STUDY OF INTRAOPERATIVE SQUASH CYTOLOGY OF INTRACRANIAL AND SPINAL CORD LESIONS WITH HISTOPATHOLOGICAL AND IHC STUDY

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ABSTRACT

BACKGROUND

The causes of discordant diagnoses achieved at squash cytology of intracranial and spinal cord tumours were ascertained. Lesions having the advantage of diagnostic accuracy by squash cytology of intracranial and spinal cord lesions was also determined.

METHODS

Squash preparations of 72 patients suspected to have neoplasia were made and stained with rapid haematoxylin and eosin stain and toluidine blue stain. The smears were classified according to the cytomorphological criteria and the squash cytodiagnoses were compared.

RESULTS

Total 72 cases were studied, 93.9% were neoplastic and 6.1% non-neoplastic on histopathology. Amongst neoplasms, Astrocytic tumours constituted 26.3% of cases followed by Meningiomas comprising 20.8%. Amongst the benign lesions, Tuberculoma was seen most frequently (6.95%). Overall diagnostic accuracy of squash was 98.65%. On statistical analysis, Sensitivity, Specificity, Positive Predictive value (PPV) and Negative Predictive Value (NPV) of squash cytology were 98.6%, 100%, 100% and 80% respectively.

CONCLUSION

Intraoperative Squash is reliable, accurate, cost effective diagnostic modality when combined with histopathological and immunohistochemical techniques.

KEYWORDS

Squash, Astrocytoma, Meningioma, Immunohistochemistry.

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INTRODUCTION: AIMS AND OBJECTIVE: Squash Cytology is a universally accepted technique in diagnosing a variety of Central Nervous System (CNS) lesions and is presently in vogue for both therapeutic and prognostic purposes.[1] This study was conducted with an aim to correlate squash smears with immunohistopathology and to compare statistical data employing sensitivity, specificity and diagnostic accuracy of squash cytology.

Pre-surgical diagnoses of a CNS lesion achieved by cytologic methods or by tissue biopsy helps a surgeon plan his surgery and alternative treatments. The diagnosis of intracranial tumours before surgery remained elusive for decades and was achieved only after its grossing and processing in surgical pathology laboratories.[1] Radio-imaging techniques such as contrast enhanced computerised tomography (CT) and magnetic resonance imaging (MRI) has considerably helped the neurosurgeons.

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The stereotactic brain biopsy requires technical knowhow, expertise and operation theatre facilities and thus could not be available to all operating neurosurgeons.[2] The reliability of frozen section examination of the tissue as an intraoperative diagnostic technique has been questioned due to the inherent artefacts, interpretation and instrumentation involved. The reason for the discrepant diagnosis on frozen section is partly due to the high water content and fat in fresh brain tissue, and the innately soft nature of brain tumour.[3]

The other alternative modality for diagnosis is squash cytology/crush smear cytology of CNS lesions obtained intra-operatively. This technique is dependable because of good preservation of the cell morphology, minimal difficulty involved and very low turnaround time. It has now been widely accepted as a standard diagnostic procedure.[4] In this present study, the cytomorphology of neoplastic lesions of CNS by intraoperative squash cytology was studied and compared with the histopathological diagnosis on surgical biopsy specimen and a correlation was established. The causes of discordant diagnoses achieved at squash cytology of CNS lesions were ascertained. Tumour types which can be of diagnostic surety by squash cytology of intracranial and spinal cord lesions was also determined.

MATERIAL AND METHODS: This is a prospective observational study conducted in the Department of Pathology, Osmania General Hospital, Hyderabad.

Inclusion Criteria: Seventy-two indoor patients of neurosurgery with space occupying lesions suggestive of space occupying lesion on clinicoradiographic assessment posted for open biopsy or excision for assessment of surgical management were included in the study.

Exclusion Criteria: Cases with insufficient tissue quantity to yield cytological diagnosis and not suitable for squash preparation were excluded from the study.

A study proforma with preliminary data of name, age, sex, address, hospital registration number and clinical diagnosis was recorded. The findings of computed tomography (CT) and/or magnetic resonance imaging (MRI) evaluation of the intracranial and spinal cord lesions were recorded for their size, location, number and radiologic diagnoses. The squash preparations were performed in the pathology laboratory on a small unfixed piece of tumour tissue, received on saline soaked gauze immediately after removal. Squash smears were made by placing another clean glass slide over it and a gentle pressure was applied as the slides were pulled apart to make the smears of uniform thickness. They were immediately put in 95% ethyl alcohol for 2 minutes for wet fixation and were subsequently stained in the pathology department with rapid haematoxylin and eosin stain. All squash smears were also dry fixed to be stained by Toluidine Blue stain.

In provisionally cytodiagnosed cases of granulomatous inflammatory lesions, a PAS fungal stain was performed routinely. Wherever necrosis was evident with granulomatous reaction ZN stain to unearth AFB was performed. An attempt was made to perform immunocytochemistry in difficult cases, but were reviewed with immunohistopathology. The squash smears were assessed for their cellularity and were typed according to the cytomorphological criteria described in the literature. The remaining left over tissue was sent for routine histopathological processing for further evaluation and correlation.

The histomorphology of the CNS lesions were reported based on the standard WHO criteria.[5] Immunohistochemical stains were done in histologically indefinite cases to come to a final diagnosis where deemed necessary using standard IHC protocols. The intraoperative cytodiagnoses of CNS lesions were correlated with the final immunohistopathologic diagnoses. Squash cytology results were classified into the following categories: True negative (Absence of malignancy correctly diagnosed); true positive (Presence of malignancy correctly diagnosed); false negative (The cytological specimen failed to diagnose as malignancy); and false positive (The cytological specimen was incorrectly considered or suspect of malignancy). The tumours were classified according to the World Health Organization classification of CNS neoplasm 2007.[5]

RESULTS: The study comprised of 72 patients. The M:F ratio was approximately seen to be 1.08:1. Maximum numbers of cases were in the age group of 31-40 years and minimum in more than 70 years. The youngest patient was 3 months old and the oldest was 76 years old.

Age	No. of Cases	Males	Females
0 to 10	11	09	02
10-20	07	05	02
21-30	11	04	07
31-40	16	05	11
41-50	10	02	08
51-60	07	05	02
61-70	07	05	02
>70	03	03	-
Total	**72**	**38**	**34**

Table 1: Age and Sex Distribution

Age	No. of Cases	Inflammatory Lesion	Neoplastic Lesion
0-10	10	01	10
11-20	07	00	07
21-30	11	01	10
31-40	16	00	16
41-50	11	01	10
51-60	07	02	05
61-70	07	00	07
>70	03	00	03

Table 2 Distribution of Age with Type of Lesion

Cytodiagnosis	No. of Cases
Astrocytoma	18
Meningioma	16
Metastatic Deposit	07
Schwannoma	06
Medulloblastoma	05
Non-neoplastic lesions	05
Craniopharyngioma	01
Oligodendroglioma	01
Papillary Ependymoma	01
Lymphomas	01
Pituitary adenoma	01
Lipoma	01
Epidermoid cyst	02
Chordoma	01
Arachnoid cyst	01
Small Round Cell tumour	01
Langerhans Cell histiocytosis	01
Choroid Plexus Papilloma	01
Pinealoblastoma	02
Total	**72**

Table 3: Distribution of Cytodiagnosis

No specific trend of occurrence of neoplastic lesions was noted as the intracranial and spinal cord malignant lesions were distributed throughout all age ranges. There were 05 inflammatory lesions (6.9%) and 67 neoplastic lesions (93.05%).

Grades	Cytological Grading	Histological Grading	No. of Discordant Cases
Grade 1	03	02	01
Grade 2	07	06	01 (SEGA Grade 1)
Grade 3	01	00	01 (Grade 4)
Grade 4	08	07	01 (Grade 3)

Table 4: Cytological Grading of Astrocytomas

No. of Discordant Cases	Cytology	Histology	Immunohistochemistry	Final Diagnosis
1	Papillary Ependymoma	Choroid plexus Papilloma	Cytokeratin 7, EMA, GFAP	Choroid plexus Papilloma
2	Astrocytoma Grade 2	SEGA	GFAP, Ki67	SEGA
3	Astrocytoma Grade 3	Glioblastoma Multiforme	GFAP, Ki67	GBM
4	Pinealoblastoma	CNS PNET	CD99, GFAP, Synaptophysin, Ki67	CNS PNET
5	Medulloblastoma	Pilocytic Astrocytoma	GFAP, Ki67	PA
6	Small round cell tumour	Diffuse Large B Cell Lymphoma	CD 20, CD 45, CD 99.	DLBCL
7	Pinealoblastoma	Immature Germ Cell Tumour	OCT2, SALL4	Immature GCT
8	Astrocytoma Grade 3	Low Grade Astrocytoma	GFAP, Ki67	LGG
9	Low Grade Astrocytoma	Oligoastrocytoma	GFAP, Ki67	LGG
10	Pilocytic Astrocytoma	Ependymoma Grade 2	EMA, GFAP	PA
11	Astrocytoma Grade 4 (GBM)	Astrocytoma G3	GFAP, Ki67	Astrocytoma grade 3
12	Chronic Granulomatous Lesion.	Glioblastoma Multiforme	GFAP, Ki67	GBM

Table 5: Comparison of Discordant Cytological Diagnosis with Histodiagnosis

FP	FN	PPV	NPV	Sensitivity	Specificity	Accuracy
-	01	100%	80%	98.6%	100%	98.65%

Table 6: Values of Correlation of Cytological Diagnosis

Microscopic Images Squash and Histopathology of Discordant Cases

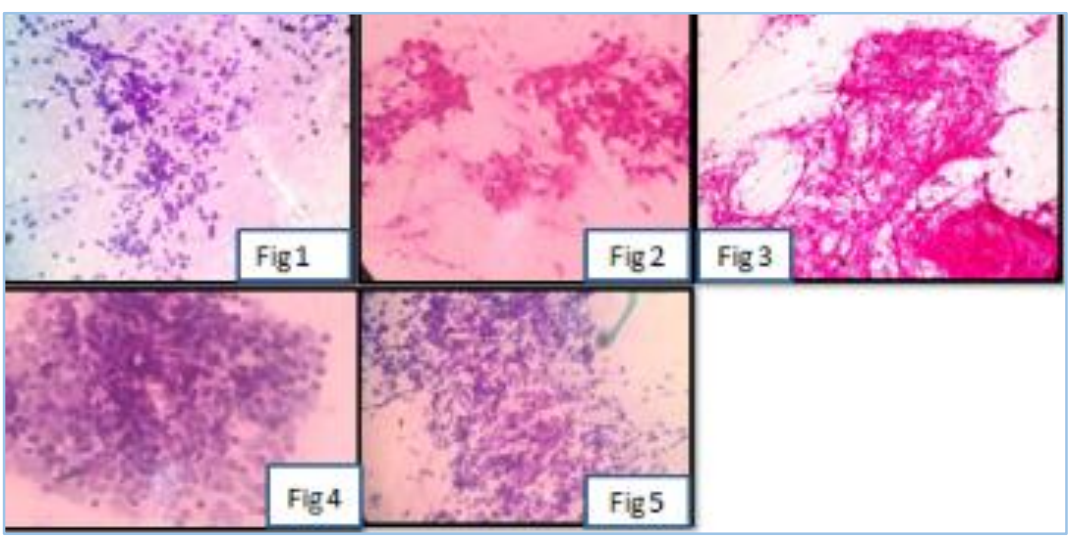

Fig. 1: Squash Smear Grade 1 Astrocytoma (Toluidine Blue Stain 10X)
Fig. 2: Squash Smear Grade 2 Astrocytoma 10X H&E Stain
Fig. 3: Squash Smear Grade 2 Astrocytoma H and E Stain 10X
Fig. 4: Squash Smear Medulloblastoma (Toluidine Blue) 40X
Fig. 5: Squash Normal Cerebellum (Toluidine Blue Stain 10x)

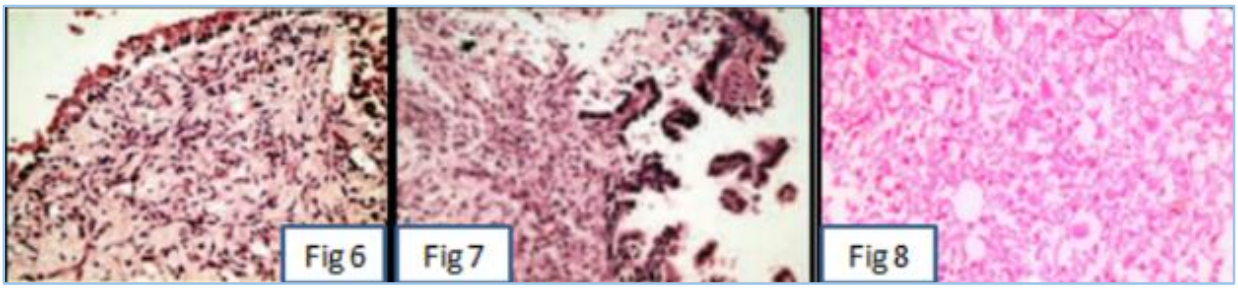

Fig. 6: Histopathology of Immature Teratoma 10X H&E Stain
Fig. 7: Histopathology of Immature Teratoma with Immature Neuroepithelial Elements 40X H&E Stain
Fig. 8: Histopathology of Pilocytic Astrocytoma with Charachterstic Rosenthal Fibres 40X H&E

DISCUSSION: In the present analysis, we have observed that there is no clear age distribution of the tumours of the CNS with lesions occurring in wide age range.[6] The present study has also observed that brain and spinal cord tumours were distributed in similar age. Although, we encountered only three cases of spinal lesions in one-year analysis. The present study; however, observed male to female ratio of 1.08:1 which is in agreement with other authors. Many studies on CNS space occupying lesions diagnosed by intraoperative squash cytology reported a predominance of neoplastic over inflammatory pathologies. The present study has a similar observation regarding distribution of the lesions of neoplastic pathology (93.86%) versus inflammatory pathology (7.14%) which is in accordance with other studies.[7,8,9,10,11,12,13,14,15]

Astrocytoma as a neoplastic lesion was commonly observed in many different studies. The cytomorphological features of Tuberculoma were characterised by presence of epithelioid granuloma comprising numerous epithelioid cells, Langhans giant cells, lymphocytes, plasma cells. The corresponding paraffin sections also showed the presence of numerous granulomata in the lesion.

At times, cytomorphological resemblance of Langhans giant cells to tumour giant cells occurring in GBM has been noted. We cytodiagnosed a case as chronic granulomatous lesion based upon extensive eosinophilic amorphous acellular material interpreted as caseous necrosis on histodiagnosis, this case turned out a GBM with extensive garland type of pseudopalisading necrosis with tumour giant cells.

A pineal region tumour cytodiagnosed as pinealoblastoma was confirmed immunohistologically as immature teratoma with presence of ectodermally derived epithelial tissue islands and mesodermally derived mature cartilage with accompanying neuroepithelial elements with mitosis. When Spindle to oval cells with eosinophilic cytoplasm, vesicular nuclei with evenly distributed chromatin with whorling pattern of arrangement were seen on squash smears, in such cases a cytodiagnosis of meningioma was confidently put forth.[7,8,13] Many studies have also reported intranuclear inclusions as a common finding associated with cytomorphology of meningioma. However, in the present study, 13/16(81.25%) cases revealed presence of intranuclear inclusions whereas two cases did not reveal which may be attributed to thick smear preparations.[10,15]

Presence of spindle cells clusters with difficulty in smearing, overlapping cell clusters and individual tumour cells having pointed spindled wavy nuclei also containing hypocellular areas with palisading of cells around collagen was observed in cases of Schwannoma.[10,15]

Cells with sharply defined cell margins having polygonal to round shapes and enlarged round to oval nuclei with dense eosinophilic finely granular cytoplasm were observed in cases of Pituitary Adenoma.[8] Many studies have reported grade-I astrocytoma on squash cytology in low to moderately cellular smears with tumour cells showing fine, slender and short projections, increased chromasia and delicate fine chromatin with minimally increased vascularity and absence of necrosis.[9,10]

Presence of smears with moderate increase in cellularity with thin cytoplasmic processes, coarse irregular nuclei and endothelial cell proliferation was observed in grade 2 astrocytomas.[9] Diagnostic cytomorphological features for grade-III astrocytoma that is anaplastic Astrocytoma on squash smears showed moderate to high cellularity, cells with prominent cytoplasmic processes, irregular and pleomorphic nuclei with dense and irregular coarse chromatin with areas of haemorrhage and increased vascularity with lack of necrosis.[9,10]

In our study, we encountered a case of Astrocytoma grade 3 which was finally reported Glioblastoma Multiforme on histopathology. Cytosmears when showing high cellularity comprising cells with short blunt cytoplasmic processes, marked pleomorphic nuclei coarsely clumped chromatin, with increased mitotic figures, endothelial cell proliferation resembling glomeruloid bodies, coagulative necrosis and tumour giant cells were diagnosed as Glioblastoma Multiforme.[9,10] Some authors have observed discordance in reporting of metastatic lesions to brain with high grade gliomas[9], we did not encounter any discordant case in our study on immunohistological comparison. The present study has cytodiagnosed 3 cases of poorly differentiated squamous cell carcinoma, 2 cases of adenocarcinoma, 2 cases of follicular thyroid carcinoma intraoperatively and later confirmed immunohistologically.

Grading of squash cytosmears using WHO grading system has been attempted by many authors, they observed difficulties in interpreting cytosmears with low grade astrocytomas with reactive gliosis. The present study also encountered a similar problem in grading of a few cases as mentioned by the other authors though there were no major fallacies in grading as down grading was done in one of the 18 cases on histopathological assessment. A study of Sharma et al[14] adversely commented on grading of astrocytoma on squash smears and imprint cytology.

However, we recommend the performance grading of squash cytosmears as total of 73.68% of grades were in agreement with the final grade on histopathology. Most of the studies attributed to the incompatible grades achieved on histopathology were due to non-representative tissue sampling for squash cytology.

Authors	Discordant Cases	Reasons for Discordance
Cahill and Hidvegi et al[8]	3/32	Limited to Type of Lesion
Shah et al[9]	6/156	Failure to Diagnose
Savargaonker and Farmer et al[6]	6	Not Mentioned
Torres and Colleco et al[10]	22/307	Not Mentioned
Kini et al[11]	14/100	Failure to Diagnose Histological Type
Mitra et al[12]	11/31	Grading Errors
Jha et al[13]	2/34	Grading Errors
Firlik et al[7]	32/316	Not Mentioned

Table 7: Discordant Cases of Various Studies and Reasons for Discordance

Twelve Discordant cytodiagnosis of various categories was confirmed on histopathology as per Table 5. In Present study, cytodiagnosis of five different grade astrocytoma were histologically classified and graded as Sub ependymal Giant Cell Astrocytoma, glioblastoma multiforme, low grade astrocytoma, ependymoma. Discordance in Subependymal Giant cell Astrocytoma can be explained by the absence of pleomorphic tumour cells and giant cells on account of sampling error. Grade discordance in various astrocytomas could be either lack of necrosis on cytosmears leading to undergrading or misconstruing of blood and plasma products as necrosis. Low grade astrocytoma on cytodiagnosis was histologically reported as oligoastrocytoma, this can be explained by lack of oligodendroglial cells (Cells with perinuclear clearing on cytosmears) which may be not evident at times in wet fixed smears.

Cytodiagnosis of pinealoblastoma reported in a five year male child and twenty eight year adult male were histodiagnosed as immature teratoma and central CNS PNET respectively. The latter was a recurrent CNS tumour with previous histodiagnosis of pinealocytoma with astroglial differentiation. On suprasellar recurrence cytodiagnosis of pinealoblastoma eventually turned as CNS PNET on immunohistology. The cytodiagnosed case of pinealoblastoma in a 5-year-old child was reported as immature germ cell tumour on immunohistology. This could be explained by differential sampling on squash cytology with absence to recognise epithelial and immature neuroectodermal structures.

Discordant cytodiagnosis of medulloblastoma was histologically reported as pilocytic astrocytoma. The plausible explanation could be selective sampling of tumour tissue during surgery by trans-vermian approach leading to initial sampling of normal cerebellar tissue with inability to recognize it on toluidine blue stain where it closely resembles the round cells characteristically seen in medulloblastoma, we observed morphology of cells on air dried smears closely simulates the round blastic immature cells seen in medulloblastoma case, this scenario can be avoided in certain cases by requesting the neurosurgeons for second biopsy sample from the tumour proper.[15] The sensitivity and specificity in present study is observed as 98.6% and 100% respectively and is compared with other studies as below.

Authors	Sensitivity	Specificity
Firlik et al[7]	96%	75%
Sharma et al[14]	90%	87.5%
Present Study	98%	100%

Table 8

Almost all studies reviewed for the present work have reported a high accuracy rate of intraoperative squash smear diagnosis of intracranial and spinal cord tumours as more than 80%.[1,3,4,6-8] The present study has an accuracy rate of 98.57% which is in agreement with the above studies.

CONCLUSION: On submission of representative sample of a CNS lesion, the cytomorphology of cellular arrangements and its architectural patterns are characteristic of specific diagnosis of varied CNS lesion.

The sensitivity, specificity and rate of accuracy for squash cytology are high with a low turnaround time. A detailed study of the discordant lesions and its reasons are understood and applied for future cases, hence intraoperative squash diagnostic technique is reliable and useful in the management of CNS lesions.

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