reported an accuracy rate of 87% when 178 squash smears were compared with histology. Pogady et al (1993) and Ili et al (1995) reported accuracy rates of 90% and 94.17% by smear techniques respectively. Imtiaz et al (2006) observed an overall diagnostic accuracy of crush smear of 93.5% in a cross sectional study including 100 intraoperative neurosurgical biopsies. Shukla et al (2006) investigated 278 patients using squash smear technique and on comparing the cytologic diagnosis with final histologic diagnosis, the diagnostic accuracy was 87.76%. Iqbal et al (2007) studied 151 cases with intraoperative crush smear cytology and observed a diagnostic accuracy of 95.36% when histology was taken as gold standard. Jaiswal et al (2010) conducted a retrospective study of 326 cases of CNS intraoperative consultations using crush smears and obtained a concordance of 83.7% between the intraoperative diagnosis and the final diagnosis.

The diagnostic accuracy of imprint smear was found to be more than the squash smear due to reasons like maintenance of architecture in the smearing process. Thus microcystic areas, rosette formation and other structural features of tumours are seen in the imprint smears and help in making more accurate diagnosis. Whereas, in squash smear these structures may be destroyed. Furthermore, thickness of the imprint smear is even and so cellularity of smear can be commented upon with certainity, but in squash smear thickness is not uniform and depends on the pressure exerted during crushing and the type of the tissue smeared. So an estimate of cellularity cannot be made with certainity and chances of misdiagnosing a high grade tumour are higher. Another advantage of imprint smear is that the tissue is not destroyed and so it can be used for subsequent histopathological sectioning. For crush smear soft portion must be available for smearing and tissue taken is a tiny part of the lesion and may not contain the representative areas leading to false positive and false negative results.

In conclusion, from the present study it is evident that both the squash and imprint smear examination of lesions of central nervous system are useful, quick and reliable diagnostic tools. However, imprint smear examination is superior to squash smears not only because of its better sensitivity, specificity and diagnostic accuracy, but also due to added advantages like reduced tissue damage in imprint technique and reuse of the same material for histological interpretation.

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