USM SHORT TERM GRANT NO. 304/PPSP/6131316

Intraoperative Crush Cytology in the Diagnosis of Neurosurgical Cases

Profesor Madya Dr. Manoharan Madhavan
Department of Pathology
School of Medical Sciences
Universiti Sains Malaysia

UNIVERSITI SAINS MALAYSIA

Intraoperative Crush Cytology in the Diagnosis of Neurosurgical Cases

UNIVERSITI SAINS MALAYSIA

Short Term Grant No. 304/PPSP/6131316

Dr. Manoharan Madhavan
Associate Professor of Pathology
Department of Pathology
School of Medical Sciences
Universiti Sains Malaysia
16150, Kubang Kerian, Kelantan, Malaysia

BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
CANSELORI
UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1)	Nama Penyelidik	:	Profesor Madya Dr. Manoharan Madhavan
	Nama Penyelidik-Penyelidik Lain (Jika berkaitan)	:	1. Dr. Gurjeet Kaur d/o Chatar Singh 2. Dr. Jain George P
2)	Pusat Pengajian/Pusat/Unit	:	Pusat Pengajian Sains Perubatan
3)	Tajuk Projek	:	Intra Operative Crush Cytology in the Diagnosis of Neurosurgical Cases

4) (a) Penemuan Projek/Abstrak

(Perlu disediakan makluman di antara 100 – 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

ABSTRACT

TITLE: Intra Operative Crush Cytology in the Diagnosis of Neurosurgical Cases

Intraoperative cytologic smears in neurosurgery are easy to perform and inexpensive and permit reasonably high diagnostic accuracy.

Even very tiny specimens, especially of soft consistency are suitable for this technique, which is extremely important in operation of tumours localized in functionally important brain areas.

The cytologic diagnosis could be made based on the presence of characteristic cytomorphological features. Very high accuracy can be archived in certain tumours like nerve sheath tumour, germinoma, meningioma, metastasis, grade II astrocytoma, pituitary adenoma and oligidendroglioma. However, the accuracy is low in high grade glioma, medulloblastoma and ependymoma. In the absence of their diagnostic cytomorphological features, the correlation with

clinicoradiological features might help in arriving at the correct diagnostic and increase their diagnostic accuracy.

Further studies involving more number of cases is needed to find out the value of crush cytology in diagnosing the rare tumours and inflammatory lesions.

In cytology examination, presence of pure normal glial tissue has high negative predictive value. This helps in guidance during targeting the lesions including steriotactic biopsy and also during the resection of infiltrative lesions for definition of tumour margins.

Keywords: *cytology, brain tumour, intraoperative.*

(b) Senaraikan Kata Kunci (Keywords) yang digunakan di dalam abstrak:

Bahasa Malaysia	Bahasa Inggeris
Sitologi	Cytology
Tumor otak	Brain tumour
Intraoperatif	Intraoperative

5) Output Dan Faedah Projek

(a) Penerbitan (termasuk laporan/kertas seminar)
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

1. M. Madhavan., Jain GP., Jaffri MA., Zamzuri I. *Intraventricular Squamous Papillay Craniopharyngioma : Report of a Cases with Intraoperative Imprint Cytology.* Acta Cytol 2005: 49; 431-434.

(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila guna kertas berasingan)

(c) Latihan Gunatenaga Manusia

i) Pelajar Siswazah: Tiada

ii) Pelajar Prasiswazah: Tiada

iii) Lain-Lain: Tiada

6. Peralatan Yang Telah Dibeli:

Video-VGA Converter
RM 388.00, No. LPO : C20054, Date of Purchased: 17/02/2005

UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

Laporan menetapi kejayaan penyelidikan

T/TANGAN PENGERUSI
J/K PENYELIDIKAN
PUSAT PENGAJIAN

PROFESSOR ABDUL AZIZ BABA
Chairman of Research & Ethics Committee
School of Medical Sciences
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan

15/2/06

Volume 49 Number 4

ACTA CYTOLOGICA

The Journal of
Clinical Cytology
and Cytopathology

July–August 2005

ISSN0001-5547

Submit manuscripts online at: www.acta-cytol.com/submit

Intraventricular Squamous Papillary Craniopharyngioma

Report of a Case with Intraoperative Imprint Cytology

Manoharan Madhavan M.B.B.S., M.D.(Path), M.I.A.C., **Jain George P.**, M.B.B.S., M.S., M.Ch. (Neurosurg), **J. Abdullah Jafri**, M.D., Ph.D., and **Zamzuri Idris**, M.B.Bch. (M. Neurosurg)

Background

Squamous papillary craniopharyngioma is a distinct entity, and its cytologic features may be misleading. Because of the rarity of this tumor, this case is being reported with a note on the cytologic features.

Case

A 56-year-old Malay man who had 1-month history of generalized lethargy was admitted for altered sensorium. On examination, he was found to have neck stiffness, bilateral papilledema and generalized atrophy of muscles, with reduced power in all limbs. Magnetic resonance imaging of the brain showed a solid mass in the third ventricle causing obstructive hydrocephalus. Intraoperative cytology of the mass diagnosed intraventricular meningioma. However, the final histopathologic examination revealed squamous papillary craniopharyngioma.

Cytology showed an abundant cellular yield composed essentially of sheets and groups of polygonal cells with a moderate amount of cytoplasm and bland, round nuclei.

Conclusion

Craniopharyngioma, squamous papillary type, is a rare entity and usually occurs in adults as an intraventricular solid tumor. Awareness of this entity will aid in arriving at the correct cytologic diagnosis. (Acta Cytol 2005;49:431–434)

Keywords: craniopharyngioma, papillary; cerebral ventricles; intraoperative period; imprint cytology.

Craniopharyngioma constitutes about 3% of brain tumors.[1] Most patients with craniopharyngioma are in the first or second decade of life. The location of the tumor is usually suprasellar, although it may occupy the sella as well. It is usually composed of adamantinomatous-type epithelium. In rare cases the tumor has a macroscopic papillary appearance, lined with pseudopapillary squa-

From the Department of Pathology and Neuroscience Unit, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia.
Dr. Madhavan is Associate Professor, Department of Pathology.
Dr. George is Lecturer, Neuroscience Unit.
Dr. Jafri is Professor, Neuroscience Unit.
Dr. Idris is Trainee Lecturer, Neuroscience Unit.
Address reprint requests to: Manoharan Madhavan, M.B.B.S., M.D.(Path), M.I.A.C., Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia (madhavan@kb.usm.my).

Financial Disclosure: The authors have no connection to any companies or products mentioned in this article.
Received for publication April 2, 2004.
Accepted for publication May 29, 2004.
0001-5547/05/4904-0431/$19.00/0

mous epithelium, and is termed *squamous papillary type*. It is considered a distinct entity as it is usually solid, occurs exclusively in adults and has a better postoperative outcome. The intraoperative cytology may be misleading, as the features are different from those of the adamantinomatous type. This case is being reported because of its rarity, with a note on the cytologic features.

Case Report

A 56-year-old Malay man who had been treated for dengue fever in another local hospital was admitted to our neurosurgical unit following a 1-month history of generalized lethargy. He became bed bound and developed altered sensorium. He appeared drowsy and did not respond to questions. This happened gradually and progressively. The patient had a history of a central headache prior to his deteriorating condition. There was no history to suggest endocrine or hypothalamic disturbances other than a previous history of intermittent fever that led to the incorrect diagnosis of dengue fever.

On the day of admission the patient was drowsy, nonresponsive and confused. His vital signs were normal. He had neck stiffness, and on cranial nerves examination there was bilateral papilledema with midsize pupils, 4 mm in diameter, which reacted sluggishly to direct light. Generalized atrophy of the muscles with reduced power in all limbs was noted. In addition, the left lower limb was slightly hypertonic, with slightly increased reflex. The Babinski sign was positive on the left side. All the basic blood investigations were normal.

Magnetic resonance imaging of the brain (Figure 1) showed a solid tumor measuring 2.9×3.4×3.5 cm situated exclusively inside the third ventricle at the level of the foramen of Monro, causing obstructive hydrocephalus. Total microsurgical excision of the tumor was performed through a pericoronal, interhemispheric, transcallosal approach. Intraoperatively the tumor appeared predominantly soft, with some firm areas and a moderate amount of vascularity, which could be easily aspirated.

Intraoperative imprint cytology smears, stained with hematoxylin-eosin, revealed a high cellular yield. The cells were predominantly polygonal, with a moderate amount of cytoplasm and uniform, bland, round nuclei. They were arranged predominantly in syncytial groups, with some lying singly (Figure 2). No evidence of keratinization, calcified debris or cellular whorling was present. With the presence of a syncytial cell arrangement, a diagnosis of intraventricular meningioma was made. However, histopathologic examination of the tumor revealed a solid sheet of squamous cells punctuated by a fibrovascular core (Figure 3). No evidence of keratinization or basaloid-type squamous cells and calcified debris was present. Hence, a diagnosis of craniopharyngioma, squamous

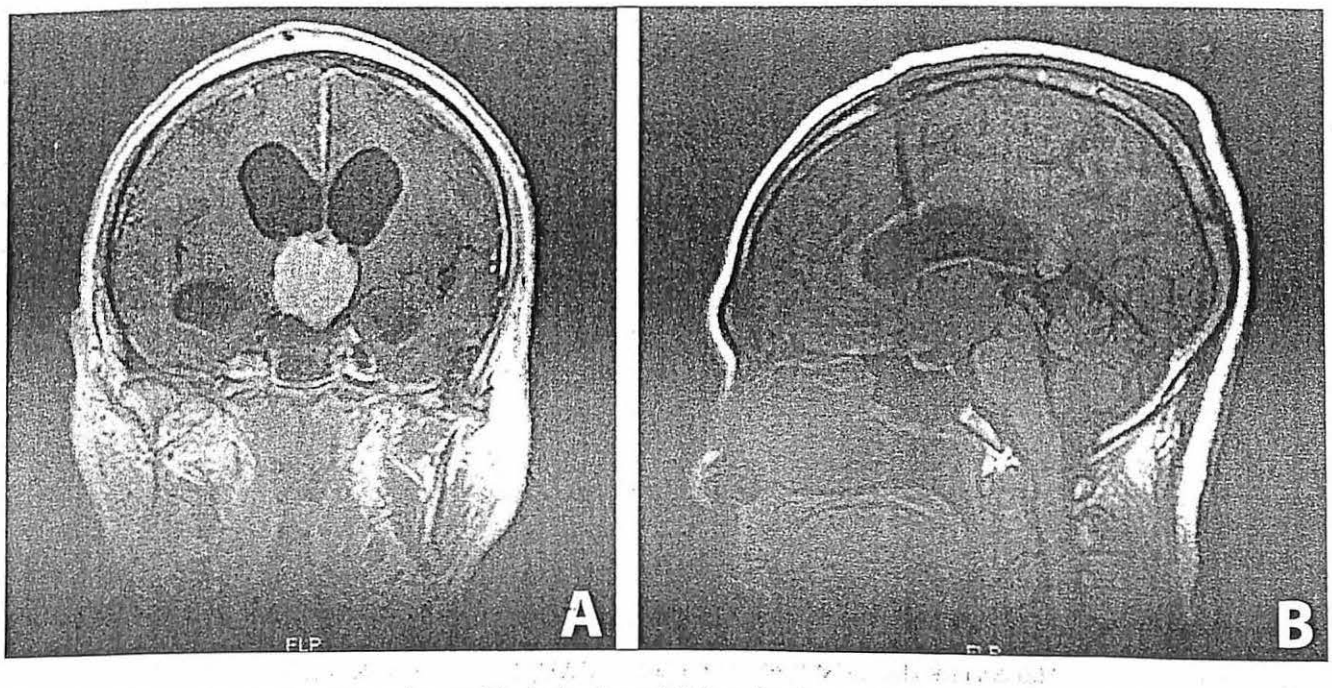


Figure 1 (A) Coronal magnetic resonance image of the brain, after gadolinium, showing an enhancing, rounded mass in the third ventricle obstructing the flow of cerebrospinal fluid at the foramen of Monro. (B) Sagittal T1-weighted image showing the mass of isointensity to the brain parenchyma occupying the anterior two-thirds of the third ventricle.

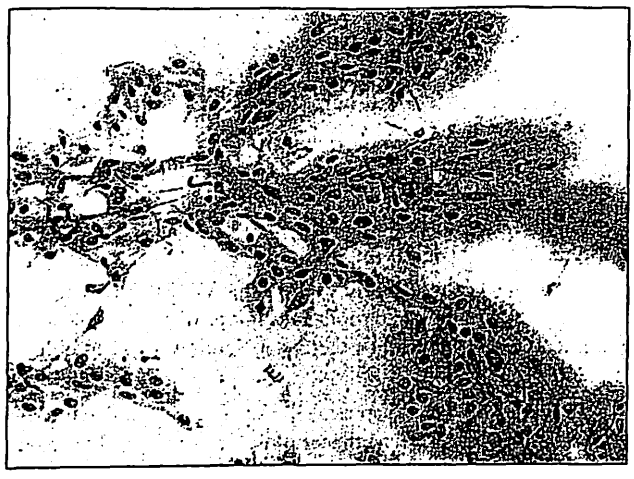


Figure 2 Imprint cytology showing polygonal cells arranged in syncytial groups (hematoxylin-eosin, ×100).

papillary type, was made.

The patient recovered uneventfully after the operation. However, at the time of discharge, on the 12th postoperative day, he died of acute myocardial infarction.

Discussion

There are 2 distinct clinicopathologic entities described as craniopharyngioma. The more common entity is the adamantinomatous type. It usually occurs during the first and second decades of life. Few cases have been observed in people in their 70s and 80s.[2] Craniopharyngiomas, 10–20% are squamous papillary type,[2,3] and it typically affects adults, as in our case. A case affecting a 10-year-old boy has been reported.[3]

The adamantinomatous type usually involves the suprasellar region and extends into the adjacent areas, including the third ventricle. A few cases have been seen in the sella and infrasellar region.[4,5] In the present case the tumor was situated in the third ventricle. Craniopharyngioma involving the third ventricle exclusively is typically the squamous papillary type, although it may affect other regions as well.

Craniopharyngioma of the adamantinomatous type is usually cystic but is sometimes solid or combined solid and cystic. In contrast, the papillary type is frequently solid.[6] A few reported cases were both cystic and solid or entirely cystic.[2]

Microscopically the squamous papillary type is composed of papillary structures and mature squamous epithelium in solid areas and attenuated cells in cystic areas.[4] The present case was entirely solid and composed of solid sheets of mature squamous cells punctuated by a fibrovascular core. Stellate reticulum, microcysts, and flaky and wet keratin were conspicuously absent. Hence, a diagnosis of squamous papillary type was made. Occasionally both types of epithelium are present together. Calcification, typical of the adamantinomatous type, is extremely rare in the squamous papillary type. If present, it occurs as microcalcification.[4]

The cytologic features have been described only for the adamantinomatous type[7] and are composed of a mixture of numerous keratinized cells, characteristic squamous cells of basal type, ghost cells, macrophages and calcified debris. In our case an intraoperative cytologic diagnosis was sought. Cytology showed an abundant cellular yield composed essentially of sheets and groups of polygonal cells with a moderate amount of cytoplasm and bland, round nuclei. In view of the syncytial arrangement of cells and small clustering of cells resembling cell whorling, a diagnosis of intraventricular meningioma was suggested. No evidence of keratinization was present. Even if it were present, it might have suggested another diagnosis, such as epidermoid cyst or Rathke's cleft cyst with squamous metaplasia.[8] The solid nature of the lesion and awareness of it might suggest the diagnosis.

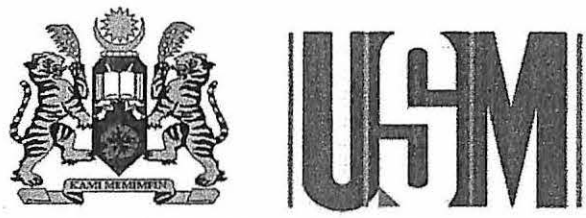
Craniopharyngiomas are usually treated with gross total resection. Radiotherapy may be given if residual tumor is present after surgical excision. Between the 2 histologic types of this tumor, no difference in resectability, efficacy of radiotherapy and overall survival has been documented.[4,9] Adamson et al[6] showed that the postoperative outcome was good in squamous papillary type, as there was clear demarcation between the tumor and adjacent brain. However, a case of meningeal seeding from surgical manipulation has been reported.[10]

Figure 3 The tumor was composed of a solid sheet of squamous cells punctuated by a fibrovascular core (hematoxylin-eosin, ×25).

References

1. Lederman GS, Recht A, Loeffler JS, Dubuisson D, Kleefield J, Schnitt SJ: Craniopharyngioma in an elderly patient. Cancer 1987;60:1077–1080
2. Crotty TB, Scheithauer BW, Young WF Jr, Davis DH, Shaw EG, Miller GM, Burger PC: Papillary craniopharyngioma: A clinicopathological study of 48 cases. J Neurosurg 1995;83: 206–214
3. Giangaspero F, Burger PC, Osborne DR, Stein RB: Suprasellar papillary squamous epithelioma ("papillary craniopharyngioma"). Am J Surg Pathol 1984;8:57–64
4. Petito CK, DeGirolami U, Earle KM: Craniopharyngiomas: A clinical and pathological review. Cancer 1976;37:1944–1952
5. Kachhara RR, Nair SS, Gupta AK, Radhakrishnan VV, Bhattacharya RN: Infrasellar craniopharyngioma mimicking a clival chordoma: A case report. Neurol India 2002;50:198–200
6. Adamson TE, Wiestler OD, Kleihues P, Yasargil MG: Correlation of clinical and pathological features in surgically treated craniopharyngiomas. J Neurosurg 1990;73:12–17
7. Smith AR, Elsheikh TM, Silverman JF: Intraoperative cytologic diagnosis of suprasellar and sellar cystic lesions. Diagn Cytopathol 1999;20:137–147
8. Parwani AV, Taylor DC, Burger PC, Erozan YS, Olivi A, Ali SZ: Keratinized squamous cells in fine needle aspiration of the brain: Cytopathologic correlates and differential diagnosis. Acta Cytol 2003;47:325–331
9. Honegger J, Grabenbauer GG, Paulus W, Fahlbusch R: Regression of a large solid papillary craniopharyngioma following fractionated external radiotherapy. J Neurooncol 1999;41:261–266
10. Elmaci L, Kurtkaya-Yapicier O, Ekinci G, Sav A, Pamir MN, Vidal S, Kovacs K, Scheithauer BW: Metastatic papillary craniopharyngioma: Case study and study of tumor angiogenesis. Neurooncol 2002;4:123–128

Intraoperative Crush Cytology in the Diagnosis of Neurosurgical Cases



UNIVERSITI SAINS MALAYSIA

Short Term Grant No. 304/PPSP/6131316

Dr. Manoharan Madhavan
Associate Professor of Pathology
Department of Pathology
School of Medical Sciences
Universiti Sains Malaysia
16150, Kubang Kerian, Kelantan, Malaysia

CONTENTS

INTRODUCTION

Rapid diagnosis is essential for intraoperative management of surgical patients. It is primarily done for diagnosing tumours and differentiating it from tumour like conditions. The other role of rapid diagnosis is to ascertain the tumour clearance of the surgical margin.

Rapid diagnosis of surgical specimen is largely achieved by 'Frozen Section' (FS) study. However, FS study is hindered by some technical difficulties, especially when applied to neurosurgical specimens. It also needs expensive special equipment - cryostat. To overcome these problems, it can be complemented by intraoperative cytological examination. The cytology is a simple technique, very cheap, and yield faster report.

If intraoperative cytology is applied to neurosurgical cases, the diagnosis can be obtained in about 10 minutes and it will permit reliable intra operative guidance during lesion targeting and resection. Only in few centres, the cytological examination is practiced because of its variable accuracy. The accuracy of cytology is predominantly influenced by the nature of the lesion, method of sampling, method of sample preparation and finally the availability of expertise.

In Universiti Sains Malaysia (USM), the 'Neuroscience Department' is an established department and the surgery is performed regularly for the neurosurgical cases. However, an intra operative cytological diagnosis has not been practiced in hospital USM (HUSM) and so its accuracy is not known.

AIM

Objective:

To evaluate cytological diagnosis against the 'gold standard' histopathological diagnosis and find out its accuracy.

METHODOLOGY

Materials:
This is a prospective evaluation study involving 134 patients who underwent surgery for intracranial and intraspinal space occupying lesions in HUSM from November 2002 to October 2005.
Exclusion criteria: Patients underwent surgery for established cases of intracranial haemorrhage, aneurysm, and arteriovenous malformation (AVM).

Method:
Whenever surgery is undertaken for the neurosurgical cases, the primary researcher visits the operation theatre. The neurosurgeon will take out the samples from the suspected area. Usually, from each patient, only one sample will be taken out. If it is found to be non representative, further samples will be taken out.
From each sample, a small piece of tissue 0.5 – 2mm in diameter is placed at one end of a glass slide. The tissue is slightly crushed between with a second slide and is moved rapidly to smear the tissue on the first slide. After the smears are prepared, the slides are immediately immersed in universal fixative (95% ethyl alcohol) for 2 minutes.
For staining, the slides are immersed in water for 1 minute and stained in haematoxylin for 2 minutes. Then the slides are washed in running tap water for 1 minute and dipped once in liquor ammonia followed by washing in running tap water till the excess dye is washed off. After that step, the smears stained in eosin for 2 min and the excess dye is removed by two changes of absolute alcohol. Finally the smears are cleared in xylene and mounted in synthetic medium (DPX).
The primary researcher examines those slides, then and there and conveyed the diagnosis to the surgeons immediately. The remaining specimens are sent to the pathology laboratory for the 'Gold standard' histopathological examination. The on call pathologist, who is blind folded for the cytology diagnosis, reports the histopathology slides.
Finally, the cytological diagnoses are evaluated against the histopathological diagnosis and its accuracy is determined.

RESULT

One hundred and thirty-four patients were included in this study. Their age ranged from 1 year to 74 years (Fig. 1). Majority (97.8%) of the patients were Malay, while the remaining 2.1% were Chinese. Male patients (74) were more in number than the female patients (60). The male: female ratio was 1.2: 1.

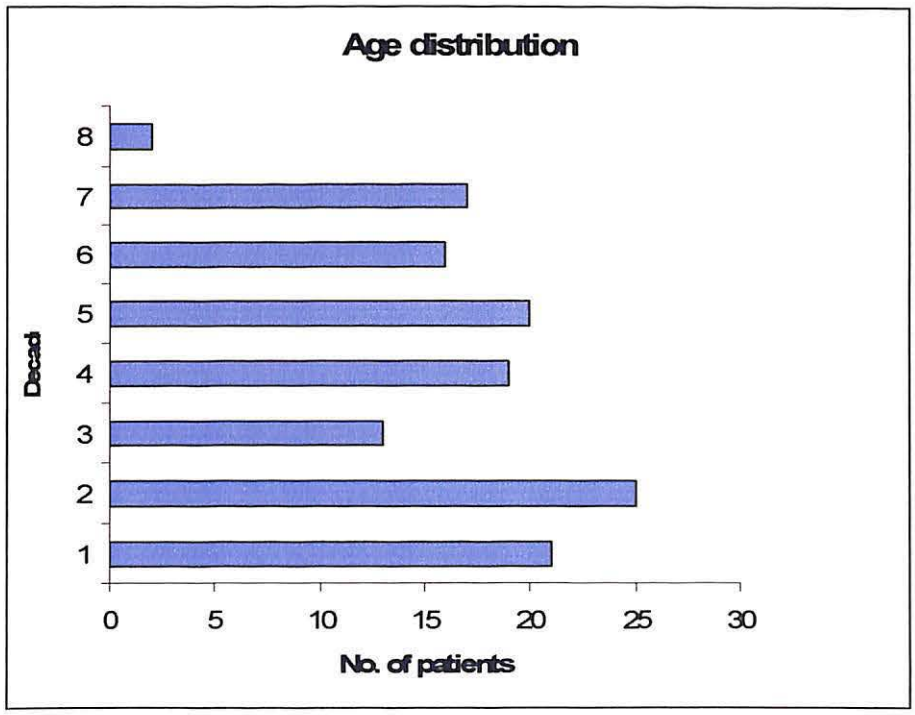


Fig. 1: Shows almost equal distribution of patients in relation to age.

Regarding topography of the lesions, majority (42.5%) were located in the cerebrum, followed by cerebellum (12%), sellar region (8.2%), spinal cord (7.5%) pineal gland (6.7%), ventricles (3%) and other sites. (Tab. 1)

Tab 1: Topography of the lesions found in 134 patients

S. No	Site of lesion	No. of cases (%)
1.	Cerebrum	57 (42.5%)
2.	Cerebellum	16 (12%)
3.	Sellar & supra sellar region	11 (8.2%)
4.	Spinal cord	10 (7.5%)
5.	Pineal gland	9 (6.7%)
6.	Ventricles	4 (3%)
7.	Others sites	27 (20.1%)
Total		134 (100%)

One hundred and eighty samples were taken from those one hundred and thirty four patients. From 88 patients, only one sample was obtained while two samples from 18 patients, three samples from six patients and four samples from four patients were taken. (Table. 2)

Tab 2: Number of samples obtained from each patient.

No. of patients	No. of samples	No. of samples obtained
102	1	104
22	2	44
6	3	18
4	4	16
Total		180

For those 180 cytology samples, the material for histopathology examination could be obtained only for 151 samples. Hence the cytology – histopathology correlation could not be made for the remaining 29 samples. (Tab. 3)

Tab. 3: Cytology results of those cases where material for HPE was unavailable.

Cytology diagnosis	No. of cases
Normal	15
Tumour	9
Necrosis	2
No opinion was possible	2
No cellular material	1
Total cases	29

Over all, complete correlation with the final diagnosis was found in 151 samples (83.9%). The histological diagnoses made in those 151 samples are shown in table 4.

Tab. 4: Histological diagnosis of 151 samples

Diagnosis	No. of cases
Meningioma	28
Normal	15
High grade glioma(III & IV)	15
Metastasis	13
Nerve sheath tumour	10
Medulloblastoma	7
Astrocytoma (grade II)	7
Pilocytic astrocytoma	6
Germinoma	5
Ependymoma	5
Pituitary adenoma	5
Necrosis	5
Oligodendroglioma	4
Abscess	2
Craniopharyngioma	2
Pineocytoma	2
Haemangioblastoma	2
Haemangioma	2
Immature teratoma	2
Cryptococcal infection	2
Inflammatory lesion	2
Tuberculoma	1
Choroid plexus papilloma	1
Colloid cyst	1
DNET	1
PNET	1
Chordoma	1
Chondroma	1
NHL	1
No tumour cells seen	1
No opinion possible	1
Total	151

Among the 151 histologically confirmed samples, 115 cytological diagnoses correlated well with the final diagnosis and thus the over all diagnostic accuracy of cytology was found to be 76.1%.

The common diagnostic entities included meningioma, normal, high grade glioma (grade III & IV), metastasis, nerve sheath tumour, medulloblastoma, astrocytoma (grade II), pilocytic astrocytoma, germinoma, ependymoma, pituitary adenoma, necrosis and oligodendroglioma (Fig. 1 to Fig. 6).

There were 15 cases of normal glial tissue diagnosed histologically. None of them were misdiagnosed in cytology. However, one case of infarction was misdiagnosed as normal glial tissue in cytology. Though its diagnostic accuracy was 100%, its positive predictive

value was only 93.75%. No false negative cases were encountered cytologically. Hence its negative predictive value was 100%

There were 28 cases of meningioma, out of which 26 were diagnosed correctly in cytology. Thus the diagnostic accuracy (92.9%) was very high for meningioma.

There were 15 cases of high grade glioma which included anaplastic astrocytoma and glioblastoma. Out of these 15 cases, nine cases were diagnosed cytologically. Among the remaining six cases, two were misdiagnosed as non Hodgkin lymphoma, other two were misdiagnosed as low grade astrocytoma and others were diagnosed as ependymoma and pineoblastoma. The diagnostic accuracy for high grade glioma was only 60%.

Metastasis was diagnosed histologically in 13 cases. Cytology revealed correct diagnosis in all but one case and thus its diagnostic accuracy was very high (92.3%). One of the gemistocytic astrocytoma and one of oligodendroglioma were misdiagnosed as metastasis. Hence its positive predictive value was 85.7%.

Histologically ten cases of nerve sheath tumours were diagnosed and all of them were diagnosed correctly in cytology also. Its diagnostic accuracy was the highest (100%). However, two cases of meningioma were misdiagnosed as nerve sheath tumour. Hence its positive predictive value is 83.3%.

The diagnostic accuracy appeared low (57.1%) for medulloblastoma. There were seven cases of medulloblastoma and only four cases were diagnosed cytologically.

On comparing high grade astrocytoma, the diagnostic accuracy appeared better (85.7%) for grade II tumours which included four cases of fibrillary astrocytoma and two cases of gemistocytic astrocytoma. Out of three cases of gemistocytic astrocytoma, one was diagnosed as metastasis.

Regarding pilocytic astrocytoma, there were six cases and only three were diagnosed under cytology examination. Two were diagnosed as fibrillary astrocytoma and one was suspected to be haemangioblastoma. So, its diagnostic accuracy was only 50%.

There were five cases of germinoma and all of them were diagnosed correctly under cytology. Hence its diagnostic accuracy was 100%. However, one case of medulloblastoma was wrongly diagnosed as germinoma under cytology. Hence its positive predictive value was 83.3%.

There were five cases of ependymoma and only one of them was correctly diagnosed. Others were misdiagnosed as low grade glioma and medulloblastoma. Two of the cases histologically diagnosed as medulloblastoma were misdiagnosed as ependymoma. Hence its diagnostic accuracy (20%) as well as positive predictive value is 71.4%.

There were five cases of pituitary adenoma and only one of them was misdiagnosed as non Hodgkin lymphoma. Hence, its diagnostic accuracy was 80%.

Five samples showed features of necrosis histologically. Four of them were diagnosed correctly under cytology while the remaining one was interpreted as normal glial tissue. Its diagnostic accuracy was 80%. Though samples from many of the tumours showed features of necrosis apart from tumour cells, the above mentioned four cases did not show any viable cells.

Four cases are diagnosed as oligodendroglioma histologically and three of them were diagnosed correctly under cytology. The remaining one case was incorrectly diagnosed as metastasis. Hence its diagnostic accuracy was 75%.

The common diagnoses and correlation between cytology and the final diagnosis are shown in table 5.

Tab. 5: Common diagnoses and correlation between cytology and the final diagnosis

Diagnosis	No. of cases	Correlation
Normal	15	100%
Nerve sheath tumour	10	100%
Germinoma	5	100%
Meningioma	28	92.9%
Metastasis	13	92.3%
Astrocytoma (grade II)	7	85.7%
Pituitary adenoma	5	80%
Necrosis	5	80%
Oligodendroglioma	4	75%
High grade glioma(III & IV)	15	60%
Medulloblastoma	7	57.1%
Pilocytic astrocytoma	6	50%
Ependymoma	5	20%

The less common entities included abscess, craniopharyngioma, pineocytoma, haemangioblastoma, haemangioma, immature teratoma, cryptococcal infection, and non specific inflammatory lesion and only two samples were available in each entity.

The very rare entities included tuberculoma, choroid plexus papilloma, colloid cyst, dysembryoplastic neuroepithelial Tumour (DNET), primitive neuroectodermal tumour (PNET), chordoma, chondroma and non Hodgkin lymphoma (NHL) and only one sample was available in each entity.
The misdiagnosed lesions are shown in table 6.

Tab. 6: Cytologically misdiagnosed lesions

Histopathology diagnosis	Number	Misdiagnosed as (Number)
High grade glioma (III & IV)	9	Low grade glioma (2) Non-Hodgkin lymphoma (2) Pineoblastoma (1) Ependymoma (1)
Medulloblastoma	7	Ependymoma (2) Germinoma (1)
Pilocytic astrocytoma	6	Fibrillary astrocytoma (2) Haemangioblastoma (1)
Ependymoma	5	Fibrillary astrocytoma (2) Medulloblastoma (2)
Haemangioma	2	Gliosis (2)
Haemangioblastoma	2	Benign spindle cell tumour (2)
Cryptococcal infection	2	Granulomatous lesion (1) Haemangioblastoma (1)

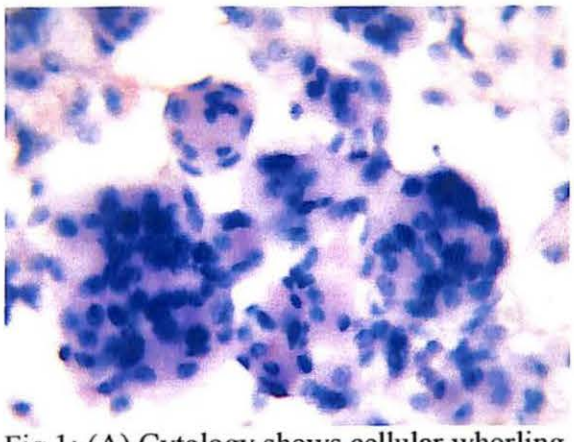
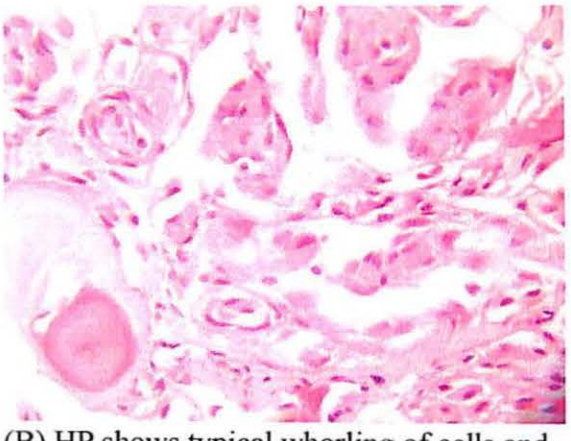


Fig.1: (A) Cytology shows cellular whorling (H&E X 40).



(B) HP shows typical whorling of cells and psammoma bodies (H&E X 10).

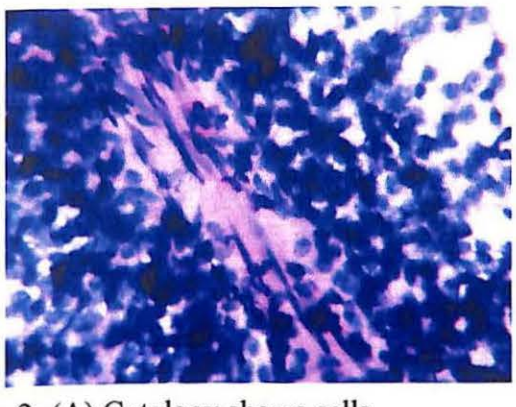
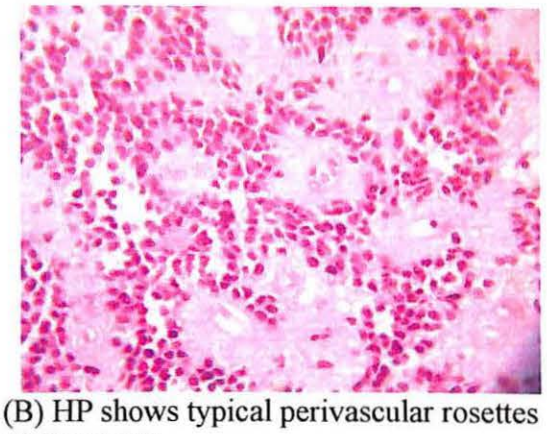


Fig.2: (A) Cytology shows cells concentrating around the vessels (H&E X 40).



(B) HP shows typical perivascular rosettes (H&E X 10).

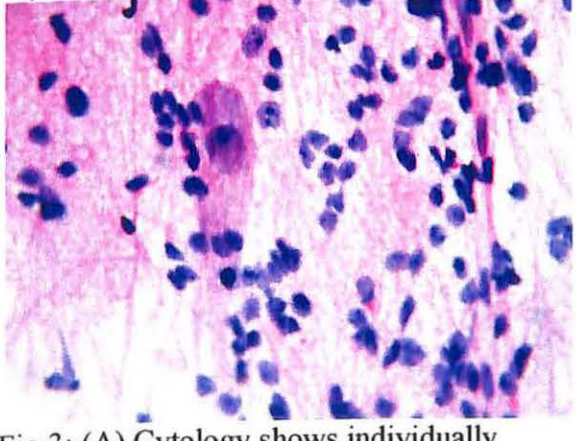
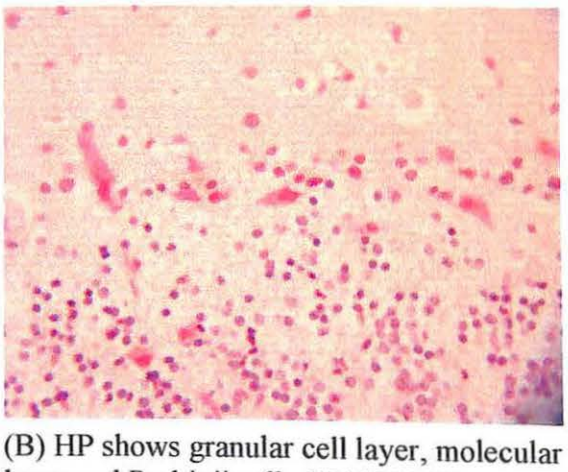


Fig.3: (A) Cytology shows individually dispersed mononuclear granular layer cells with occasional scattered Purkinji cells (H&E X 40).



(B) HP shows granular cell layer, molecular layer and Purkinji cells (H&E X 10).

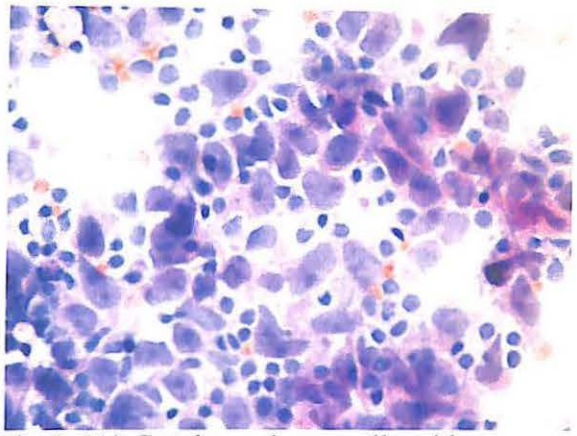
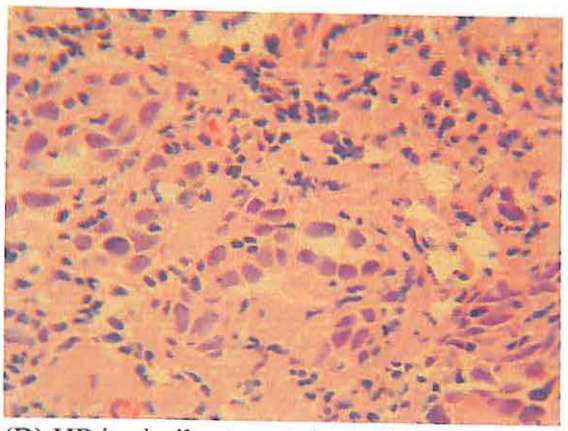


Fig.4: (A) Cytology shows cells with vesicular nuclei containing prominent nucleoli interspersed with lymphocytes (H&E X 40).



(B) HP is similar to cytology (H&E X 40).

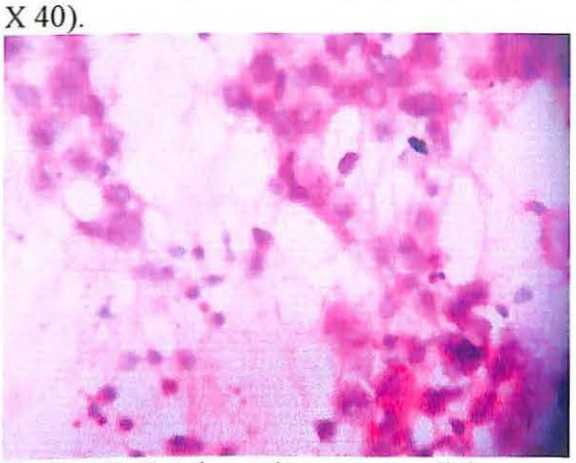
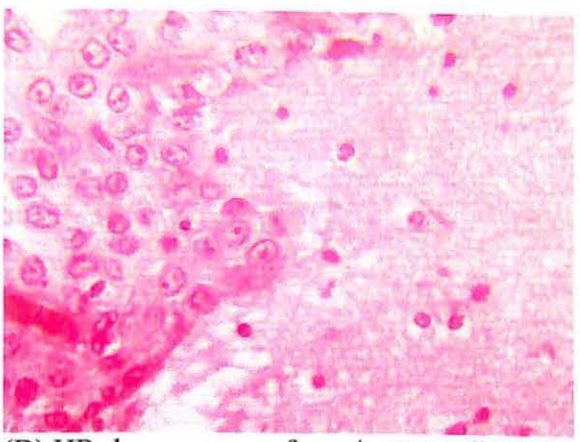


Fig.5: (A) Cytology shows neuroglial background and groups of polygonal cells with pleomorphic nuclei (H&E X 40).



(B) HP shows group of carcinoma cells infiltrating the glia (H&E X 40).

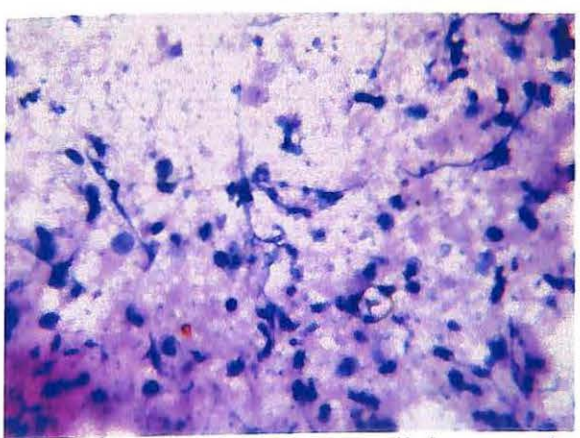
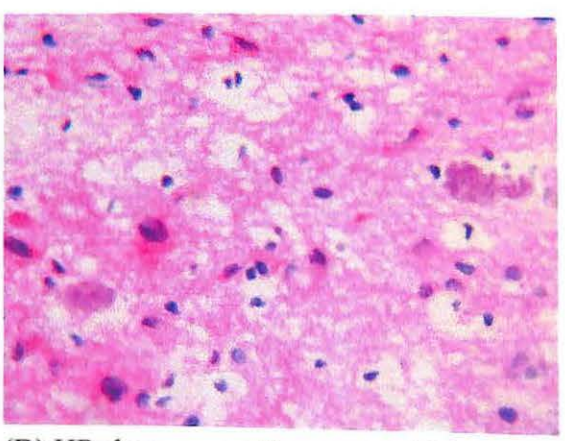


Fig.6: (A) Cytology shows acellular necrotic debris (H&E X 40).



(B) HP shows necrotic neurons (H&E X 40).

Discussion

The purpose of this prospective study was to determine the diagnostic accuracy of intraoperative crush cytology in neurosurgical procedures at a university hospital. In most of the samples, only one sample was taken. When the first sample showed normal glial tissue, further two to four samples were taken till a diagnostic area was obtained. In some cases, the neurosurgeon collected multiple samples, when the lesion appeared heterogeneous.

The cytological smears are prepared from the samples by different methods (Firik KS 1999). Touch preparation yields less cellularity and is found to be less accurate (Marshall LF et al 1973). In our study, the smears were prepared by crushing between two slides as described in other study (Karl R 2002). This method has advantageous in firm tissues like fibrous meningioma and nerve sheath tumours because, it yielded high cellularity. Even this method did not yield suitable smears in case of mesenchymal tumours such as haemangioma and chondroma.

The cytological smears are mostly stained by H&E stain, sometimes by toluidine blue and rarely by papanicoloau stain (Firlik KS1999, Slowinski J 1999). In our study, H&E stain was used, as this is routinely used in histopathology examination. This will enable the pathologist to appreciate the histomorphological features in cytology smears also.

Out of 180 samples processed for cytology, HP examination was not available for 29 samples. Because of small size, some of the samples got exhausted on making smears and could not be processed for HP examination. Of these 29 samples, about 50% were reported as normal and about 25% were reported as tumour. These cytology results helped the surgeon in deciding whether they are dealing with normal or pathological area. The true identities of the remaining 151 samples were ascertained by gold standard HP examination. It showed wide variety of lesions. Some of the lesions were common while others were rare as described in other studies. (Karl R 2002).

In various studies, the diagnostic accuracy of intraoperative cytology has been investigated. The accuracy of cytologic diagnosis as compared to the final histological diagnosis ranges from 76% to 94% (Marshall LF et al 1973, Slowinski J 1999, Asha T1989, Berkeley BB 1978, Firlik KS 1999, Bleggi-Torres LF, Karl R 2002). In our study, the overall accuracy was on lower side i.e., 76.1%. This low accuracy could be multifactorial. First of all, the cytological diagnoses were made solely based on morphological features. The clinicoradiological features were deliberately avoided by the pathologist to assess how much the cytology features alone are helpful in arriving at a diagnosis. The next deterring factor could be the samples being non representative. Some specimens like mesenchymal tumours are hard to crush and get monolayer sheets of cells. This hinders proper evaluation of smears and arriving at a correct diagnosis. Firlik KS (1999) has also reported 100% inaccuracy in vascular malformations. Moreover, certain tumours like ependymoma, non Hodgkin lymphoma and medulloblastoma may have similar looking cytology features. Similar result was obtained in other study too (Karl R 2002). Lastly, the pathologist has a limited experience in cytology of neurosurgical specimens. This study was conducted over a period of 2 years, while other studies conducted over 15 years to 19 years (Karl R 2002, Firlik KS 1999).

The cytological diagnosis was immediately made known to the surgeon while the HP result took considerable time to reach the surgeon. Hence, the intraoperative diagnosis was found very useful to the operating surgeon.

CONCLUSION

Intraoperative cytologic smears in neurosurgery are easy to perform and inexpensive and permit reasonably high diagnostic accuracy.

Even very tiny specimens, especially of soft consistency are suitable for this technique, which is extremely important in operations of tumours localized in functionally important brain areas.

The cytological diagnosis could be made based on the presence of characteristic cytomorophological features. Very high accuracy can be achieved in certain tumours like nerve sheath tumour, germinoma, meningioma, metastasis, grade II astrocytoma, pituitary adenoma and oligodendroglioma. However, the accuracy is low in high grade glioma, medulloblastoma, and ependymoma. In the absence of their diagnostic cytomorophological features, the correlation with clinicoradiological features might help in arriving at a correct diagnosis and increase their diagnostic accuracy.

Further studies involving more number of cases is needed to find out the value of crush cytology in diagnosing the rare tumours and inflammatory lesions.

In cytology examination, presence of pure normal glial tissue has high negative predictive value. This helps in guidance during targeting the lesions including stereotactic biopsy and also during the resection of infiltrative lesions for definition of tumour margins.

References

Asha T, Shankar SK, Rao TV, Das S. Role of squash-smear technique for rapid diagnosis of neurosurgical biopsies-a cytomorphological evaluation. Indian J Pathol Microbiol 1989;32:152-60

Berkeley BB, Adams JH, Doyle D, Graham DI, Harper CG. The smear technique in the diagnosis of neurosurgical biopsies. N Z Med J 1978;87:12-5

Bleggi-Torres LF, de Noronha L, Schneider Gugelmin E, Martins Sebastiao AP, Werner B, Marques Maggio E, Queiroz Telles JE, Martins Collaco L. Accuracy of the smear technique in the cytological diagnosis of 650 lesions of the central nervous system. Diagn Cytopathol 2001;24:293-5

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;91:454-8

Karl R, Wolfgang D, and Klaus K. High Diagnostic Accuracy of Cytologic Smears of Central Nervous System Tumors – A 15 year Experience Based on 4,172 Patients. Acta Cytol 2002;46:667-674

Marshall LF, Adams H, Doyle D, Graham DI. The histological accuracy of the smear technique for neurosurgical biopsies J Neurosurg 1973;39:82-8.

Slowinski J, Harabin-Slowinska M, Mrowka R. Smear technique in the intra-operative brain tumor diagnosis: its advantages and limitations Neurol Res 1999;21:121-4

Publication:

M. Madhavan, Jain GP., Jaffri MA, Zamzuri I. 'Intraventricular Squamous Papillary Craniopharyngioma: report of a case with intraoperative imprint cytology' Acta Cytol 2005: 49; 431 – 434.

Acknowledgement:

- USM short term grant 304/PPSP/6131316
- All the neurosurgeons who helped me providing the samples for cytology examination.
- All the pathologists who reported the neurosurgical samples.