

Evaluation of Incidence and Diagnostic Accuracy of Squash Cytology with Histopathology of Various CNS Lesions

Teena Eugene^{1*}, Vijayashree Raghavan², Rajesh Kann³

¹Department of Pathology, SRM Medical College & Research Centre, SRM Institute of Science & Technology (SRMIST), Chennai, Tamilnadu, India.

^{2,3}Department of Pathology, Chettinad Academy of Research & Education, Kelambakkam, Chennai, Tamilnadu, India.

Email: teena.eugene19@gmail.com¹, drvjshree@gmail.com², rajeshthuva@gmail.com³

*Corresponding Author E-mail: teena.eugene19@gmail.com

Article History	Abstract
Received: 06 June 2023 Revised: 07 Sept 2023 Accepted: 29 July 2023	<p><i>Background: Central nervous system lesions continue to be one of the most diverse and difficult to research for neuropathologists. Accurate assessment of the damaged tissue is essential for the diagnosis and treatment of disorders of the central nervous system (CNS). Historically, the "squash" or "crush" approach has been used for intraoperative diagnosis of CNS tumours. The purpose of this research was to evaluate the efficacy of squash preparation for diagnosing central nervous system tumours in comparison to histology. Materials and Methods: In this retrospective study, sixty neuropathological samples were analysed. During the proper surgical process, fresh tissue samples of 0.5-1mm² were taken and submitted for squash cytology. There were supposedly frozen and squash samples. Fast staining and paraffin-embedded tissue staining were both used to create cytology smears from squash; the results of these smears were reported, and they were correlated with slides from the histopathology lab. Squash cytology tumour grade was correlated with histopathology tumour grading. Results: CNS Neoplasms were found in 58 out of 60 patients (96.6 percent). Meningiomas, schwannomas, and small round cell tumours were also common cytological diagnosis alongside gliomas. There was a connection between the cytological and histological findings. The overall diagnosis accuracy of cytology for squash was 93%. Between the ages of 40 and 50, people had the highest prevalence of central nervous system lesions. Conclusion: Squash smear cytology is an effective and rapid stand-alone diagnostic procedure that can help surgeons make judgments regarding intracranial lesions during surgery when a frozen section facility is not available.</i></p>
CC License CC-BY-NC-SA 4.0	Keywords: CNS, diagnostic accuracy, intraoperative, squash

1. Introduction

Central nervous system (CNS) tumours may only be properly diagnosed and treated if the affected tissue is properly evaluated ¹. Pathologists have always been concerned about central nervous system malignancies because of the considerable variability in their appearance. Tumor grading is the gold standard for making prognosis and treatment decisions. However, inadequate tumour material is typically obtained, making it difficult to perform histological grading of central nervous system cancers despite standards specified by WHO. Similarly, it is important to make an early diagnosis of non-neoplastic lesions, notably infective lesions such tuberculomas, brain abscesses, fungal infections, and neurocysticercosis. However, if mistreated or if the diagnosis is delayed, lifelong neurological impairments might result.

The intraoperative identification of CNS malignancies has traditionally relied on the "squash" or "crush" smear method. Since the development of stereotactic biopsies, diagnostic tissue sampling has been severely constrained.²

For a cytological diagnosis of central nervous system (CNS) abnormalities, two options exist. Cryostat sections and squash cytology are two examples. Fast and accurate diagnosis of lesions in the neurological system may be made using squash cytology. It has the potential to achieve a high level of diagnostic accuracy when used by trained professionals. In addition, a cryostat is not required for squash cytology. No skilled microtome operator is required for sectioning. It's easy to do and replicate. While most of the tissue is preserved and processed quickly and optimally for immunohistochemistry, the smear approach allows for selective examination of numerous locations from a tiny biopsy material. There is no longer any need to risk distorting or losing critical diagnostic material by performing intraoperative frozen sections on tiny priceless specimens thanks to smear preparations. Smear methods are well-established and employed in many neuro-oncology centres because they are an accurate and effective way for intraoperative evaluation of CNS biopsies. Histochemistry, immunohistochemistry, and fluorescence in situ hybridization may all be performed successfully on these samples after proper preparation².

Eisenhardt and Cushing first utilized the smear technique in 1930, and the present approach, developed by Russell et al., is based on their work³⁻⁴. Neuropathologists rely on the frozen section method and the smear technique for fast diagnosis. The smear technique is a simple, rapid, cost-effective, and accurate alternative to the more time-consuming and expensive frozen section biopsy, which requires more technical expertise as well as larger and firmer tissue and often leaves barely any tissue for final histopathological confirmation, especially in the case of stereotactic biopsy procedure⁵. To further prepare the tissue for diagnostic assessment, such specimens should be crushed or squashed. When frozen sections cannot be obtained because to a lack of resources (such as power, gas, or skilled workers), squash cytology can be an effective alternative. In recent years, it has surpassed frozen method as the gold standard in diagnostics. It is being utilized to determine whether or not a tumor is present, as well as to identify the specific neoplastic cell type and tumor grade⁶.

Since 1980, several reports on this method have appeared in various parts of the world. Squash cytology was shown to be 95% accurate in a 2002 study by Karl Roessler et al., published out of Vienna, Austria⁶. The steady change in modern pathology is reflected in the fact that the WHO 2007 classification was edited by two neuropathologists (Drs. Louis and Weistler) and two molecular pathologists (Drs. Ohgaki and Cavenee). Approximately 86 primary CNS tumour types and their variations are described.⁵ There have been some breakthroughs in squash cytology, but the field is not without its caveats. Calcified tissue prevents its use. Crush artefacts, which make reading the smear difficult, occur when too much force is applied between two slides. Squash cytology has certain limits, but it is generally reliable, risk-free, quick, and easy to use. However, histopathology is the best standard in diagnosis and must always be correlated with cytology results in squash. The aim of the present study is to examine data from the Department of Pathology on the frequency of occurrence of various central nervous system tumours. Squash cytology was correlated with histology to evaluate its diagnostic efficacy. The purpose of this study is to identify the age and gender breakdown of CNS neoplasms of varying grades. In order to assess the prevalence of different types of paediatric brain tumours, it is necessary to histologically classify these diseases according to WHO 2007 standards.

2. Materials And Methods

This was a Retrospective study, conducted in the Department of Pathology, Chettinad Hospital & Research Institute, Kelambakkam. The study was conducted after obtaining approval from the Ethical Review Committee. In this study, we analysed 60 samples of Neuropathological specimens that were received between July 2012 and July 2016. Patients hospitalised to the Department of Neurosurgery with injuries of the brain or spinal cord were used as research participants. Fresh tissue samples (0.5-1mm²) were obtained during each procedure and sent on saline-moistened gauze to a pathology lab for squash cytology. Cytology and frozen sections were performed on squash in accordance with established departmental protocols. Squash and frozen parts were reported to the neurosurgeon in charge of surgery and the information was immediately sent to them. If there were any frozen leftovers left, they were placed in formalin and sliced routinely. Histopathology was performed on the residual tissue that OT sent over after the operation. The tissue was preserved in 10% formalin. Histopathology reports and correlations might be established with the use of the squash cytology/frozen section report.

Preparation of Squash Cytology Smears

All the information on the request and the specimen was double-checked when they were received. The material was first examined on a superficial level. Next, a clean glass slide was labelled and a 0.5-1mm² tissue sample was excised and put at one end. The smear was then formed by pushing down on the tissue with a second slide and rapidly dragging that slide over the first slide, creating an even layer of tissue. The amount of force used was carefully calculated. Isopropyl alcohol was used to quickly fix the smear preparation for five minutes. A quick Haematoxylin and eosin stain was used to examine the smear.

Procedure of Rapid Staining

- ❖ Staining in haematoxylin for 5 minutes.
- ❖ Washing in running tap water till sections turn blue.
- ❖ Differentiation in 1% acid alcohol for 5 seconds.
- ❖ Washed in water.
- ❖ Stained in Eosin for 1 min.
- ❖ Washed in water.
- ❖ Washed in absolute alcohol.
- ❖ Cleared in xylol.
- ❖ Mounted in DPX

Staining of Paraffin Embedded Tissue

Procedure

- ❖ Sections brought to water.
- ❖ Stained in haematoxylin for 15 minutes.
- ❖ Sections washed in running tap water.
- ❖ Differentiated in 1% acid alcohol – 3 to 4 quick dips.
- ❖ Washed in running tap water for 10-20 minutes till sections were blue.
- ❖ Stained with eosin for 15 seconds.
- ❖ Washed in running tap water
- ❖ Dehydrated in 95% alcohol.
- ❖ Absolute alcohol – at least 2 changes.
- ❖ Sections in Xylene – 2 changes.
- ❖ Mounted in DPX mountant

Squash smears were recorded, and histopathology slides were correlated with them. Tumor grading in squash cytology was associated with histopathology tumour grading. Special stains and immunohistochemistry were employed to supplement histology in situations when a definitive diagnosis was not possible with just that technique alone.

3. Results and Discussion

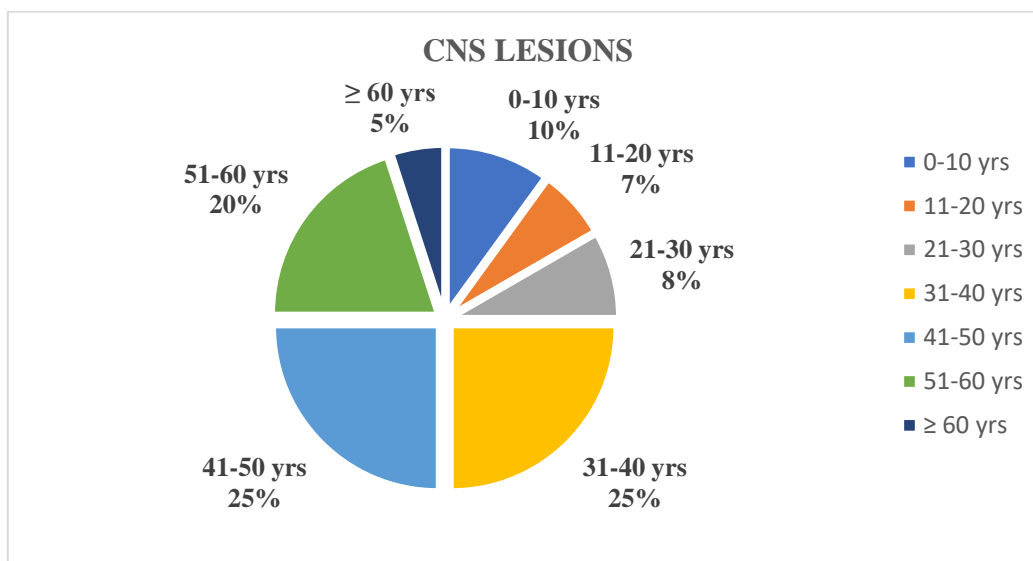


Figure- 1: classification of CNS lesions in age group wise

Notably, there were 15 cases each in the 31-40 and 41-50 age groups, making them the most affected categories. The 51-60 age group had 12 cases, while individuals aged 60 and above had 3 cases. In contrast, the 0-10 and 11-20 age groups had 6 and 4 cases, respectively, reflecting a distribution of CNS lesions among different age ranges.

Table-1: Gender distribution of CNS lesions

Sr. No.	Cns Tumors	Male	Female
1	Astrocytoma	19	3
2	Meningioma	3	3
3	Neurofibroma/Schwannoma	3	3
4	Oligodendroglioma	3	-
5	Small round cell tumor (PNET/Ewings/Medulloblastoma)	4	2
6	Ependymoma	2	-
7	Pituitary adenoma	3	-
8	CNS Lymphoma	1	1
9	Hemangioblastoma	1	-
10	Metastatic deposits	3	-
11	Gliosarcoma	1	-
12	Plasmacytoma	1	-
13	Infective lesions	1	-
14	Inflammatory Pseudo tumor	-	1
15	Others (AV Malformation, Ganglioglioma)	2	-
	TOTAL	47	13

Among the various tumor types, Astrocytoma was the most prevalent, with 19 cases in males and 3 cases in females. Meningioma and Neurofibroma/Schwannoma were evenly distributed between genders, each with 3 cases in both males and females. Oligodendroglioma affected 3 males but no females. Small round cell tumors (PNET/Ewings/Medulloblastoma) had 4 cases in males and 2 in females. Ependymoma and Pituitary adenoma exclusively occurred in males, with 2 and 3 cases, respectively. CNS Lymphoma was diagnosed in 1 case for both genders, while Hemangioblastoma was found in 1 male but not in females. Metastatic deposits and Gliosarcoma were observed in males, with 3 and 1 case(s), respectively.

Table 2: Diagnostic accuracy of squash cytology in grading astrocytoma

S.No	Grade	Diagnostic Accuracy
1	Grade I	75%
2	Grade II	90%
3	Grade III	50%
4	Grade IV	66%

Grade I tumors, the least severe, are correctly identified by medical professionals in 75% of cases. Grade II tumors, somewhat more advanced, enjoy a higher accuracy rate of 90%. Conversely, Grade III tumors, often moderately severe, pose a diagnostic challenge with a 50% accuracy rate. Lastly, Grade IV tumors, the most severe and aggressive, maintain a 66% diagnostic accuracy.

Table 3: grade and age wise incidence of astrocytoma

GRADE	1-10 Yrs	11-20 Yrs	21-30 Yrs	31-40 yrs	41-50 Yrs	51-60 Yrs	>60yrs	TOTAL
I	2	-	-	1	1	-	-	4
II	1	1	1	3	1	3	-	10
III	-	-	-	1	1	-	-	2
IV	1	-	-	1	3	1	-	6
Total	4	1	1	6	6	4	-	22

Tumor grades are categorized from I to IV, indicating varying degrees of malignancy or severity. Among individuals aged 1-10 years, there were 2 cases of Grade I tumors and 1 case of Grade II tumors. In the 11-20 years age group, there was 1 case each of Grade II and Grade IV tumors, while the 21-30 years group had 1 case of Grade II. Among individuals aged 31-40 years, there was 1 case of Grade I, 1 case of Grade III, and 1 case of Grade IV tumors. The 41-50 years age group had 1 case each of Grade I, Grade II, and Grade IV tumors. In the 51-60 years age group, there were 3 cases of Grade II tumors and 1 case each of Grade I and Grade IV tumors. Lastly, for individuals aged over 60 years, there were 3 cases of Grade II tumors.

Table-4: Correlation of squash cytology with histopathology

Sr. No.	CNS Lesions	Correlation with HPE		Diagnostic Accuracy
		Correlation	No Correlation	
1	Astrocytoma	21	1	95.45%
2	Meningioma	6	-	100%
3	Schwannoma	6	-	100%
4	Oligodendroglioma	3	-	100%
5	Small round cell tumor (PNET/Ewings/Medulloblastoma)	6	-	100%
6	Ependymoma	2	-	100%
7	Pituitary adenoma	3	-	100%
8	CNS Lymphoma	1	1	50%
9	Hemangioblastoma	1	-	100%
10	Plasmacytoma	1	-	100%
12	Gliosarcoma	-	1	0
13	Infective Lesions	1	-	100%
14	Inflammatory pseudotumor	1	-	100%
15	Others (AV Malformation, Ganglioglioma)	1	1	50%
TOTAL NO OF CASES		56	4	93%

Among the various CNS lesion categories, Meningioma, Schwannoma, Oligodendroglioma, Small round cell tumors (PNET/Ewings/Medulloblastoma), Ependymoma, Pituitary adenoma, Hemangioblastoma, Plasmacytoma, Infective Lesions, and Inflammatory Pseudotumor showed a strong correlation with HPE, achieving a diagnostic accuracy of 100% in identifying these lesions. On the other hand, CNS Lymphoma displayed a 50% diagnostic accuracy, with one case correlating with HPE

and one case not. Gliosarcoma, a rare tumor, had no correlation with HPE, resulting in a 0% diagnostic accuracy. Additionally, among other CNS lesions like AV Malformation and Ganglioglioma, half of the cases correlated with HPE, yielding a 50% diagnostic accuracy. In total, out of 56 cases, 4 did not correlate with HPE, resulting in an overall diagnostic accuracy of 93%.

Table-5: Squash cytology results were classified into the following categories

S. No	Final histopathological Diagnosis	Concordance	Discordance	Initial squash diagnosis for the Discordant cases	TP (a)	FP (b)	FN (c)
1	Astrocytoma	21	1	Medulloblastoma	21	1	
2	Meningioma	6	-		6		
3	Schwannoma	6	-		6		
4	Oligodendroglioma	3	-		3		
5	Ganglioglioma	1			1		
6	Small round cell tumor (PNET/EWINGS/MEDULLOBLASTOMA)	6	-		6		
7	Ependymoma	2	-		2		
8	Pituitary adenoma	3	-		3		
9	CNS Lymphoma	1	1	Inflammation(chronic)	1		1
10	Hemangioblastoma	1	-		1		
11	Plasmacytoma	1	-		1		
12	Gliosarcoma		1	GBM		1	
13	Metastatic lesions	3			3		
14	Inflammatory pseudotumor	1			1		
15	Infective lesions	1	-		1		
16	A.V Malformation,	-	1	Astrocytoma		1	
	TOTAL	56	4		56	3	1

In our comparative and descriptive study, it's important to note that statistical significance is primarily associated with the overall sensitivity of diagnosing CNS lesions using squash cytology when compared to the gold standard histopathology, which stands at 98.2%. Additionally, we have calculated the Positive Predictive Value, which is found to be 94.9%. Squash and histopathological results were seen as a result of present study as follows:

Figure 1: squash cytology in grade ii astrocytoma

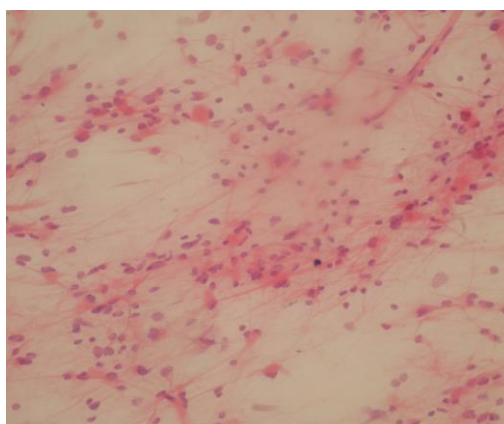


Figure 1: Infiltrative tumor with gemistocytes on a fibrillary background

Figure 2: Histopathology of Grade II Astrocytoma

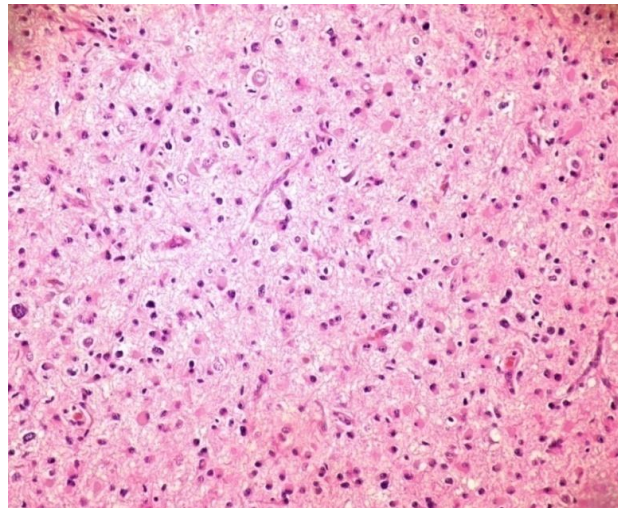


Figure 2: Grade II Gemistocytic Astrocytoma showing gemistocytes with ample eosinophilic cytoplasm having eccentrically placed nucleus on a fibrillary background

Figure 3: Squash Cytology in Grade III Astrocytoma

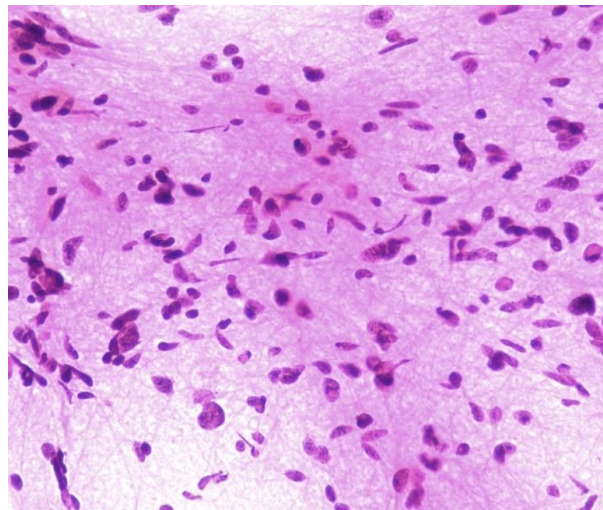


Figure 3: Anaplastic astrocytoma with moderate cellularity, pleomorphic nuclei & increased N:C ratio.

Figure 4: Histopathology in Grade III Astrocytoma

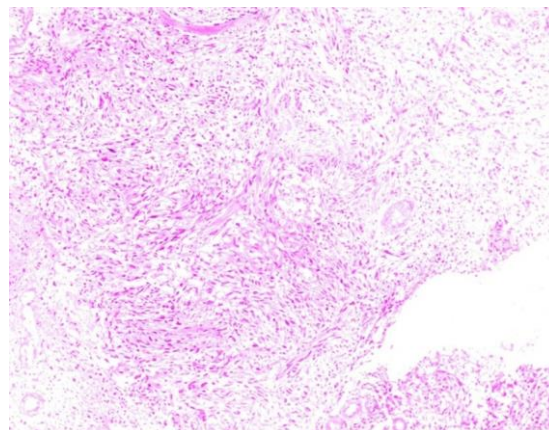


Figure 4: Anaplastic astrocytoma showing cellular tumor exhibiting diffuse anaplasia, nuclear pleomorphism and increased mitosis.

Figure 5: Squash Cytology In Grade IV Astrocytoma

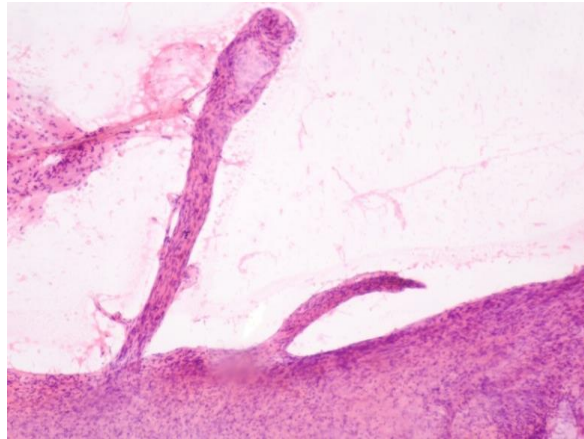


Figure 5: Grade IV Astrocytoma showing pleomorphic astrocytes with endothelial proliferation

Figure 6. Histopathology In Grade Iv Astrocytoma

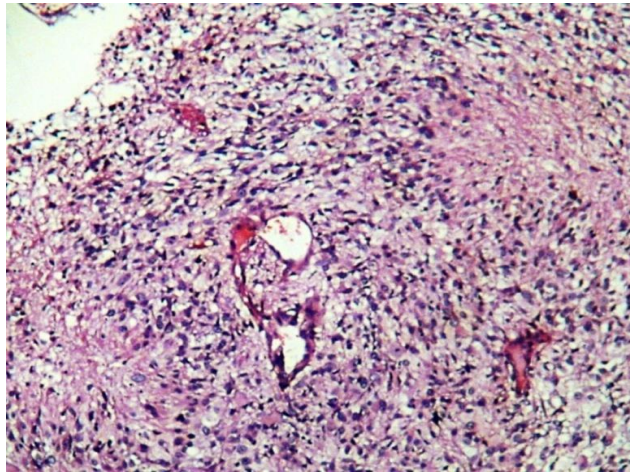


Figure 6: Grade IV Astrocytoma GBM, showing pleomorphic Astrocytes, endothelial proliferation with pseudo palisading necrosis.

Figure 7: Squash Cytology Inpsammomatous Meningioma

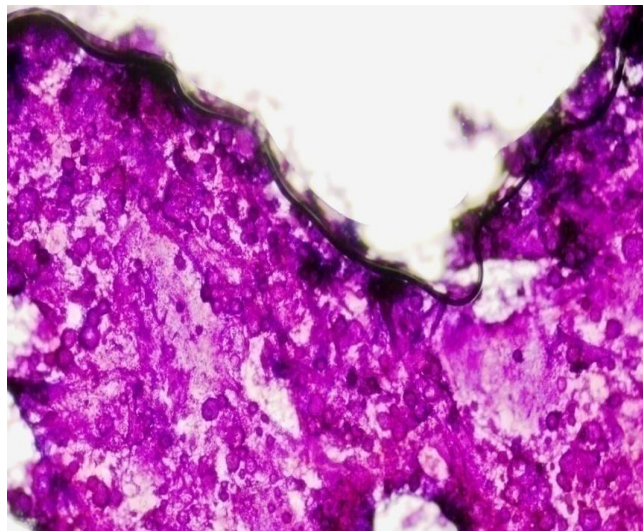


Figure 7: Psammomatous meningioma showing psammomatous calcification.

Figure 8: Histopathology In Psammomatous Meningioma

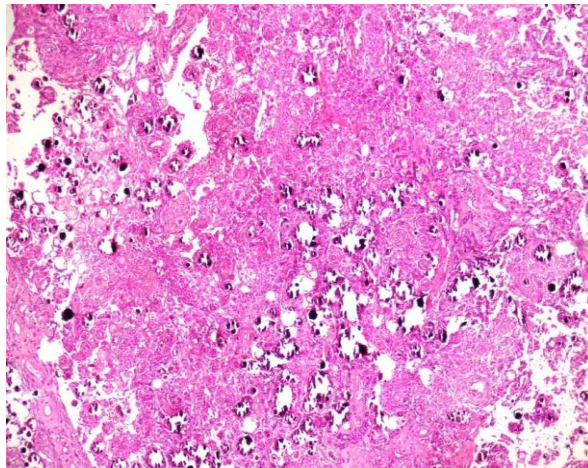


Figure 8: Histopathology in Grade I Psammomatous meningioma showing intact cellular whorls & psammomatous calcifications

Figure 9. Squash Cytology in Anaplastic Meningioma

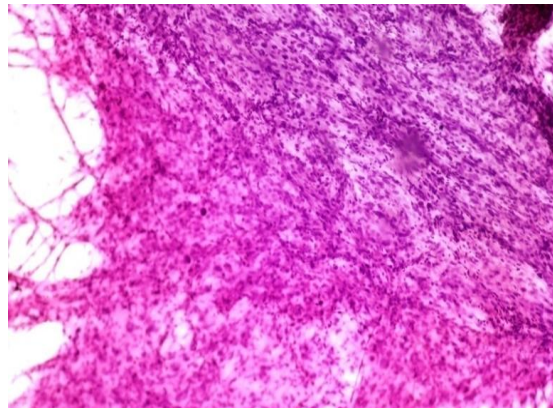


Figure 9: Grade III Anaplastic meningioma showing anaplastic features with increased mitotic activity

Figure 10: Histopathology in Anaplastic Meningioma

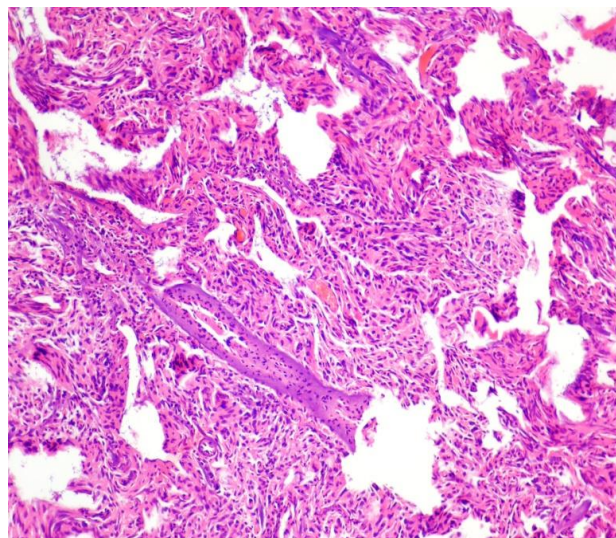


Figure 10: Grade III Anaplastic meningioma showing pattern less arrangement of anaplastic cells with increase in mitosis

Figure 11. Squash Cytology in Schwannoma

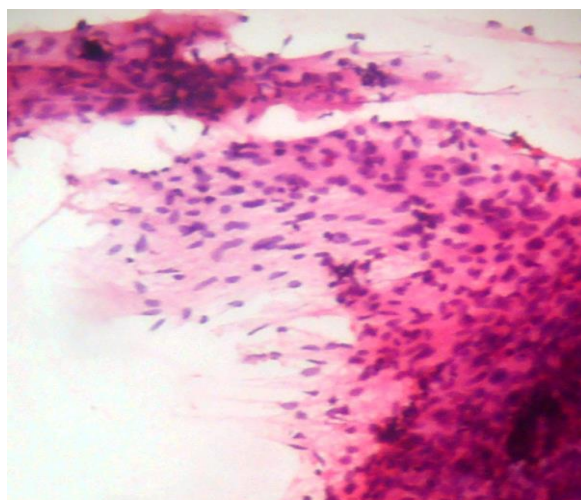


Figure 11: Schwannoma showing cohesive spindle cells forming frayed edges.

Figure 12: Histopathology in Schwannoma

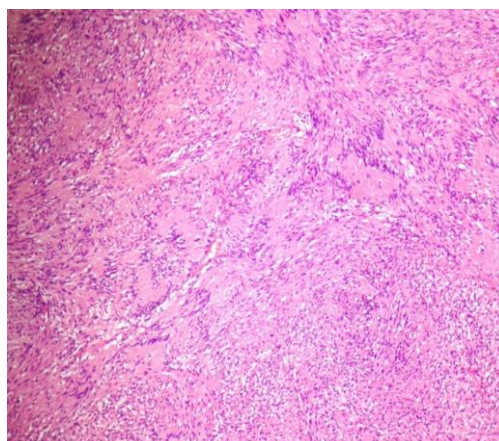


Figure 12: Schwannoma showing swirls of compact Antoni A & loose Antoni B areas with characteristic Verocay bodies

Figure 13: Squash Cytology in Medulloblastoma

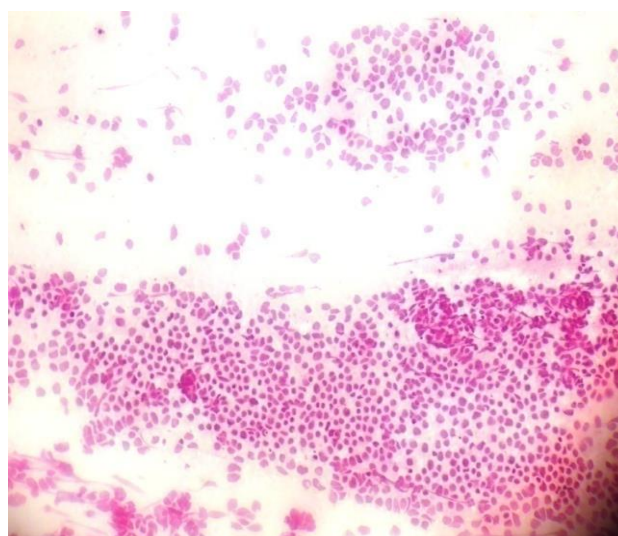


Figure 13: Medulloblastoma showing sheets of undifferentiated small round cells with minimal cytoplasm, with moderate anisonucleosis & rosetoid formation.

Figure 14: Histopathology in Desmoplastic Medulloblastoma

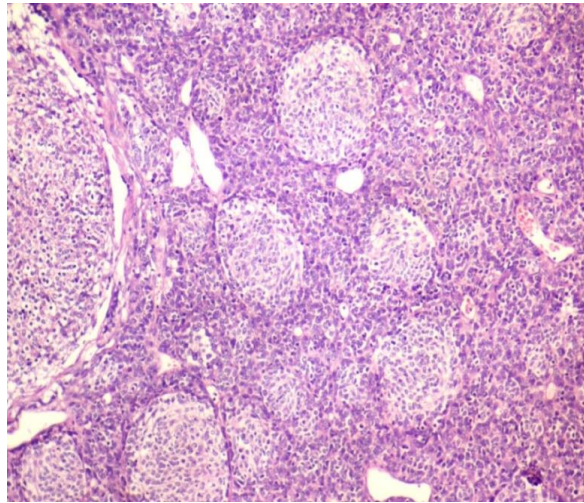


Figure 14: Desmoplastic medulloblastoma showing nodules with small round cells having hyperchromatic nuclei & nuclear moulding

Figure 15: Reticulin Staining in Desmoplastic Medulloblastoma

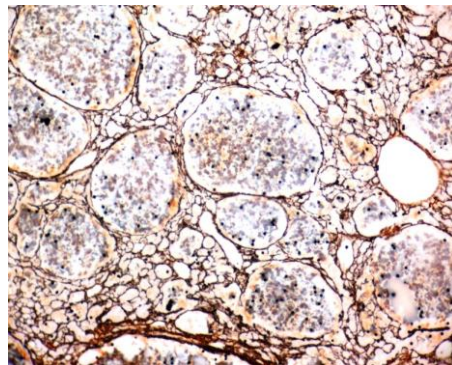


Figure 15: Reticulin stain demonstrating deposition of reticulin around the individual and nests of tumor cells.

Figure 16: Squash Cytology in Tancytic Ependymoma

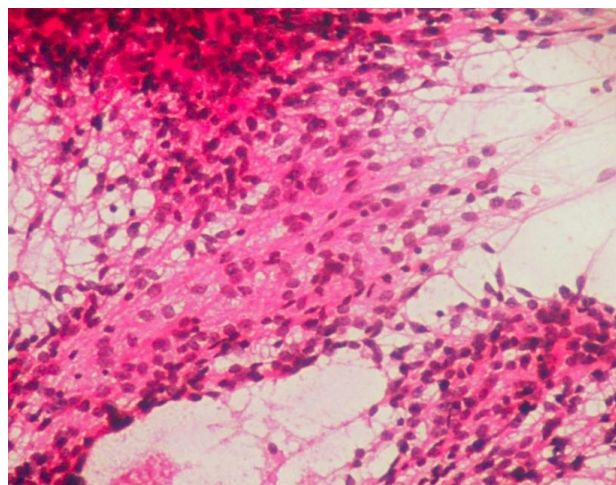


Figure 16: Tancytic ependymoma showing elongated spindle cells with oval nuclei with dark chromatin in a fibrillary background

Figure 17: Histopathology in Tancytic Ependymoma

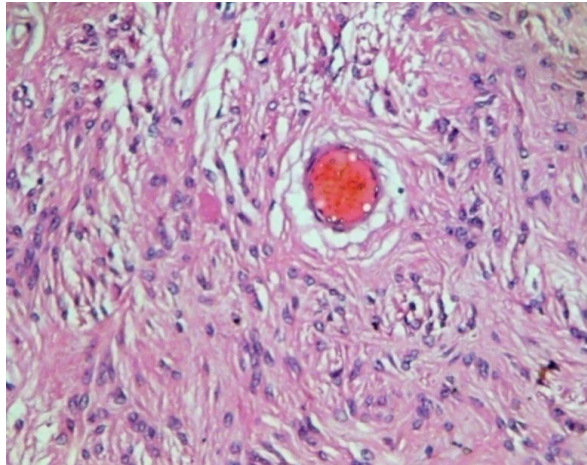


Figure 17: Perivascular pseudorosette consisting of ependymal tumor cells oriented around a central blood vessel with long fibrillar processes that extend radially towards the vessel.

Figure 18: Squash Cytology in Metastatic Deposits

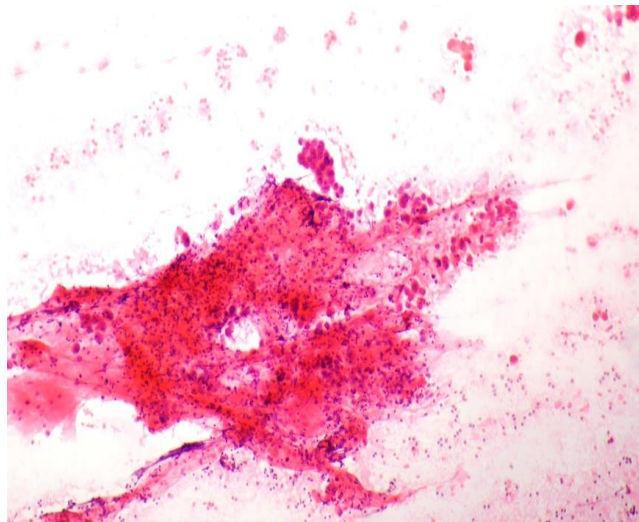


Figure 18: Squash cytology showing singly scattered clusters & branching sheets of large atypical cells

Figure 19: Histopathology in Metastatic Pappillary Adenocarcinoma

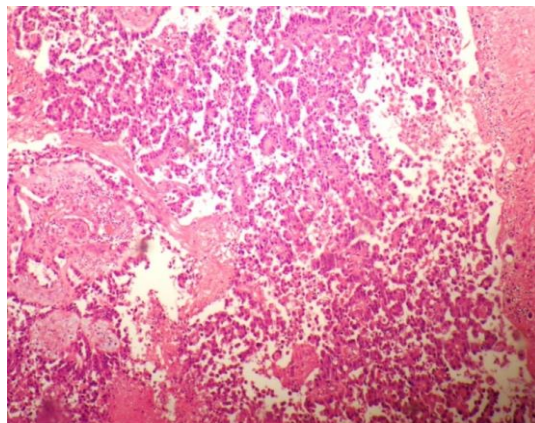


Figure 19: Section showing diffuse infiltration of malignant cells with marked pleomorphism arranged in papillary, glandular & nest like pattern

Figure 20: Squash Cytology in Ganglioglioma

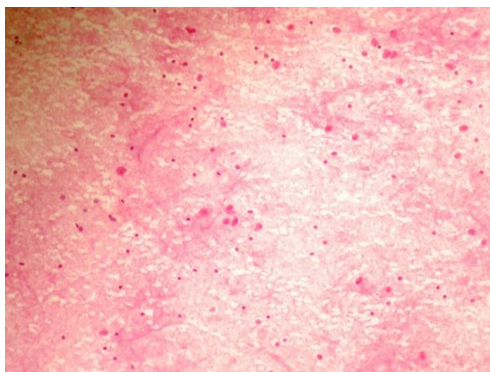


Figure 20: Ganglioglioma showing astrocytes & marked increase in ganglion cells

Figure 21: Histopathology in Ganglioglioma

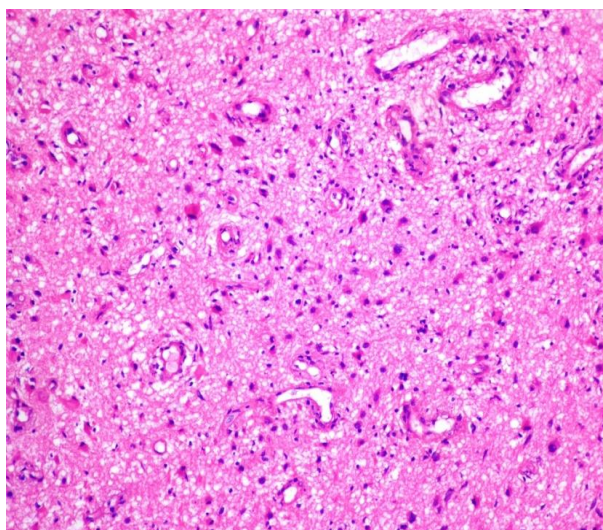


Figure 21: Ganglioglioma showing scattered large ganglion cells with lymphocyte cuffing

Figure 22: Squash Cytology in Plasmacytoma

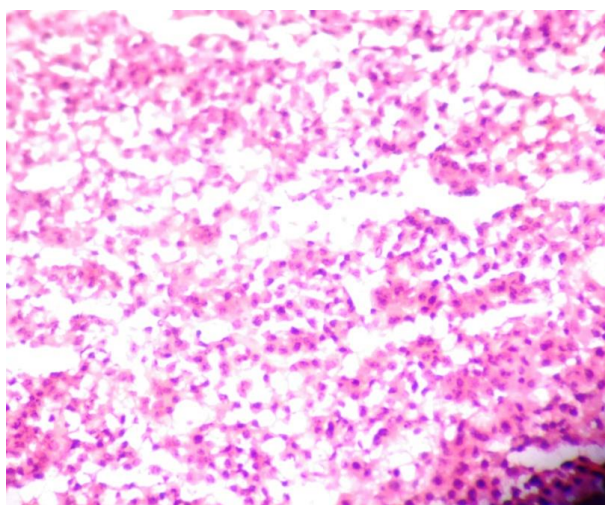


Figure 22: Plasmacytoma showing sheets of uniformly distributed plasmacells

Figure 23: Histopathology in Plasmacytoma

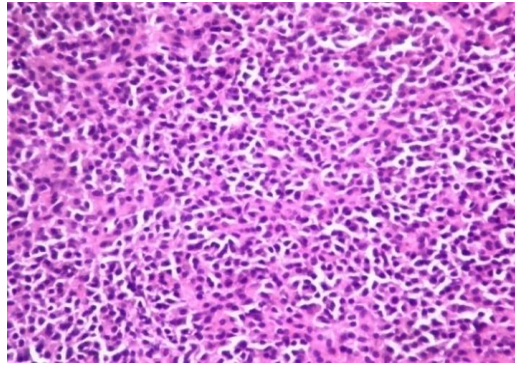


Figure 23: Plasmacytoma showing sheets of monomorphic plasmacytoid cells with abundant cytoplasm & eccentrically placed nucleus.

Figure 24: Squash Cytology In Pituitary Adenoma

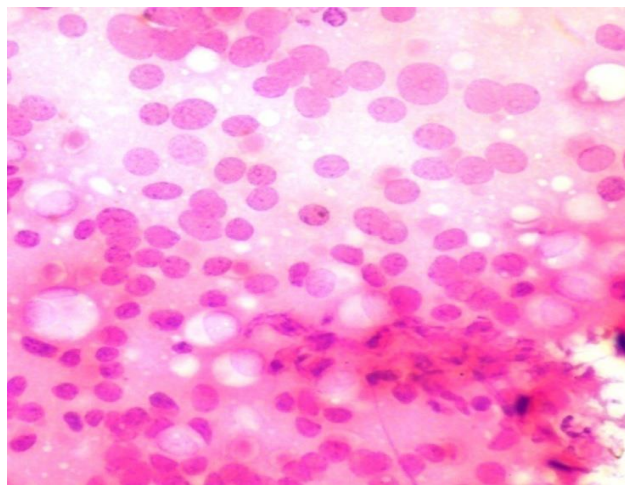


Figure 24: Pituitary adenoma showing monotonous population of round cells having salt & pepper chromatin with prominent nucleoli

Figure 25: Histopathology In Pituitary Adenoma

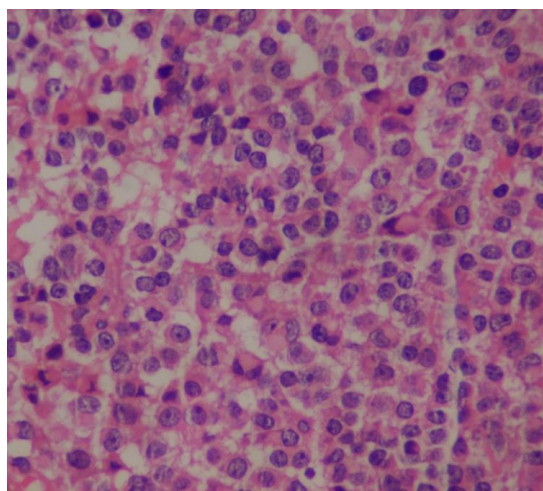


Figure 25: Pituitary adenoma showing short chains & sheets of round cells having salt & pepper chromatin with eosinophilic cytoplasm

Figure 26: Squash Cytology in Anaplastic Oligodendroglioma

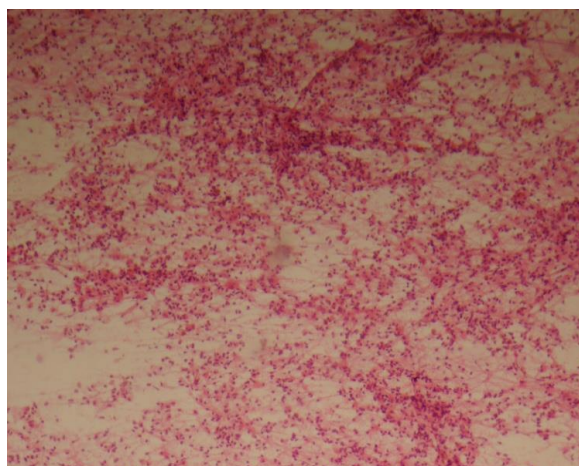


Figure 26: Anaplastic oligodendroglioma showing monomorphic round cells with vesicular nuclei & few microgemistocytes.

Figure 27: Histopathology in Anaplastic Oligodendroglioma

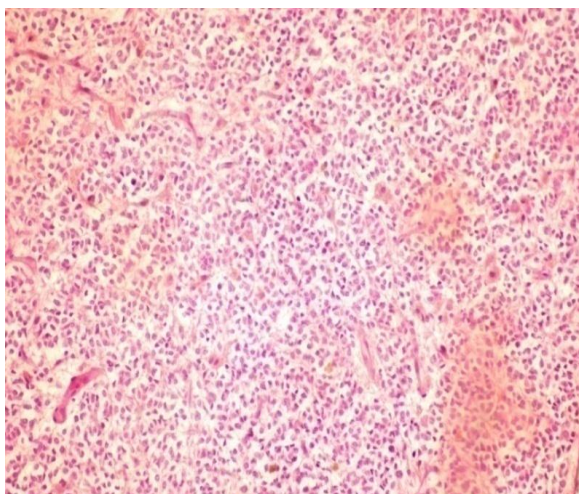


Figure 27: Anaplastic oligodendroglioma showing cells with uniform round vesicular nuclei & perinuclear halo with marked proliferation of blood vessels

Figure 28: Squash Cytology in Necrotizing Granulomatous Lesion

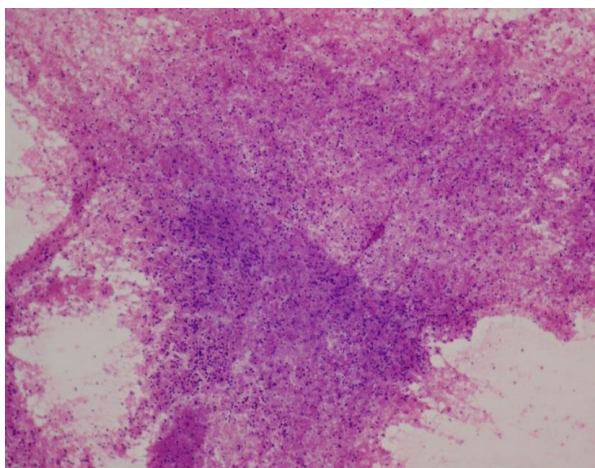


Figure 28: Squash cytology showing epithelioid cell granuloma on a necrotic background.

Figure 29: Histopathology in Necrotizing Granulomatous Lesion

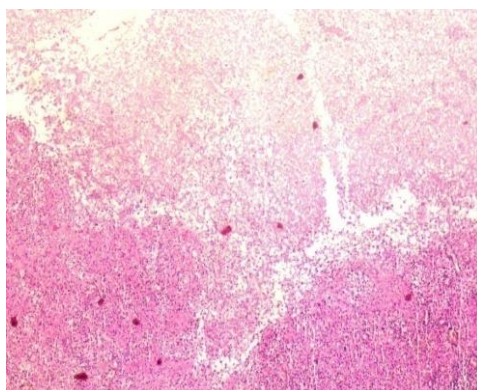


Figure 29: Section showing large area of necrosis surrounded by chronic inflammatory infiltrate & epithelioid cell collection

Figure 30. Histopathology In Av Malformation

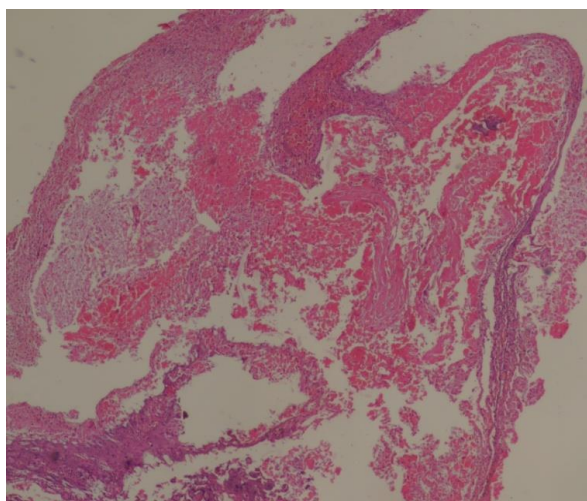


Figure 30: AV Malformation showing network of anastomosing abnormal ectatic veins & muscular arteries

Squash cytology's strengths resides in its ease of use, speed, reliability, cellular detail, tissue preservation for paraffin embedding, and minimal requirement for specialized equipment and trained staff. It is readily available at the point of surgery. Surgery from intracranial lesions that are situated in functionally essential parts of the brain benefits greatly from the fact that even very small specimens are appropriate for smear preparation⁸. In addition, it is a great resource for cytopathologist educators. Because the tissue is slightly deformed and none is lost, the most frequent touch preparations and smear preparations are appropriate for quick intraoperative identification of stereotactic brainbiopsy specimens.⁹

About 60 central nervous system lesions were investigated in this study, all of which were reported to the Pathology department at Chettinad Hospital & Research Institute. Between July 2012 and July 2016, 1.1% of people were diagnosed with a central nervous system tumour. The incidence of central nervous system cancers is around 19.34 per 100,000 people per year, as shown by the CBTRUS (Central Brain Tumor Registry of the United States) 29 database. Chang Hyun et al.⁴² report that it was 11.69 per 100,000 people-years. According to the results of the current study, those between the ages of 31 and 40 and those between the ages of 41 and 50 had the highest prevalence of CNS lesions (25 percent). Twenty percent CNS lesions were detected in the 51-60 age range.

The percentage of people with CNS lesions increased from 0% to 10% between the ages of 11 and 20, and from 7% to 8% between the ages of 21 and 30. Ages 60 and up had the lowest incidence (5%) of lesions. Sixty instances of brain and spinal cord lesions taking up a lot of space were studied here utilizing squash cytology, and the results were compared to the gold standard, histopathology. Sixty cases were examined, 58 of which were tumours and 2 were non neoplastic abnormalities. The current study's diagnostic accuracy of squash cytology among cancers agrees well with previous research. The following were the findings from a retrospective investigation of 60 CNS lesions evaluated using endoscopic and histological sections.

A total of 1.1% of patients at CHETTINAD HOSPITAL AND RESEARCH INSTITUTE WAS recorded having central nervous system lesions. CNS neoplasms are more common in men, with a male to female ratio of 3.6:1, and their prevalence increases with age. Most brain and nervous system tumours are astrocytomas (36.6 percent). Ten percent of the tumours in this research were either meningiomas, schwannomas, or small round cell tumours. The diagnostic accuracy of squash cytology was 93%. (56 out of 60).

Squash cytology has a 95.45% success rate in diagnosing astrocytomas. The biggest percentage of astrocytomas (45.45%) were classified as Grade II tumours, followed by Grade IV tumours (27.3%). Grading of astrocytomas reduced diagnostic certainty to 72%. (Grade II- 90 percent; Grade I-75 percent; Grade IV- 66 percent; Grade III- 50 percent) Our results show that squash cytology is 98.2% as sensitive as histology for diagnosing central nervous system lesions. The accuracy of the test is 94.8 percent positive.

Gender distribution of CNS lesions results showed Astrocytoma seen in 19 males. Meningioma Both men and women were diagnosed with CNS lymphoma and neurofibroma/schwannomas at the same rates. Only men were discovered to have the tumours of oligodendroglioma, pituitary adenoma, hemangioblastoma, metastasis, gliosarcoma, plasmacytoma, infection, av malformation, ganglioglioma, and ependymoma. PNET/Ewings/Medulloblastoma, a small round cell tumour, was more common in males than females.

Squash cytology was shown to provide a diagnostic accuracy of 75% for astrocytoma grades I and II, 90% for grades III and IV, and 50% for grades II and III. Squash cytology was able to offer grading in the majority of the lesions, which was a key result of our investigation. Nearly all intracranial tumours, including neurilemmoma, astrocytoma, craniopharyngioma, glioblastoma multiforme, and pituitary adenomas, showed a strong association between histology and tumour grade. According to Powell et al.¹⁰, a pathologist can benefit from both cytology and histology when examining a squash cytology made with a sufficient volume of tissue. Neurons, oligodendroglial cells, and astrocytes may all be distinguished from one another at the cellular level using this method¹¹.

In the cases of meningioma, schwannoma, oligodendroglioma, small round cell tumour (PNET/Ewings/Medulloblastoma), ependymoma, pituitary adenoma, plasmacytoma, infectious lesions, and inflammatory pseudotumor, squash cytology correlated well with histology. Some studies that compared squash cytology with histopathology found that it had a diagnosis accuracy of 87% to 97%. When we compared squash cytology to the gold standard of histology, we found that it was 95% accurate, which is on par with or better than the results of another research¹²⁻¹⁴.

Squash cytology has been compared to frozen section in a small number of investigations, with similar accuracy found for both methods¹⁵⁻¹⁷. When frozen section proved inconclusive or when frozen-section examination was not possible [for example, due to an insufficient sample size, intra-operative cytology has been found to be a valuable diagnostic tool.¹⁸ It has been suggested by Nigam et al.¹⁹ that a well prepared squash smear can replace frozen section or significantly alter its interpretation. Squash cytology is preferable to frozen section²⁰⁻²² because it eliminates freezing artefact and reduces cryostat contamination from possibly diseased tissue. Even with extremely little amounts of biopsy material, several researchers have found that cytological preparation yields more reliable morphology than frozen section²³. While squash cytology was formerly thought to be a useful adjunct to frozen section²⁴, recent evidence suggests that it may be used well on its own to inform neurosurgeons throughout the intra-operative time.

When compared to tumours in other main bodily systems, those in the central nervous system have the most subcategories (approximately 86, not counting their variations).²⁵ Histopathologists worry about central nervous system cancers since their morphology varies widely and they have a hard time appropriately evaluating them.²⁶ Due to incomplete reporting of newly diagnosed cases of CNS tumours to local cancer registries, the true tumour burden in poor nations like India is underreported.²⁷ Prevalence data collected from hospitals is crucial for determining the level of medical support needed for the treatment and management of these diseases, and serves as the foundation for tumour burden estimates.

This has potential implications for brain tumour research, particularly in assessing regional variations in molecular and genetic profiles that may inform the design of targeted, tailored medicines and the formulation of effective treatment protocols and procedures.²⁸ When it comes to identifying lesions in the brain and spinal cord, squash cytology is one of the most reliable methods. The process takes very little time and is quite cheap.²⁹⁻³⁰ Squash cytology allows for a clear view of cellular morphology in all its detail. Since the price of a cryostat is prohibitive in impoverished countries like India, and since squash cytology, unlike a cryostat, does not necessitate the use of power for slide preparation, it may be utilised as a viable diagnostic technique³¹. Additionally, cryostat sections must be cut by a skilled microtome, and freezing artefacts might lead to misdiagnoses.

Squash cytology, with all its benefits, should only be used as a preliminary investigation, with final confirmation by Histopathology as the gold standard. Only for medicinal or diagnostic use; never otherwise.³³ Squash cytology allows for quick diagnosis and evaluation of tissue adequacy, both of which are becoming increasingly important as stereotactic biopsies become more common. The quick cytological identification of CNS diseases using squash cytology is an accurate and reliable approach in the hands of a pathologist with good exposure to neuropathology.³⁴

A hard tissue is more difficult to compress than a soft and friable one. Because of the slippery nature of the tissue, we had trouble getting a decent cellular squash smear ready in case of a hydatid cyst. A moist preparation of the cyst fluid is also being examined at the same time. However, rather than providing a precise diagnosis and exact grading for each instance, intra-operative pathologists should offer adequate basic information for effective operation²⁵. Our study's small sample size prevents us from drawing firm conclusions on squash cytology's efficacy as a stand-alone intra-operative treatment. However, our results once again support the validity of squash cytology, and future multicenter research with a broader patient population may provide light on the question of whether or not to employ it in clinical practise.³⁵

4. Conclusion

Providing excellent nuclear and cytoplasmic features of intracranial lesions in low-resource settings, squash smear cytology provides an accurate, reliable, quick, safe, cost-effective intraoperative diagnostic technique. The diagnostic accuracy of intraoperative squash cytology can be increased with acquaintance with the cytomorphological aspects of CNS lesions, as well as their clinical and radiological correlate.

References:

- 1). Silverberg SG, DeLellis RA, Frable WJ, LiVolsi VA, Wick MR. Silverberg's principles and practice of surgical pathology and cytopathology. 4th ed. Philadelphia: Churchill Livingstone; 2006.
- 2). Fletcher CDM. Diagnostic histopathology of tumors. 3rd ed. Philadelphia: Churchill Livingstone; 2007.
- 3). Eisenhardt L, Cushing H. Diagnosis of intracranial tumors by supravital technique. *Am J Pathol* 1930;6(5):541-52.
- 4). Russell DS, Krayenbuhl H, Cairns H. Wet film technique in histological diagnosis of intracranial tumors- Rapid method. *J Pathol Bacteriol* 1937;45(3):501-5.
- 5). Powell SZ. Intraoperative consultation, cytologic preparations, and frozen section in the central nervous system. *Arch Pathol Lab Med* 2005;129(12):1635-52.
- 6). Roessler K, Dietrich W, Kitz K. High diagnostic accuracy of cytologic smears of CNS tumors. *Acta Cytol* 2002;46(4):667-74.
- 7). Firlik KS, Martinez AJ, Lunsford LD. Use of Cytological preparations for the intraoperative diagnosis of stereotactically obtained brain biopsies: a 19 year experience and survey of neuropathologists. *J Neurosurg* 1999;91(3):454-8. (Also referenced as #20)

- 8). Goel D, Sundaram C, Paul TR, Uppin SG, Prayaga AK, Panigrahi MK, et al. Intraoperative cytology (squash smear) in neurosurgical practice - pitfalls in diagnosis experience based on 3057 samples from a single institution. *Cytopathology*. 2007;8:300–8. (Also referenced as #10)
- 9). Bleggi-Torres LF, de Noronha L, Schneider Gugelmin E, Martins Sebastiao AP, Werner B, Marques Maggio E, et al. Accuracy of the smear technique in the cytological diagnosis of 650 lesions of the central nervous system. *Diagnostic Cytopathology*. 2001;24(4):293-95. (Also referenced as #21)
- 10). Asha T, Shankar SK, Rao TV, Das S. Role of squash-smear technique for rapid diagnosis of neurosurgical biopsies--a cytomorphological evaluation. *Indian Journal of Pathology & Microbiology*. 1989;32(3):152-60.
- 11). Guarda LA. Intraoperative cytologic diagnosis: evaluation of 370 consecutive intraoperative cytologies. *Diagnostic Cytopathology*. 1990;6(4):235-42. (Also referenced as #23)
- 12). Liu Y, Silverman JF, Sturgis CD, Brown HG, Dabbs DJ, Raab SS. Utility of intraoperative consultation touch preparations. *Diagnostic Cytopathology*. 2002;26(5):329-33.
- 13). S cucchi LF, Di Stefano D, Cosentino L, Vecchione A. Value of cytology as an adjunctive intraoperative diagnostic method. An audit of 2,250 consecutive cases. *Acta Cytologica*. 1997;41(5):1489-96.
- 14). Kim SH, Lee KG, Kim TS. Cytologic characteristics of subependymal giant cell astrocytoma in squash smears: morphometric comparisons with gemistocytic astrocytoma and giant cell glioblastoma. *Acta Cytologica*. 2007;51(3):375-79.
- 15). Nigam J, Misra V, Dhingra V, Jain S, Varma K, Singh A. Comparative study of intra-operative cytology, frozen sections, and histology of tumor and tumor-like lesions of nose and paranasal sinuses. *Journal of Cytology / Indian Academy of Cytologists*. 2013;30(1):13-17.
- 16). Wilson I. A method for the rapid preparation of fresh tissues for the microscope. *JAMA*. 1905;45:1737. (Also referenced as #30)
- 17). Krishnani N, Kumari N, Behari S, Rana C, Gupta P. Intraoperative squash cytology: accuracy and impact on immediate surgical management of central nervous system tumors. *Cytopathology : Official Journal of the British Society for Clinical Cytology*. 2012;23(5):308-14. (Also referenced as #25)
- 18). Parkin DM WS, Ferlay J, Raymond L, Young J. Cancer incidence in five continents. VII [143] ed. IARC. L, editor1997.
- 19). Kumar V AA, Fausto N, Aster JA. Pathologic basis of disease. 7th ed. ed. Cotran R, editor. Philadelphia: Elsevier; 2004.
- 20). Kondziolka D, Lunsford LD, Martinez AJ. Unreliability of contemporary neurodiagnostic imaging in evaluating suspected adult supratentorial [low-grade] astrocytoma. *Journal of Neurosurgery*. 1993;79[4]:533-36.
- 21). Olasode BJ, Ironside JW. The brain smear, a rapid affordable intraoperative diagnostic technique for brain tumors appropriate for Africa. *Tropical Doctor*. 2004;34(4):223-25. (Also referenced as #31)
- 22). Mitra S, Kumar M, Sharma V, Mukhopadhyay D. Squash preparation: A reliable diagnostic tool in the intraoperative diagnosis of central nervous system tumors. *Journal of cytology / Indian Academy of Cytologists*. 2010;27(3):81-85. (Also referenced as #32)
- 23). Chen L, Zou X, Wang Y, Mao Y, Zhou L. Central nervous system tumors: A single center pathology review of 34,140 cases over 60 years. *BMC ClinPathol*. 2013;13:14. (Also referenced as #33)
- 24). Yachnis AT. Intraoperative consultation for nervous system lesions. *Semin Diagn Pathol*. 2002;19:192–206. (Also referenced as #34)
- 25). Balsimelli LBS, Oliveira JC, Adorno FÁ, Brites CA, Bublitz GS, Tavares LCC, et al. Accuracy of intraoperative examination in central nervous system lesions: A study of 133 cases. *Acta Cytol*. 2019;63:224–32. (Also referenced as #35)
- 26). Parkin DM WS, Ferlay J, Raymond L, Young J. Cancer incidence in five continents. VII [143] ed. IARC. L, editor1997.
- 27). Kumar V AA, Fausto N, Aster JA. Pathologic basis of disease. 7th ed. ed. Cotran R, editor. Philadelphia: Elsevier; 2004.
- 28). Sundaram C. Diagnostic utility of squash [smear] technique in the inflammatory lesions of central nervous system. *Indian Journal of Pathology & Microbiology*. 2003;46(4):569-72.
- 29). Kondziolka D, Lunsford LD, Martinez AJ. Unreliability of contemporary neurodiagnostic imaging in evaluating suspected adult supratentorial [low-grade] astrocytoma. *Journal of Neurosurgery*. 1993;79[4]:533-36. (Also referenced as #20)
- 30). Wilson I. A method for the rapid preparation of fresh tissues for the microscope. *JAMA*. 1905;45:1737. (Also referenced as #16)
- 31). Olasode BJ, Ironside JW. The brain smear, a rapid affordable intraoperative diagnostic technique for brain tumors appropriate for Africa. *Tropical Doctor*. 2004;34(4):223-25. (Also referenced as #21)

- 32). Mitra S, Kumar M, Sharma V, Mukhopadhyay D. Squash preparation: A reliable diagnostic tool in the intraoperative diagnosis of central nervous system tumors. *Journal of cytology / Indian Academy of Cytologists*. 2010;27(3):81-85. (Also referenced as #22)
- 33). Chen L, Zou X, Wang Y, Mao Y, Zhou L. Central nervous system tumors: A single center pathology review of 34,140 cases over 60 years. *BMC ClinPathol*. 2013;13:14. (Also referenced as #23)
- 34). 34. Yachnis AT. Intraoperative consultation for nervous system lesions. *Semin Diagn Pathol*. 2002;19:192–206. (Also referenced as #24)
- 35). Balsimelli LBS, Oliveira JC, Adorno FÁ, Brites CA, Bublitz GS, Tavares LCC, et al. Accuracy of intraoperative examination in central nervous system lesions: A study of 133 cases. *Acta Cytol*. 2019;63:224–32. (Also referenced as #25)