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Original Research Article

Study of assessing diagnostic efficacy of squash smear technique and frozen section by comparing with histopathology in CNS lesions, with special reference to IHC

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ABSTRACT

Introduction: Central nervous system (CNS) squash cytology (CSC) has established itself as a technically simple, rapid, inexpensive, fairly accurate, and dependable intraoperative diagnostic tool. It helps neurosurgeons immensely when management is dependent on it.
Aims: To asses the efficacy of squash cytology in the intraoperative diagnosis of CNS lesions by comparing with histopathological diagnosis.
Materials and Methods: The present study is a prospective study done for one and a half year includes 43 patients who presented to the department of neurosurgery with complaints of a headache, backache, epilepsy and radiologically diagnosed to have a space occupying lesion. Only the cases where either squash cytology or frozen section was performed were included in the study.
Results: In the present study, it is observed as Out of 43 cases, squash cytology is performed in 42 cases in which histopathological diagnosis is correlated in 36 cases with a diagnostic accuracy of 85.7%. The diagnostic accuracy of squash cytology in common CNS tumors gliomas and meningiomas was 92.8% and 100% respectively. Out of the 43 cases, the frozen section is performed in 42 cases of which histopathological diagnosis is correlated in 37 cases with a diagnostic accuracy of 87%. The diagnostic accuracy of frozen section in common CNS tumors gliomas and meningiomas was 85.7% and 100% respectively. The sensitivity and specificity of frozen section in the identification of tumor was 94.7% and 100%. The positive predictive value and negative predictive value of the frozen section in the identification of the neoplastic lesion was 100% and 66.6% respectively.
Conclusion: Squash cytology and frozen section are preliminary diagnostic methods, which should always be followed by a definitive histopathological diagnosis.

1. Introduction

Brain tumors account for 2% of cancers and most of them being primary tumors. They account for 20% of childhood neoplasms. The annual incidence of central nervous system (CNS) tumors is 10 to 17 persons per 100,000 for intracranial neoplasms and 1 to 2 per 100,000 for intraspinal neoplasms.[1] The various CNS lesions range from the neoplastic gliomas to non-neoplastic infectious lesions like tuberculosis

Intraoperative pathologic consultation plays an important role not only in the diagnosis of the lesion but also

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in guiding the neurosurgical biopsies and their resection. Stereotactic surgeries are the commonly used methods in dealing the brain tumors due to their location. The neurosurgeon will have minimal feasibility in localization and demarcation of the tumor when compared to other sites. Approximately 33% of the stereotactic biopsies may yield nondiagnostic tissue in the first specimen, and intraoperative evaluation of this material assures that a sufficient number of diagnostic biopsy samples are obtained. Discrepancies between intraoperative diagnosis and final histopathological diagnosis were seen in 3% of cases. [1]

With the advances in the neuroradiologic techniques, preoperative understanding of the neurosurgical lesion was achieved, and it aids the pathologist in forming an intraoperative diagnosis. Nonneoplastic lesions like infections including tuberculosis, fungal infections, a brain abscess can be diagnosed early, as they are potentially curable, which may sometimes lead to neurological deficits when there is a delay in diagnosis.

Intraoperative rapid diagnostic methods include cytological examination, frozen sectioning and touch imprint preparations. The high water content of the brain tissue and small amount of tissue requirement makes this crush cytology more feasible on CNS lesions. A small piece of tissue bit about 1mm size is crushed under the microscopic slides and made into smears. Then they are stained and examined. This method is easy to perform and, no special equipment is needed. Frozen section is a special histotechnique method which uses instrument cryostat, with the help of which sections are prepared and stained with rapid hematoxylin and eosin staining method. Due to the high water content, in frozen section ice crystal artifacts are formed commonly, but the pathologist is more familiar with the tissue morphology in case of frozen section compared to cytology smears. Further, the frozen section is more feasible in CNS lesions like meningiomas, schwannomas, neurofibroma where the tissue is firm in consistency.

2. Materials and Methods

The present study is a prospective study done for one and a half year (December 2016 – May 2018) in the Department of pathology. This study was done after taking the ethical approval from the ethical review committee of Narayana medical college, Nellore. This study includes 43 patients who presented to the department of neurosurgery with complaints of a headache, backache, epilepsy and radiologically diagnosed to have a space occupying lesion. Only the cases where either squash cytology or frozen section was performed were included in the study.

Patient details, presenting complaints and radiological findings were noted. During surgery, small tissue bits of size 1-3 mm are taken in saline and squash slides were prepared. The remaining tissue was used for frozen sectioning and then fixed in 10% formalin for histopathological examination.

2.1. Preparation of squash smear

After correlating the patient details on the container and in the requisition form, firstly the specimen is inspected grossly. Viable soft tissue is selected and is dissected into small 0.5- 1 mm^2 tissue bits with the help of forceps and scalpel. Hard and firm areas, necrotic and hemorrhagic areas are not selected. By pressing the tissue against the two microscopic slides, smears are prepared. Care was taken in applying pressure so that uniform smear is produced. Smears are then rapidly fixed by using alcohol and stained with hematoxylin and eosin.

2.2. Preparation of frozen section smears

By using cryostat first the tissue are frozen, and then sections are made with the help of microtome, and then slides are fixed in alcohol. These slides are then stained with rapid hematoxylin and eosin method

The squash cytology smears and frozen sections are studied, and the diagnosis was made separately. This diagnosis is then compared with the final histopathological diagnosis. In some cases, the diagnosis is inconclusive in histopathological slides and immunohistochemistry is used as the adjuvant.

The results of the study were then statistically analyzed by using the chi-square test, Fisher exact test.

The following formulae calculate the sensitivity, specificity, positive predictive value and negative predictive value:

Sensitivity - A/A+C X 100
Specificity – D/B+D X 100
Positive predictive value – A/A+B X 100
Negative predictive value – D/C+D X 100

3. Results

Table 1: Demographic distribution of cases

Age group	**Males**	**Females**
0-30 years	7 (16.27%)	3 (6.98%)
31-50 years	3 (6.98%)	8 (18.60%)
51-70 years	6 (13.95%)	16 (37.20%)
Total (n=43)	16 (37.21%)	27 (62.79%)
Lesions		
Neoplastic	13(30%)	26(60.4%)
Non – neoplastic	3(6.9%)	1(2.3%)
Location		
Intracranial	38	88.3%
Spinal	5	11.6%
Intracranial lesions		
Anterior cranial fossa	5	13.16%
Middle cranial fossa	22	57.89%
Posterior cranial fossa	11	28.95%

Table 2: Comparision of squash cytology diagnosis with histopathological diagnosis

Histopathological diagnosis	Squash cytology	
	With correlation (%)	No correlation (%)
Astrocytoma (8)	7 (87.50%)	1 (12.50%)
Oligodendroglioma (6)	6 (100%)	0 (0%)
Meningioma (8)	8 (100%)	0 (0%)
Schwannoma (7)	7 (100%)	0(0%)
Ependymoma (1)	1 (100%)	0 (0%)
Chordoma (1)	0 (0%)	1 (100%)
Choroid plexus papilloma (1)	1 (100%)	0 (0%)
Pituitary adenoma (1)	1 (100%)	0 (0%)
Metastatic deposit (2)	2 (100%)	0 (0%)
Tuberculoma (3)	2 (66.66%)	1 (33.33%)
Others (4)	1 (25%)	3 (75%)
Total cases	36 (85.71%)	6(14.29%)

In all the cases of meningiomas and schwannomas squash diagnosis is correlated with histopathological diagnosis. Squash cytology diagnosis in this study is comparable to histopathology with a p-value of 0.002, which is statistically significant.

In the case of chordoma, mixed glioma, ganglioglioma, gliosarcoma there is no correlation of squash diagnosis with histopathology.

Table 3: Comparision of frozen section diagnosis by with histopathological diagnosis.

Histopathological diagnosis	Frozen section(%)	
	With correlation	No correlation
Astrocytomas (8)	7(87.50%)	1 (12.50%)
Oligodendrogliomas(6)	5 (83.33%)	1 (16.66%)
Meningioma (8)	8(100%)	0 (0%)
Schwannoma(7)	7(100%)	0 (0%)
Ependymoma (1)	1 (100%)	0 (0%)
Choroid plexus papilloma (1)	1 (100%)	0 (0%)
Pituitary adenoma (1)	1 (100%)	0 (0%)
Tuberculoma (3)	3 (100%)	0 (0%)
Metastatic deposits (2)	2 (100%)	0 (0%)
Others (5)	2 (40%)	3 (60%)
Total cases	37 (88.09%)	5 (11.91%)

Out of the 43 cases, the frozen section is performed in only 42 cases. In case of chordoma due to inadequate sample, frozen sectioning is not done. Out of the total 42 cases, correlation to frozen section and histopathology is seen in 37 cases with an accuracy of 88.09%. Diagnosis is correlated in 12 cases of gliomas, all cases of meningiomas and schwannomas in the present study, frozen section diagnosis is comparable to histopathological diagnosis with a p-value of 0.016, which is statistically significant.

Table 4: Lesions with a disparity between squash cytology diagnosis and histopathology diagnosis

Squash cytology diagnosis	Histopathology Diagnosis
Low-grade astrocytoma	Tuberculoma
Reactive gliosis	Low-grade astrocytoma
Low-grade astrocytoma	Chordoma
Low-grade astrocytoma	Mixed glioma
Low-grade astrocytoma	Ganglioglioma
High-grade glioma	Gliosarcoma

The disparity in the squash cytology diagnosis and histopathological diagnosis is seen in 6 cases with a discordance rate of 14.29. Out of the 6 cases the differentiation of neoplastic and non- neoplastic lesions is not accurately done in 2 cases.

Table 5: Lesions with a disparity between frozen section diagnosis and histopathology diagnosis

Frozen diagnosis	Histopathology Diagnosis
Reactive gliosis	Low-grade astrocytoma
Reactive gliosis	Oligodendroglioma
Low-grade astrocytoma	Mixed glioma
Low-grade astrocytoma	Ganglioglioma
High-grade glioma with a spindle cell component	Gliosarcoma

The disparity in the diagnosis of frozen section and histopathology is seen in 5 cases. 2 cases of neoplastic lesions were diagnosed as non-neoplastic lesions in the frozen section.

Table 6: Age wise distribution of different grades of astrocytomas.

Age group	Grade I	Grade II	Grade III	Grade IV
0-20 years	2	1	1	0
20-50 years	0	0	0	1
>50 years	0	0	1	2

Out of the eight astrocytomas, 3 cases are grade 4 accounting to 37.50%. Low-grade astrocytomas are seen in younger age group, and high-grade astrocytomas are seen in elderly age group. The disparity in the grade of astrocytomas by squash cytology is seen in 2 cases and by frozen section is seen in 1 case. Both by squash cytology and frozen section 1 case of low-grade astrocytoma is reported as a non-neoplastic lesion.

Out of the 6 cases of oligodendrogliomas, 4 cases are grade II, and 2 cases are grade III. The age group of the cases ranges from 20-60 years. All the cases of the oligodendrogliomas were diagnosed by squash cytology accurately without disparity in grade. In frozen sectioning, the disparity in the diagnosis is observed in 1 case. Five cases were diagnosed accurately without disparity in grade.

Table 7: Disparity in the grades of astrocytomas between squash cytology, frozen sectioning, and Histopathology

Method	Astrocytomas studied	Accurate diagnosis with the grade	Discordance in grade	Discordance in diagnosis
Squash cytology	8	5(62.50%)	2(25%)	1(12.50%)
Frozen section	8	6(75%)	1(12.50%)	1(12.50%)

Table 8: Cordinance and discordance of diagnosis of oligodendrogliomas in respect to grade by Squash cytology, Frozen section with the Histopathological diagnosis

Method	Histopathological diagnosis			
	Oligodendrogliomas studied	Diagnosed with grade	Discordance in grade	Discordance in diagnosis
Squash cytology	6	6(100%)	0	0
Frozen section	6	5(83.33%)	0	1(16.66%)

All the 8 cases of meningiomas in this study are WHO grade I meningiomas. Out of the 8 cases, 7 were transitional meningiomas, and 1 case was a meningotheliomatous meningioma. All the cases were observed in female patients and their most common location is parasagittal region.

In the present study sensitivity, specificity, positive predictive value and negative predictive values of squash smear are 97.36%, 75%, 97.36%, and 75% respectively.

The sensitivity, specificity, positive predictive value and negative predictive values of the frozen section are 94.73%, 100%, 100%, and 66.66% respectively. In the case of gliosarcoma, glial component showed GFAP positivity, and sarcomatous component showed vimentin positivity. In one case of anaplastic astrocytoma, ki-67 immunostaining was used for grading which showed 7%. All the cases of schwannomas showed S-100 positivity. All the cases of meningiomas showed EMA positivity. The two cases of metastatic deposits showed positivity for cytokeratin and negativity for GFAP.

3.1. Squash cytology

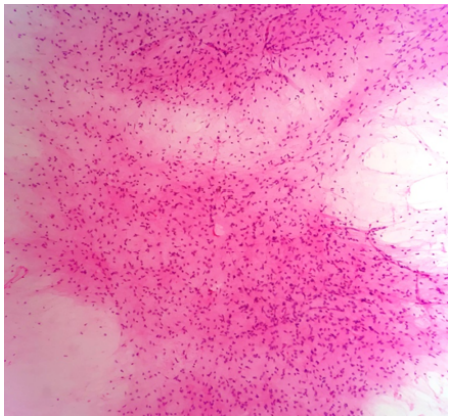

Fig. 1: Low grade astrocytoma with fibrillary background (H&E X100)

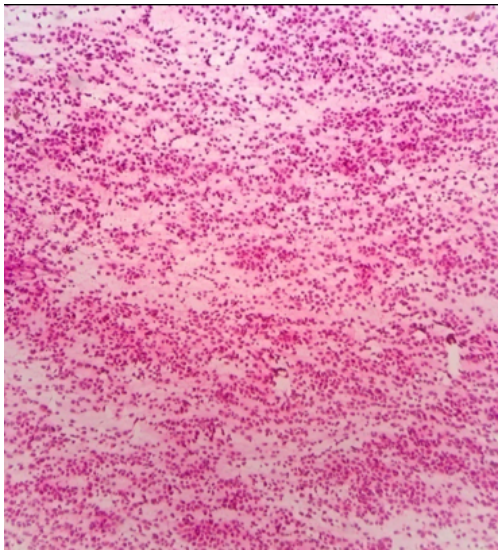

Fig. 2: Anaplastic oligodendroglioma showing increased cellularity and atypia (H&E X100)

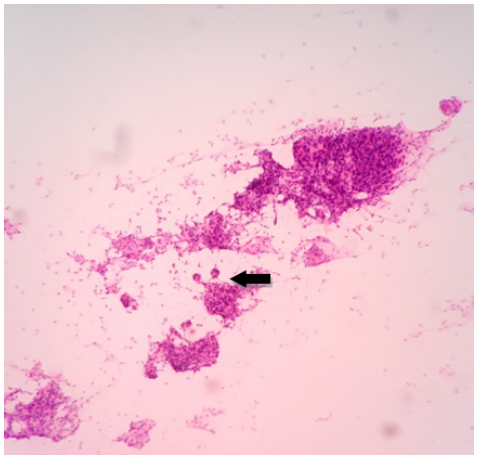

Fig. 3: Smear showing whorls of meningothelial cells (arrow) (H&E X100)

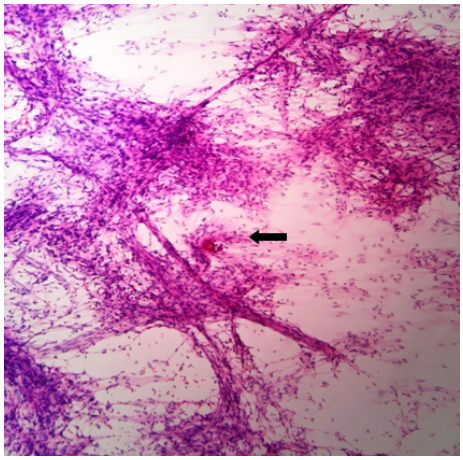

Fig. 4: Smear showing spindled meningothelial cells along with a psammoma body (arrow) (H&E X 100).

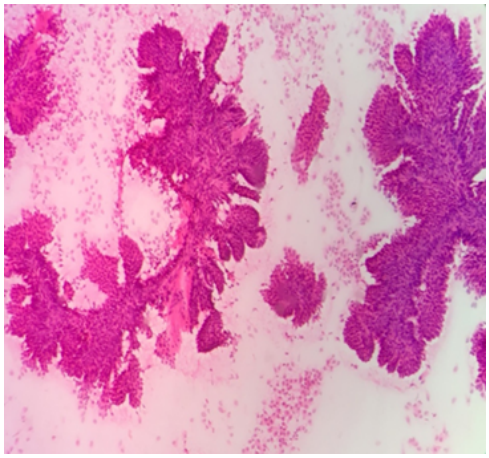

Fig. 5: Choroid plexus papilloma (H&E X100)

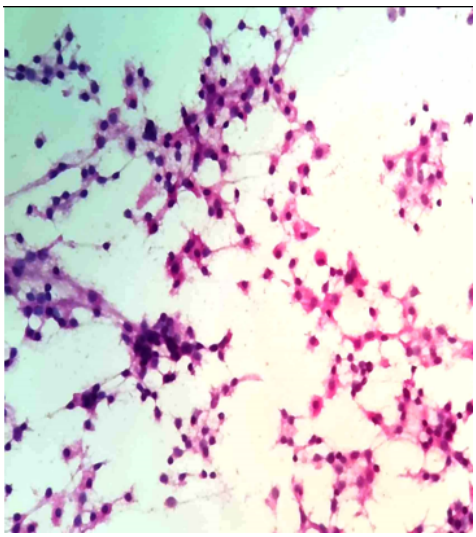

Fig. 6: Pituitary adenoma (H&E X400)

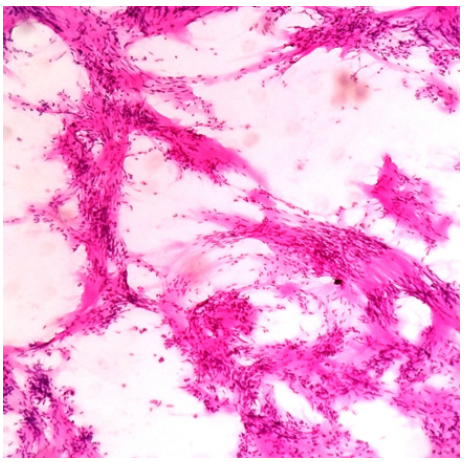

Fig. 7: Schwanoma showing verocay bodies (H&E X100)

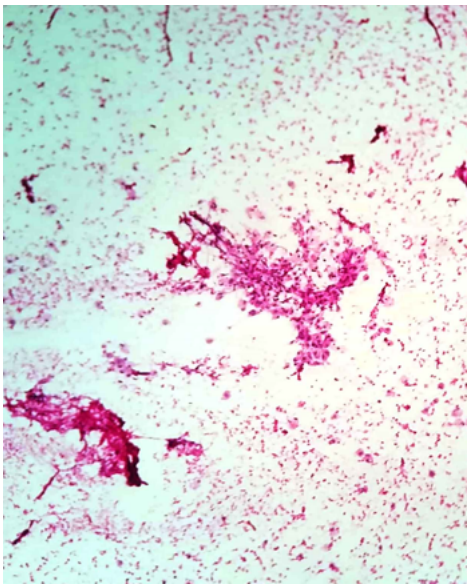

Fig. 8: Carcinomatous metastatic deposits with necrotic background (H&E X400)

4. Discussion

Central nervous system tumors are the second most common neoplasms in children and sixth most common tumors in adults.[1] With the introduction of stereotactic surgical procedures, there is a significant change in the approach to the intracranial space-occupying lesions. Many times there is a significant overlap in the clinical presentation of neoplastic tumors with non – neoplastic lesions like tuberculoma, fungal infection, parasitic infection and pyogenic abscess.[2] Intraoperative diagnostic methods like squash cytology and the frozen section not only helps in differentiating these lesions but also provides a diagnosis, which helps in guiding further management plan.

The inherent soft nature of the brain tissue makes it more susceptible to the squash smear technique. It is an important diagnostic tool for CNS lesions where cryostat is not available. There are several advantages to squash smear technique as it is easy, less cost, less time consuming and less technical skill is required.

Frozen sectioning is an intraoperative diagnostic tool not only in the neurosurgical biopsies but also in other systems. The architectural similarity between the frozen section and final biopsy is the most important advantage of frozen section, which makes a pathologist more comfortable with frozen section. Ice crystal artifacts are the most important challenges faced in the frozen section of brain specimen. But it is more feasible in firm lesions like meningioma & schwannoma and inflammatory lesions.[3]

In the present study, squash cytology and the frozen section was performed in 43 neuropathological specimens, and the intraoperative diagnosis is then correlated with the final histopathological diagnosis. The age group in this study ranges from 13 – 75 years with the maximum number of cases occurring in the elderly age group of > 50 years. The maximum peak is seen in the 5^{th} decade which is similar to that of Seema acharya et al.[4] study. In a study conducted by Jamunarani et al.,[5] the maximum peak is seen in the 4^{th} decade. Among astrocytomas, glioblastoma multiforme is occurring in the 5^{th} decade which is similar to the studies conducted by Sumit et al.[6]

Meningiomas show a bimodal age distribution with the first peak in the 2^{nd} decade and the second peak in the 4^{th} decade. The results of our study also showed the same age distribution. In general, the incidence of the CNS lesions usually shows a slight male preponderance. Similar results are observed in studies conducted by Savargaonkar et al.[7] and H Rani et al.[8] But here, there is a female preponderance. This discrepancy is observed because meningiomas are the maximum number of cases in our study along with astrocytomas. All the cases of meningiomas reported in our study are observed in females. In general, the incidence of non-neoplastic lesions is less in CNS compared to the neoplastic lesions. Similar results are observed in our study which is correlated with other studies conducted by Vikram et al., Swagatika et al., Manish et al.[9–11]

Glial tumors are the most common primary CNS tumors. Meningiomas are the second commonest CNS tumors. Our study also showed similar results with the maximum number of cases being astrocytomas and meningiomas. Similar results are observed in studies conducted by Vikram et al. and Swagatika et al.[9,10]

In the present study, 8 cases of astrocytoma were diagnosed. Out of these, squash diagnosis was made in 7 cases, and the diagnosis was made by frozen section in 7 cases. But correct grading was possible in only 5 cases by using squash cytology and in 6 cases by using frozen section. Hence the diagnostic accuracy of both squash cytology and the frozen section was 87.50% without grading. This accuracy is reduced to 62.50% by squash cytology and 75% by frozen section with grading. Astrocytomas vary significantly in grade from one area to other within a single tumor.[20] Due to their heterogeneous areas, even it is inappropriate to grade them by frozen section.

Squash smears showed increased cellularity compared with normal brain tissue with a fibrillary background. Cells have an elongated and irregular nucleus with stippled chromatin. Naked nuclei are also seen in few foci. Cellular atypia is increased with the increase in grade of the tumor. Granular eosinophilic bodies are noted in a case of pilocytic astrocytoma. Increased atypia with mitotic activity, vascular network and necrosis are noted in the case of glioblastoma.

In the frozen section, the neoplastic area clearly showed increased cellularity compared to the adjacent normal brain tissue in case of infiltrating astrocytomas. In one case, the area showing the cellular atypia is missed and is diagnosed as Grade II astrocytoma, and in final histopathology, it is diagnosed as an anaplastic astrocytoma. Vascular proliferation with a glomeruloid pattern is easily appreciated in the frozen section in case of glioblastoma.

All the cases of glioblastoma in the biopsy sections showed an adjacent low-grade astrocytic component indicating probably all are secondary glioblastomas. In one case, the squash smear and frozen section showed a little increase in cellularity without any atypia. On squash smear, the cells are characteristically showing fibrillary processes. It is intraoperatively diagnosed as reactive gliosis by both squash cytology and frozen section. In biopsy, by the evaluation of the complete specimen, there is increased cellularity with mild nuclear atypia along with microcystic changes. KI-67/MIB index showed <5 % of the positivity, and it is diagnosed as a low-grade astrocytoma. The diagnostic accuracy of gliomas by squash cytology are 100%, 88.5% and 93.3% by H Rani et al.,[8] Jindal A et al.,[12] and Jamunarani et al.,[5] respectively and correlated well with the present study which is 87.51%.

In 2 cases, both by squash cytology and frozen section, the intraoperative diagnosis is low-grade astrocytoma, but in histopathology one tumor showing low-grade astrocytoma features along with plenty of ganglion cells which is diagnosed as ganglioglioma. In another case, there were mixed features of both astrocytoma and oligodendroglioma which is diagnosed as mixed glioma (Grade II).

In one case, squash diagnosis is anaplastic astrocytoma, and the frozen diagnosis was high-grade astrocytoma with spindle cell component. Biopsy showed islands of astrocytic tissue embedded in an anaplastic mesenchymal component. IHC with GFAP showed positivity only in the areas of astrocytic tissue, and the diagnosis of gliosarcoma was made. In our study, a total of 6 cases of oligodendrogliomas were reported which constitute to 13.95%. Out of the 6

Table 9: Comparision of the accuracy of squash cytology in different CNS lesions of the present study with other studies

Histopathological diagnosis	Squash cytology		
	Present study (n=42)	H Rani[8] et al. (n=110)	Jindal A[12] et al. (n=150)
Astrocytoma	7/8(87.50%)	36/36(100%)	31/35(88.57%)
Oligodendroglioma	6/6(100%)	1/3(33.33%)	13/13(100%)
Meningioma	8/8(100%)	21/24(87.5%)	20/20(100%)
Schwannoma	7/7(100%)	12/12(100%)	22/22(100%)
Ependymoma	1/1(100%)	4/4(100%)	7/9(77.77%)
Choroid plexus tumors	1/1(100%)	-	-
Pituitary adenoma	1/1(100%)	4/4(100%)	12/12(100%)
Metastatic deposits	2/2(100%)	4/4(100%)	8/8(100%)
Tuberculoma	2/3(66.66%)	9/10(90%)	1/1(100%)
Others	1/5(20%)	12/13(92.30%)	27/30(90%)
Total	36/42(85.71%)	103/110(93.63%)	141/150(94%)

Table 10: Comparision of the accuracy of squash smear in common CNS lesions gliomas and meningiomas in different studies

Histopathological diagnosis	Seema Acharyaet al.[4]	Tilgneret al.[13]	Patty et al.[14]	Present study
Gliomas	74.39% (61/82)	92.43% (3078/3330)	79.24% (42/53)	92.85% (13/14)
Meningioma	95.16% (59/62)	81.63% (40/49)	93.54% (29/31)	100% (8/8)
Overall squash accuracy	83.78%	81.30%	87.02%	85.71%

Table 11: Cordinance and discordance of squash cytology in various studies – Overview

Various studies	Squash cytology		
	Cases	Cordinance rate	Discordance rate
Savargaonkar p et al.[7]	103	94.0	6.0
Rao S et al.[3]	120	96.0	4.0
Kini JR et al.[15]	100	86.0	14.0
Sundaram S et al.[16]	3057	89.0	11.0
H Rani et al.[8]	110	96.36	2.63
Swagatika et al.[10]	61	80.33	19.67
Present study	42	85.71	14.29
Comparision of cordinance and discordinance rates of frozen section in various studies			
Plesec et al.[17]	2156	97.30	2.70
Vikram et al.[9]	75	75.30	24.70
Swagatika et al.[10]	60	81.67	18.33
Khoddami et al.[18]	273	91.20	8.80
Present study	42	88.09	12.01

Table 12: Comparision of the sensitivity, specificity, positive predictive value and negative predictive value of squash smear technique and frozen section in different studies

Squash cytology	Swagatika et al.[10]	Savita s patil et al.[19]	Present study
Sensitivity	94.4%	90.47%	97.36%
Specificity	85.7%	82.76%	75%
Positive predictive value	98.07%	79.17%	97.36%
Negative predictive value	66.67%	92.32%	75%
Frozen section	Swagatika et al.[10]	Khoddani et al.[18]	Present study
Sensitivity	96.15%	91.4%	94.73%
Specificity	75%	99.7%	100%
Positive predictive value	96.15%	88.4%	100%
Negative predictive value	75%	99.8%	66.66%

cases, 4 cases were grade II, and 2 cases were grade III. All the 6 cases were diagnosed by squash cytology with an accuracy of 100%, and 5 cases were diagnosed by frozen section with an accuracy of 83.33%. When grading is taking into consideration, the accuracy remained the same.

Squash smears showed uniform round tumor cells arranged against amorphous eosinophilic neuropil background. Atypia is identified in high-grade lesions. Thin capillary structures are identified which are characteristic of oligodendroglioma. The frozen section also showed uniform round cells having a round nucleus. Characteristic fried egg appearance that is seen in histopathology is absent in the frozen section. In one case, due to ice crystal artifact, the lesion is considered as non-neoplastic reactive gliosis. The accuracy in diagnosing oligodendrogliomas by squash cytology is 33.3% and 100% in H Raniet al.,[8] and Jindal A et al.,[12] study. Our study is well correlated with Jindal A et al. study. The accuracy of frozen section in the diagnosis of oligodendroglioma in our study is 83.3% which is well correlated with khoddami et al.,[18] study which is 100%

In our study, meningiomas are the second most common CNS tumors which are similar to many studies in India. In general, the incidence of meningiomas is common in females, in our study all the cases were reported in female patients. The incidence of meningioma has a bimodal peak, one at the 2^{nd} decade and other at 4^{th} decade which observed in our study.

Out of the 8 cases, 7 cases are transitional meningioma, and 1 case is meningotheliomatous meningioma. Meningiomas are usually firm and are difficult for smear preparation,[3] but in our study smears are prepared from all the cases easily. Squash smears of all the 8 cases showed the characteristic whorling pattern of the round to oval meningothelial cells with wispy cytoplasm. In some cases, spindle cells are also noted, but the diagnosis of schwannoma is excluded by the presence of whorls of meningothelial cells in all the cases. Psammoma bodies are also noted in a few cases. Frozen sections were similar to histopathology, and the two histological patterns were more clearly observed in the transitional meningiomas. The diagnostic accuracy of squash cytology in our study in diagnosing meningiomas is 100%. Our study results are similar to that of H Rani et al.[8] and Jindal A et al.,[12] which are 87.5%, and 100% respectively.

The diagnostic accuracy of frozen section in diagnosing meningioma is 100% by our study which is well correlated with khoddami et al.[18] study. In our study, schwannoma accounts for 7 cases, out of which 5 cases are intracranial, and two were spinal. Squash smears showed the characteristic twisted rope appearance of schwannomas, having spindled cells having buckled nucleus. Verocay bodies are also appreciated in some squash smears. Frozen section showed the characteristic Antoni A and Antoni B areas along with verocay bodies. The accuracy of squash and frozen section in the diagnosis of schwannoma in our study is 100% which is well correlated with H Raniet al.,[8] Jindal A et al.[12] and Khoddami et al.[18]

Two cases of metastatic deposits were reported in our study, both the cases were diagnosed by squash cytology and frozen section. In one case the squash smears showed pleomorphic squamous cells with arranged in clusters and background showing keratin material with tumor diathesis. In another case, pleomorphic cells arranged in clusters and individual pattern, but biopsy sections showed glandular formations. In one case, frozen section showed neoplastic tumors cells arranged in islands and nests along with adjacent areas showing necrosis. In the other case, the tumor cells are arranged in a glandular pattern.

Biopsy of one case showed keratin pearls, and the diagnosis is made as metastatic carcinomatous deposits with a possible primary of squamous cell carcinoma. In the second case, neoplastic cells were arranged in a glandular pattern, and the diagnosis of adenocarcinomatous metastatic deposits was made. The diagnosis is confirmed by immunohistochemistry which showed positive for cytokeratin and negative for GFAP.

A total of 3 cases of tuberculoma are reported in our study, and biopsy evaluation of all of them showed caseating granulomas with reactive gliosis. In one case squash smears showed caseous necrosis, and epithelioid cell clusters and the diagnosis is readily made. In one case, necrosis is not present but epithelioid cell aggregates are present. In one case, squash smear showed astrocytes showed atypical changes and the diagnosis is made as a low-grade astrocytoma. The discrepancy is most possibly due to sampling error which made us to misinterpret reactive gliosis as a low-grade astrocytoma. A similar error is also observed in Kini JR et al.[15] study. In frozen sections, all the three cases showed well-formed granulomas, and the caseous necrosis is readily observed in 2 cases.

One case of pituitary adenoma was reported in our study and is diagnosed both by squash cytology and frozen section. Both squash smears and frozen section showed uniform round cells having a round nucleus and granular eosinophilic cytoplasm. The diagnosis of pituitary adenoma is made by correlating with radiological imaging studies.

One case of Choroid plexus papilloma is reported in our study which is diagnosed by both squash cytology and frozen section. Both squash cytology smears and frozen section showed the characteristic papillae lined by a single row of cuboidal cells without any nuclear pleomorphism.

One case of clival chordoma is reported in our study. Due to the tiny sample, the frozen section is not performed. Squash smears examined showed fibrous tissue strands which were misinterpreted as glial background. The characteristic physaliferous cells were not observed which made us misdiagnose it as a low-grade astrocytoma. The biopsy showed characteristic myxoid

areas and even in biopsy physaliferous cells are not appreciated. The diagnosis is confirmed by performing immunohistochemistry outside.

One case of cavernous hemangioma is reported in our study. The intact vascular tissue is sent to us intraoperatively, and an only frozen section is performed as squash smear has no role. Frozen section showed large vascular spaces lined by endothelial cells and the diagnosis of cavernous hemangioma is made.

In this study, the sensitivity and specificity of squash cytology was 97.36% and 75% respectively. The sensitivity and specificity of the frozen section was 94.73% and 100% respectively. This shows that there is a significant association between squash cytology and the frozen section with histopathology. Both squash cytology and frozen section are important intraoperative diagnostic methods.

Hence both squash cytology and frozen section can be employed as rapid intraoperative diagnostic methods in CNS lesions. Rather than employing a single diagnostic method, squash cytology, and the frozen section when used combined has more accuracy. However, only a provisional diagnosis is made by squash cytology and the frozen section which should always be confirmed with histopathology and should not be used as a definitive diagnostic methods.

5. Conclusion

Squash smear technique and frozen section are the commonly used methods intraoperative diagnostic methods in the CNS lesions. In addition to the preliminary diagnosis, these methods help in the assessment of the sample diagnostic value, differentiation of non-neoplastic infectious from the neoplastic lesions. Squash smear technique is an easy, rapid, inexpensive technique which provides clear details of the cellular morphology. It does not require electricity and experienced microtomist, unlike frozen sections. Hence it can be used as an important diagnostic tool in centers where cryostat is not available.

Frozen sections provide the tissue morphology similar to that of the biopsy section which makes the pathologist more familiar with frozen sections. It has similar diagnostic efficacy for gliomas as to that of squash cytology and can be used as a routine intraoperative diagnostic method. Both squash cytology and the frozen section have their own advantages as well as disadvantages in the intraoperative diagnosis of neuropathological lesions. Hence instead of using a single method, the combined use of squash cytology and frozen section helps the pathologist to overcome the disadvantage of individual methods and in providing an intraoperative diagnosis. But both the methods, squash cytology and frozen section are only preliminary diagnostic methods, which should always be followed by a definitive histopathological diagnosis.

6. Conflict of Interest

None.

7. Source of Funding

None.

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