DIAGNOSTIC ACCURACY AND PITFALLS OF SQUASH SMEAR CYTOLOGY VERSUS PARAFFIN SECTIONS OF GLIAL LESIONS

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ABSTRACT Diagnosis of central nervous system lesions is always a challenging task for neurosurgeon and neuropathologist. There is varied spectrum of CNS lesions, starting from inflammatory lesions to neoplastic lesions, traumatic injuries and degenerative changes. Intraoperative diagnosis of these lesions is of utmost importance as further management relies on it. Total 42 cases of glial tumors were received by the department of Pathology for intra-operative pathological reporting in four year period. Unfixed tissue was received and first examined grossly.

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INTRODUCTION:

Diagnosis of central nervous system lesions is always a challenging task for neurosurgeon and neuropathologist. There is varied spectrum of CNS lesions, starting from inflammatory lesions to neoplastic lesions, traumatic injuries and degenerative changes. Intraoperative diagnosis of these lesions is of utmost importance as further management relies on it. Recent advancement of radiological techniques like MRI and CT scan has helped a lot in diagnosis of intracranial lesions, even small lesions which are clinically silent can now be detected but still the role of intraoperative pathological diagnosis is of great value. Overall diagnostic accuracy of preoperative radiological findings is good, but still 10% - 30% cases are misdiagnosed. (1)

Decision regarding proper sampling of lesion, margin and nature of lesion, are all dependent on intraoperative pathological diagnosis. Though histopathology is always the gold standard technique for diagnosis but for intraoperative pathological opinion, frozen section provides best architectural details. Frozen sectioning technique used for intraoperative diagnosis is not always available in many centers of our country. Intraoperative diagnosis in CNS lesions can be done by cytological evaluation of tissue. Crush cytology has emerged as a very helpful tool in intraoperative pathological reporting of CNS lesions. Opinion can be given even with small tissue obtained by stereotactic biopsies.

Overall incidence of intracranial tumors account for 10-17 per 1,00,000 persons [2-3]. Of various CNS lesions, glial tumors are most common lesions that we come across. The correct intraoperative assessment is necessary as their nature varies from milder form of pilocytic astrocytoma to aggressive Glioblastoma.

We have conducted this study so as to share our experience of squash cytology of glial tumours and to find its diagnostic accuracy & shortcomings.

MATERIAL AND METHODS:

This study was conducted in the Department of Pathology, Bhopal Memorial Hospital and Research Centre, Bhopal. It was approved by the Institutional ethical committee. Total 42 cases of glial tumors were received by the department of Pathology for intra-operative pathological reporting in four year period. Unfixed tissue was received and first examined grossly. The apparently viable tissue was then placed between two glass slides. With sufficient pressure between the tips of thumb and index finger, thin smears were made. They were then immediately fixed in 95% ethyl alcohol for 1-2 minutes and stained by rapid Hematoxylin and eosin (H&E) method. Targeted turn-around time was 15 to 20 minutes for intraoperative diagnosis. Microscopic features of squash smears were noted based on cellular, cytoplasmic, nuclear and background features. Findings were correlated with the available clinical and radiological findings and also with the final histopathological diagnosis. Cytological findings were co-related with histopathological diagnosis which is accepted as gold standard. To avoid observer bias, two pathologists independently evaluated all the cases. Statistical analysis was done and diagnostic accuracy for squash cytology was calculated. Observations were recorded and detailed re-evaluation of discordant cases was done.

Deviations in the grade of tumour or altogether different diagnosis on squash smear cytology were taken as discordant whereas the cases with the same diagnosis and grade on cytology and histopathology were taken as concordant. The tumours were classified according to the 2007 World health organization classification of CNS neoplasms.(4)

RESULTS:

Total 42 cases were studied. Out of these, 26 were male and 16 were female. Glioblastoma is the most common finding (57.14%) followed by astrocytoma (26.19%), ependymoma (9.52%), oligodendroglioma (4.76%) and ganglioglioma (2.38%). (Fig:1)

Most of the tumors were grade IV (57.14%) followed by grade III (16.66%), I (14.28%) and II (11.90%). On crush cytology, overall 39 cases were reported correctly whereas 03 cases were misdiagnosed. (Fig:1)
Two cases of Glioblastoma and one case of astrocytoma were diagnosed incorrectly on crush cytology. Overall diagnostic accuracy of crush cytology in diagnosing glial tumor came out to be 92.85%.

DISCUSSION:

Intraoperative cytological preparation was first introduced by Cushing and Eisenhardt in year 1930 and by Badt in 1937. (5), Even in apex centers of our country cryostat facility is not always available for intraoperative pathological diagnosis. Crush cytology can solve the same purpose with high precision in lesions of CNS, as there is no or very little fibrous tissue. Smears can easily be made and spread is even whereas in tissue from other region there is always a difficulty due to large amount of connective tissue. Squash cytology also brings challenges of crushing or pressure artifact, loss of characteristic histological pattern and other misleading findings which can be overcome with experience of crush cytology reporting.

We found diagnostic accuracy of 92.85% which is comparable to the study done by Pawar et al (6) where they found 88% accuracy. Other authors found accuracy ranging from 86-97%. Similar findings were obtained in a study done in Brazil which reported specificity and positive predictive value of 35% and 99.1% respectively. Glial tumors were identified by their fibrillary background along with other specific subtype findings. We misdiagnosed two Glioblastoma cases on squash cytology.(Table-1) One was wrongly reported as granulomatous inflammation as large areas of necrosis were seen but no surrounding glial tissue seen. Mitotic activity was indeterminate. The capillary endothelial cells were entangled and gave impression of epithelioid cells. (Fig-2)Another case was misdiagnosed as gemistocytic astrocytoma, as several gemistocytes were observed in absence of areas of necrosis and mitotic activity. (Fig-3)Although we diagnosed it as astrocytic tumor however we missed the grade of tumor so taken as misdiagnosed case. A case of astrocytoma was misdiagnosed as oligodendroglioma because several thin walled vascular channels are seen with foci of calcification.(Fig-4) Many a times the tissue edema is misinterpretated as peri-nuclear halo and newly proliferating blood vessels were taken as chicken wire appearance leading to misdiagnosis as oligodendroglioma. Mouriquand et al and other authors also observed similar findings (7).

The tumour may be under graded because of sampling error or non-availability of the tissue having representative areas or avoidance of necrotic tissue deliberately while preparing cytosmears. Thus sampling from adequate areas plays very important role in correct diagnosis and cases were often misinterpreted when not adequately sampled.

Crush cytological preparation can serve as a cheap diagnostic tool in experienced hands. Very less tissue is required for diagnosis and the remaining tissue can be used for further processing and other modalities to help in final diagnosis (8). Squash preparation is also useful in processing samples from patients of AIDS and other slow virus diseases, considering the contamination of instruments used by fresh unfixed tissues(9,10).The squash technique is useful in centers where facility for frozen section is unavailable or in case of power breakdown or lack of trained technical personnel(11)

CONCLUSION

We highly recommend use of squash smear cytology, as it is quite accurate, simple, cheap and reliable method for intraoperative diagnosis of glial lesions.As cryostats are not available in every center and it also requires trained technical staff, so crush cytology is a better established method for intraoperative neuropathological diagnosis. In experienced hands crush cytology attains high degree of accuracy in presence of proper clinico-radiological findings and adequate sampling.

Table 1: Misdiagnosed cases on squash cytology.

S.No	Squash Diagnosis	Histopathology
1	Granulomatous Inflammation	Glioblastoma
2	Gemistocytic astrocytoma	Glioblastoma
3	Oligodendroglioma	Diffuse astrocytoma

Figure 1: Statistical data
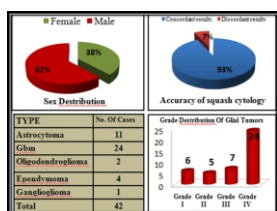
Figure 2: Squash cytology (H&E): GBM misdiagnosed as granulomatous inflammation.
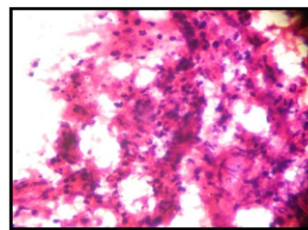
Figure 3: Squash cytology(H&E) : GBM misdiagnosed as gemistocytic astrocytoma
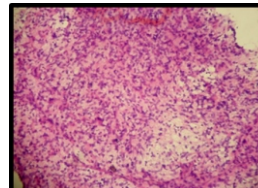
Fig-4Figure 4 : Squash cytology(H&E): Diffuse astrocytoma misdiagnosed as oligodendroglioma.
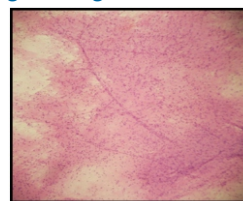
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