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Comparative study of frozen section and squash preparation for intraoperative diagnosis of CNS lesions and their correlation with paraffin sections in a tertiary care hospital, Vadodara

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Abstract
Background: The main usefulness of intraoperative consultation on lesions of the CNS is to confirm or rule out the presence or absence of neoplasia and to define the neoplastic cell type and histologic grade of the tumor. Two main techniques for the intra-operative diagnosis of specimens are available: cryostat (frozen) sections and smear (squash,crush) preparations. The choice of the most suitable investigative technique depends on individual experience and preference.
Aims and objectives: This study aims to assess the sensitivity / diagnostic accuracy by squash preparation and frozen section, by comparing it with paraffin sections.
Materials and Methods: This study comprised of 65 cases of CNS tumours received and analysed. Intraoperative squash smears and frozen sections were prepared and intraoperative diagnosis were compared with paraffin sections.
Results: Out of total 65 cases, 18 cases were of Meningioma, 12 cases were of Glial Tumour and 7 cases were of Schwannoma. Out of total 65 cases, 32 (49.23%) cases were of more than 40 years old. In the present study, male to female ratio was 0.55: 1. Sensitivity of squash method was 90.74% and of frozen section was 88.88%. Combination of both methods resulted in increased sensitivity to 94.44%.
Conclusion: In conclusion, squash preparation and frozen section techniques are complementary to each other for intraoperative diagnosis of CNS lesions. Combined utility of both these methods yield high diagnostic accuracy.

Keywords: CNS lesions, frozen section, histopathology, intraoperative diagnosis, squash preparation

Introduction

Pathological results are needed in urgency during the ongoing operative procedures in surgical field. Neurosurgery is aided by the use of intra-operative diagnostic modalities, particularly for biopsy specimens of suspected neoplasms. The main usefulness of intraoperative consultation on lesions of the CNS is to confirm or rule out the presence or absence of neoplasia [1, 2] and to define the neoplastic cell type and histologic grade of the tumor according to consensus classification. CNS tumors, with their huge variety of types and sub-types, each with its own clinical peculiarities and different cytohistologic characteristics, presented a great challenge from the start.

Two main techniques for the intra-operative diagnosis of specimens are available: cryostat (frozen) sections and smear (squash, crush) preparations [3]. The choice of the most suitable investigative technique depends on individual experience and preference, and many neuropathologists prefer, if possible, to use both techniques on biopsy specimens if sufficient tissue is available. In experienced hands, these, highly accurate sampling methods allow a tissue diagnosis to be made on lesions previously considered unsuitable for biopsy, particularly deep seated lesions [4]. Intraoperative evaluation of lesions in the CNS requires the clinical, radiological and histologic data and knowledge of clinicopathologic entities and their common locations.

The frozen section procedure as practiced today in medical laboratories is based on the description by Dr. Louis B. Wilson in 1905.The preparation of smears from brain biopsy material is a well-established technique for rapid diagnosis in neuropathology. In 1930, Eisenhardt and Cushing described the use of cytologic examination of touch preparations

stained with supravital dyes for the rapid diagnosis of brain tumors at the time of surgery [5-8].

The soft texture, however, aids in smear preparation, often revealing exquisite cytological details. Intraoperative cytologic smears in neurosurgery are rapid, easy to perform, inexpensive [2, 9], permitting high diagnostic accuracy allowing reliable intraoperative diagnoses and guidance during targeting and resecting lesions in neurosurgery. Also the amount of the tissue required is lesser by which more amount can be preserved for paraffin sections. Cytological methods are ideal for a speedy intraoperative diagnosis of stereotactic CNS biopsy samples because the tissue is minimally distorted and none is wasted [7, 10, 11].

Frozen sections are mainly useful for the more firm, rubbery neoplasms such as meningiomas, ependymomas, and most metastatic tumours in which it is difficult to prepare good cytology smears [12, 13]. Some microscopic features are better in one than the other among the FS and cytologic smears. Because of this, some studies have also been shown the combined utility of both the procedures for intraoperative purpose.

Although, the choice of the method depends on individual experience and preference, and these methods were considered as complementary procedures to assist neurosurgeon in diagnosis [14]. The studies have also shown the combined use of frozen section and squash smear resulting in overall increased diagnostic accuracy compared to that using a single procedure only [15, 16].

Though the neuro imaging and neurosurgical techniques have been revolutionized, in order to plan rational treatment of intracranial lesions, a certain amount of diagnostic certainty is still required that only pathology can offer. Infectious and inflammatory processes must be distinguished from neoplastic processes, while defining the type of the neoplastic process involved is a crucial factor in determining surgical strategy. In addition, intraoperative consultation is essential to avoid non- diagnostic biopsies by confirming that the sample obtained is representative of the lesion.

Intraoperative diagnostic techniques help in forming the decisions that are made with regard to triaging of the remaining tissue that is not submitted for intraoperative consultation evaluation, as well as deciding what test may need to be employed to further evaluate the lesion and differential diagnosis.

Aims and objectives

This study aims to analyze the view of ovarian tumors with respect to clinical presentation, gross and microscopic characteristics and also to study the frequency and histopathological patterns of ovarian tumours.

Materials and methods

Study Design: The present study was carried out at histopathology section of pathology department of Government Medical College and S.S.G. General Hospital, Vadodara, a tertiary care centre in Vadodara district of Gujarat state, India during the period of March 2015 to February 2016.

Type of study: Prospective

Data collection procedure: In present study, we analysed all 65 cases which received for histopathology examination under the diagnosis of CNS tumours from Neurosurgical department.

Inclusion criteria: All the intraoperative calls as well as post-surgical biopsy specimens fron Neurosurgical Department were included in this study.

Exclusion criteria: Only post-surgical biopsy specimens without intraoperative calls fron Neurosurgical Department were excluded from the study.

Detailed clinical history along with radiology (CT/MRI) reports were available in most of the cases along with clinically suspected diagnosis. The radiology images were scrutinized as to the intra or extra axial location of the lesions and accompanying features like perilesional edema, tumor necrosis, post contrast enhancement, ring enhancing lesions, midline shift and so forth.

All the intraoperative biopsy specimens were examined as soon as possible after they had been removed. To make the smear preparation, a tissue fragment of up to 5 mm diameter was placed at one end of a pre labelled glass slide. The tissue sample was removed from the biopsy specimen with minimal trauma and crushing with forceps was avoided as this would seriously impair cytological preservation. Individual tissue samples on the labelled glass slides were gently crushed by a second slide held at right angle. This slide was gently rotated to spread the specimen evenly across the base of the labeled slide. It was important to ensure an even distribution of cells across the labelled slide. Once the smears were prepared, they were fixed immediately in methanol before drying. In a few cases, air dried smears were also made. The methanol fixed smears were stained by haematoxylin and eosin. The air dried smears were stained by Giemsa stain.

For the frozen sections, approximately 1x1 cm tissue section from the specimen is chosen followed by putting the tissue section in cryostat at -20 °C for 10 minutes and then approximately 10µ thick sections were cut. Once the sections were cut, the sections were put in 10% formalin for 5 minutes and then stained by haematoxylin and eosin stain.

The remaining sample was submitted for routine paraffin sections following fixation in 10% formalin. In some cases additional specimen with 10% formalin were also sent by the neurosurgeon.

To overcome the sampling errors, multiple areas were sampled for both squash and frozen sections. The differential diagnosis of CNS lesions by smear preparations and frozen section were based on nuclear, cytoplasmic, background and architectural features. The nuclear features studied included shape, chromicity, pleomorphism, membrane, nucleoli, mitotic activity and pseudo inclusions. Cytoplasmic features consisted of processes, vacuolation, granularity and scarcity. Fibrillar nature of tissue, necrosis, collagen, myxoid matrix, psammoma bodies, rosenthal fibres and cellular infiltrates were assessed as the background features. Some pathognomonic architectural features like cell whorls, papillae and cell rosettes were also looked for.

Results

Table 1: Distribution of number of cases in different CNS lesions.

Sr. No.	Name of Lesion	No. of cases	Percentage
1	Meningioma	18	27.7
2	Craniopharyngioma	05	7.7
3	Pitutary Adenoma	03	4.6
4	Glioma	12	18.5
5	Infection / Inflammation	04	6.1
6	Cysts	06	9.2
7	Schwannoma	07	10.8
8	Neurofibroma	01	1.5
9	MPNST	01	1.5
10	Extraskeletal Osteosarcoma	01	1.5
11	Hemangioblastoma	02	3.0
12	Lymphoma	01	1.5
13	Chordoma	02	3.0
14	Metastatic Adenocarcinoma	02	3.0
Total		65	100

Out of total 65 cases, 18 (27.7%) were of meningioma, 12 (18.5%) were of glioma and 07 (10.8%) were of schwannoma.

Table 2: Age wise distribution of CNS lesions.

Sr. No.	Name of Lesion	< 12 Years	13 – 40 Years	>40 Years	Total
1	Meningioma	00	07	11	18
2	Craniopharyngioma	01	04	00	05
3	Pitutary Adenoma	00	02	01	03
4	Glioma	00	05	07	12
5	Infection / Inflammation	00	01	03	04
6	Cysts	01	04	01	06
7	Schwannoma	00	04	03	07
8	Neurofibroma	00	00	01	01
9	MPNST	00	01	00	01
10	Extraskeletal Osteosarcoma	00	00	01	01
11	Hemangioblastoma	00	01	01	02
12	Lymphoma	00	01	00	01
13	Chordoma	01	00	01	02
14	Metastatic Adenocarcinoma	00	00	02	02
Total		03 (4.6%)	30 (46.2%)	32 (49.2%)	65 (100%)

Out of total 65 cases, 32 (49.2%) were of >40 years old, 30 (46.2%) were between 13 – 40 years old and only 03 (4.6%) cases were < 12 years old.

Table 3: Sex wise distribution of CNS lesions

Sr. No.	Name of Lesion	Male	Female	Total
1	Meningioma	03	15	18
2	Craniopharyngioma	02	03	05
3	Pitutary Adenoma	01	02	03
4	Glioma	08	04	12
5	Infection / Inflammation	01	03	04
6	Cysts	02	04	06
7	Schwannoma	01	06	07
8	Neurofibroma	00	01	01
9	MPNST	00	01	01
10	Extraskeletal Osteosarcoma	01	00	01
11	Hemangioblastoma	01	01	02
12	Lymphoma	01	00	01
13	Chordoma	01	01	02
14	Metastatic Adenocarcinoma	01	01	02
Total		23 (35.4%)	42 (64.6%)	65 (100%)

Out of total 65 cases, majority 42 (64.6%) cases were female and 23 (35.4%) cases were male with male to female ratio was 0.55: 1.

Table 4: Site / Region wise distribution of CNS lesions.

Sr. No.	Name of Lesion	No. of cases	Percentage
1	Supratentorial	39	60
2	Infratentorial	13	20
3	Spinal Canal	13	20
Total		65	100

Out of total 65 cases, majority 39 (60%) cases were located in supratentorial region followed by 13 (20%) cases each in infratentorial region and spinal canal.

Table 5: Comparative sensitivity of Squash, Frozen and Combined Techniques in CNS lesions (54 Cases)

Sr. No.	Name of Lesion	Paraffin Block	Squash	Frozen	Combined
1	Meningioma	14	13	13	14
2	Craniopharyngioma	05	05	05	05
3	Pitutary Adenoma	02	02	02	02
4	Glioma	11	11	09	11
5	Infection / Inflammation	3	2(1)	2(1)	2(1)
6	Cysts	6	5	6	6
7	Schwannoma	5	5	5	5
8	Neurofibroma	1	1	1	1
9	MPNST	1	1	1	1
10	Extraskeletal Osteosarcoma	1	1	1	1
11	Hemangioblastoma	2	1(1)	1(1)	1(1)
12	Lymphoma	1	0(1)	0(1)	0(1)
13	Chordoma	1	1	1	1
14	Metastatic Adenocarcinoma	1	1	1	1

Note: No. shown in '( )' are the discrepant cases.

Sensitivity of individual methods – for squash was 90.74%, for frozen section was 88.88%. Combination of both of these methods resulted in increased sensitivity to 94.44%.

Discussion

In the past two decades, the increased need for rapid diagnosis and the advent of stereotactic procedures have resulted in either supplementing or replacing the use of frozen section (FS) study by the cytology technique in intraoperative consultations [15]. The specimen obtained during operative procedure will allow for the use of cytologic techniques coupled with frozen section and can usually provide a specific and definitive diagnosis.

The soft and edematous nature of neurosurgical specimens makes it easier to prepare squash smears, and results in FS being suboptimal due to freezing artifacts. On the other hand, firm, inflammatory and most metastatic lesions are better visualized on frozen sections [17]. However, several studies have noted that both squash smears and frozen section complement each other and the combination of the two techniques is beneficial in the intraoperative diagnosis [18].

Over a period of 12 months, the collected data is analysed and compared with other studies to determine relative frequency and distribution of CNS lesions in the present setup along with the accuracy of squash preparation & frozen section individually as well as when they are used in combination.

Study by Verma *et al*. [9] & present study showed meningiomas as the most common encountered lesions with gliomas being the second most common lesions. Exactly opposite results were found by Rao *et al*. [17] and Mitra *et al*.

[19]. Pratima *et al*. [18] found gliomas as the most common encountered lesions followed by reactive lesions.

In the present study, neoplastic lesions formed the major category (55/65 cases) and nonneoplastic lesions formed the minor one (10/65 cases). Similarly, in the study by Narang *et al.* [15], neoplastic lesions formed the major group with 62 cases with 13 nonneoplastic cases. Pornsuk *et al*. [20] found that 85.5% of cases were neoplastic.

As with Verma *et al*. [9] and Modi *et al*. [21], in the present study, majority of the cases fall among patients over 13 years. Similarly Pratima *et al*. [18] study showed 17 of the total 103 cases among patients <20 years and 86 cases among the patients >20 years of age. In the present study, the male:female ratio showed preponderance of females. Similar results were found in the study by Nishant *et al*. [22], while Pratima *et al*. [18], Narang *et al*. [15] and Pornsuk *et al*. [20] found predominance of males. In this study majority of the tumors are located in the supratentorial region, which is consistent with the results of the study done by Verma *et al*. [9] and Modi *et al*. [4].

When used in combination, the sensitivity of intraoperative techniques (squash & frozen section) increased as compared to use of either technique at a time in the present study. Similar results were found in studies by Narang *et al*. [15] & Rani *et al*. [16]. Pratima *et al*. [18] found that using the combination of cytology and frozen section was beneficial and concordance of intraoperative consultation with final diagnosis was found in 94% of cases. Similarly Rao *et al*. [17] showed 94% and Di Stefano *et al*. [23] showed 95.29% correlation of intraoperative diagnosis with final diagnosis. Nasir Ud Din *et al*. [24] & Pornsuk *et al*. [20] using both frozen section & cytology smears for intraoperative diagnosis found accuracy of 94.8% and 89% respectively.

Conclusion

The intraoperative squash smears and frozen sections were performed from open biopsies and the diagnosis submitted in each case enabled the neurosurgeons to obtain adequate resection margins and ultimately establish the post - operative therapeutic protocol as well.

All the cases were compared with the paraffin sections of their respective cases for the purpose of assessing sensitivity of both (squash cytology & frozen section) individually and their combined sensitivity. Sensitivity of Squash cytology technique was 90.74% whereas sensitivity of Frozen section technique was 88.89% and sensitivity of combined both techniques was 94.44%. A high diagnostic accuracy was achieved in the major tumor categories including, meningomas, gliomas, schwannomas, craniopharyngiomas and pituitary adenoma.

To conclude, we recommend squash preparation and frozen section techniques are complementary to each other for intraoperative diagnosis of CNS lesions, and combined utility of both these methods yield high diagnostic accuracy.

Importance of this study

This is important to know the purpose of assessing sensitivity of both (squash cytology & frozen section) individually and their combined sensitivity.

Author contribution

First author Dr. S.P. Hiryur has given full guidance regarding study design and draft of manuscript. Second and corresponding author Dr. Ushma Patel has collected all data and done study in his own institute and prepare the manuscript in presentable manner for publication. Third author Dr. Sivaranjini N assisted in preparing master data of this study.

References

1. Sharma N, Misra V, Singh PA, Gupta SK, Debnath S, Nautiya A. Comparative Efficacy of Imprint and Squash Cytology in Diagnosing Lesions of the Central Nervous System. Asian Pac J Cancer Prev. 2011; 12(7):1693-6. PMID:22126546
2. Roessler K, Dietrich W, Kitz K. High diagnostic accuracy of cytologic smears of central nervous system tumors. A 15-year experience based on 4,172 patients. Acta Cytol 2002; 46(4):667-74. doi:10.1159/000326973.
3. Patil SS, Kudrimoti JK, Agarwal RD, Jadhav MV, Chuge A. Utility of Squash Smear Cytology in Intraoperative Diagnosis of CNS Tumors. J Cytol. 2016; 33(4):205-9. doi: 10.4103/0970-9371.190442
4. Ahluwalia CK, Chandrasoma PT. Cytomorphology of subependymal giant cell astrocytoma. A case report. Acta Cytol. 1993; 37(2):197-200. PMID: 8465641.
5. Pawar N, Deshpande K, Surase S, D'costa C, Balgi S, Goel A. Evaluation of the Squash Smear Technique in the Rapid Diagnosis of Central Nervous System Tumors: A Cytomorphological Study. The Internet Journal of Pathology. 2009; 11(1):5853.
6. Shrestha S, Thapa BK, Bhattarai B. Smear technique for intraoperative diagnosis of central nervous system neoplasms. Journal of Pathology of Nepal. 2014; 4:544-7. DOI: https://doi.org/10.3126/jpn.v4i7.10296
7. Kini JR, Jeyraj V, Jayaprakash CS, Indira S, Naik CN. Intraoperative consultation and smear cytology in the diagnosis of brain tumours. Kathmandu Univ Med J. 2008; 6(24):453-7. PMID: 19483425.
8. Krishnani N, Kumari N, Behari S, Rana C, Gupta P - Intraoperative squash cytology: accuracy and impact on immediate surgical management of central nervous system tumours. Cytopathology. 2012; 23(5):308-14. doi: 10.1111/j.1365-2303.2011.00905.x.
9. Verma SK, Kumar R, Srivani J, Arnold J– Diagnostic Accuracy of Squash Preparations in Central Nervous System Tumors. Iran J Pathol. 2013; 8(4):227-34.
10. Firlik KS, Martinez AJ, Lunsford LD. Use of cytological preparations for the intra-operative diagnosis of stereotactically obtained brain biopsies: a 19 year experience and survey of neuropathologists. Journal of Neurosurgery. 1999; 91(3):454-8. Doi: 10.3171/jns.1999.91.3.0454
11. Powell SZ. Intraoperative consultation, cytologic preparations and frozen section in the central nervous system. Arch Pathol Lab Med. 2005; 129(12):1635–52. Doi: 10.1043/1543-2165(2005)129[1635:ICCPAF]2.0.CO:2
12. Mollah N, Baki A, Afzal N, Hossen A. Clinical and pathological characteristics of brain tumor. BSMMU Journal. 2010; 3(2):68-71.
13. Folkerth RD. Smears and frozen sections in the intraoperative diagnosis of central nervous system lesions. Neurosurg Clin N Am, 5(1):1-18. PMID: 8124086.

14. Wilson LB. A method for the rapid preparation of fresh tissues for the microscope. JAMA. 1905; XLV(23):1737. doi:10.1001/jama.1905.52510230037003c
15. Nanarng V, Jacob S, Mahapatra D, Mathew JE. Intraoperative diagnosis of central nervous system lesions: Comparison of squash smear, touch imprint, and frozen section. J Cytol. 2015; 32(3):153-8. doi: 10.4103/0970-9371.168835
16. Samal S, Kaira R, Sharma J, Singh I, Panda D, Ralli M. Comparision of squash smears and frozen sections versus paraffin sections in intraoperative diagnosis of central nervous system lesions. Oncol J India. 2017; 1(2):25-30. DOI: 10.4103/oji.oji_21_17
17. Rao S, Rajkumar A, Ehtesham MD, Duvuru P. Challenges in neurosurgical intraoperative consultation. Neurol India. 2009; 57(4):464-8. Doi: 10.4103/0028-3886.55598
18. Savargaonkar P, Farmer PM. Utility of intra-operative consultations for the diagnosis of central nervous system lesions. Ann Clin Lab Sci. 2001; 31(2):133-9. PMID: 11337901.
19. Mitra S, Kumar M, Sharma V, Mukhopadhyay D. Squash preparation: A reliable diagnostic tool in the intraoperative diagnosis of central nervous system tumors. J Cytol. 2010; 27(3):81-5. Doi: 10.4103/0970-9371.71870
20. Cheunsuchon P, Punyashthira A, Tanboon J, Sangruchi T. Accuracy of intraoperative consultation in central nervous system lesions in Siriraj Hospital. Siriraj Med J. 2014; 66:113-9.
21. Bleggi-Torres LF, Gasparetto EL, Faoro LN, Hanel R, Grande CV, de Carvalho Neto A. de Noronha L. Pleomorphic Xanthoastrocytoma: report of a case diagnosed by intraoperative cytopathological examination. Diagn Cytopathol. 2001; 24(2):120-2. PMID: 11169891.
22. Teo JG, Ng HK. Cytodiagnosis of pilocytic astrocytoma in smear preparations. Acta Cytol 1998; 42(3):673-8. DOI: 10.1159/000331825.
23. Di Stefano D, Scucchi LF, Cosentino L, Bosman C, Vecchione A. Intraoperative diagnosis of nervous system lesions. Acta Cytol. 1998; 42(2):346-56. DOI: 10.1159/000331614.
24. Ud Din N, Memon A, Idress R, Ahmed Z, Hasan S. Central nervous system lesions: Correlation of intraoperative and final diagnosis, six year experience at a referral centre in a developing country, Pakistan. Asian Pac J Cancer Prev. 2011; 12(6):1435-7. PMID: 22126477.