A STUDY ON THE RELIABILITY OF PER OPERATIVE FROZEN / SMEAR EXAMINATION IN COMPARISON TO THE FINAL HISTOPATHOLOGICAL EXAMINATION OF NEUROSURGICAL LESIONS

PROJECT REPORT

SUBMITTED FOR M.Ch NEUROSURGERY

by

Dr. Venkata Srinivasa Rao Nooti

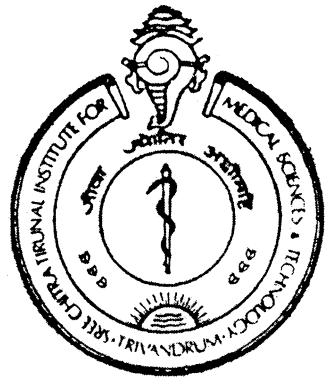
November 2005

DEPARTMENT of NEUROSURGERY

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

TRIVANDRUM-695011

PROJECT REPORT



SUBMITTED FOR M.Ch NEUROSURGERY

by

Dr. Venkata Srinivasa Rao Nooti

November 2005

DEPARTMENT of NEUROSURGERY

SREE CHITRA TIRUNAL INSTITUTE
FOR
MEDICAL SCIENCES AND TECHNOLOGY

TRIVANDRUM-695011

PROJECT TITLE

A STUDY ON THE RELIABILITY OF PER OPERATIVE FROZEN / SMEAR EXAMINATION IN COMPARISON TO THE FINAL HISTOPATHOLOGICAL EXAMINATION OF NEUROSURGICAL LESIONS

Name : Dr. Venkata Srinivasa Rao Nooti

Programme : M.Ch. Neurosurgery (3 years)

Month & Year of Submission : November 2005

CERTIFICATE

I, Dr. Venkata Srinivasa Rao Nooti hereby declare that I have actually performed or assisted all the procedures listed under the report.

Place : Thiruvanathapuram

Date : 05:11:2005

Venkata Srinivasa Rao Nooti

Forwarded :

Dr. Venkata Srinivasa Rao Nooti has carried out the minimum requirement of procedures/etc.

Dr. R.N.Bhattacharya.
Professor & Head,
Department of Neurosurgery,
SCTIMST, TVM – 11.

ACKNOWLEDGEMENT

At the outset I would like to offer my prayers to "Lord Ganesha" without whose blessings this work would have never been accomplished.

I am grateful to Dr. K. Mohandas Director of the Institute, for giving permission to carry out this work and for all the institutional help required.

I sincerely thank Prof. R.N. Bhattacharya, Head of Department Neurosurgery for his faith in me. This dissertation work would never have been possible without his active guidance and support.

I am deeply indepted to Prof. V.V. Radhakrishnan for his fatherly guidance and the support extended by him to carry out this work smoothly and efficiently.

I am also deeply indebted to Prof Suresh Nair for his valuable counsel during my Neurosurgical training and during the period of this study.

I also wish to place on the record my immense gratitude to Dr. Ravi Mohan Rao & Dr Girish Menon who has always been a constant source of inspiration to me.

I am grateful to Dr Rajesh B.J. for his painstaking effort of going through the work and giving this dissertation a final meaningful shape.

I am thankful to all other faculty members in my department namely Dr. Mathew Abraham, Dr. Muthuretanam T and Dr Easwer H.V for all the support and guidance.

I cannot forget the help and directions provided by Dr Mukund Prasad who has been more than a friend to me.

I also like to mention my sincere thanks to all my colleagues, friends and juniors who had helped me to accomplish this project.

I also wish to thank all the staff of Neurosurgery and Pathology personnel for all the required help and support.

I remember with reverence my parents who were coonstat source of inspiration in my neurosurgical venture. I also remember with love, and thank my wife Dr. Ramani for her constant support and above all the Neurosurgical patients for making the journey worth while.

(Dr. Venkata Srinivasa Rao Nooti)

CONTENTS

INTRODUCTION

Despite considerable advances in neuroimaging it is still not possible to accurately diagnose the histological nature of the disease in the pre operative period in some cases. These are some of the issues, which concern the neurosurgeon and necessitate the need for seeking a preliminary intraoperative histological diagnosis and the main indications for this are as follows:

1. Where knowledge of the precise nature of a tumor will affect the surgeon's intra operative management during a definitive surgical procedure. This often involves making a decision on how radical an excision should be attempted.

2. Where the object of the surgical procedure is to obtain tissue for a definitive paraffin section diagnosis. This is often in the context of a free hand or a guided needle biopsy. The intra operative result safeguards the surgeon against being in the wrong location, or inadvertently sampling purely necrotic or reactive tissue associated with the lesion. The main point of exercise lies in achieving a definitive histological diagnosis rather than altering immediate surgical management. It is therefore worth noting that the smear or frozen section in these circumstances does not necessarily have to give a precise diagnosis in its own right,

merely the information that the tissue sampled is likely to prove diagnostic in paraffin sections.

3. Where a radical excision is being performed on a tumor of known diagnosis & the surgeon needs help in defining the margins of the lesion with adjacent brain tissue. The diagnosis may have been confirmed in a previous biopsy or by a sample taken earlier in the same procedure. This is usually more relevant to infiltrating gliomas, where the margins of solid tumor with more diffusely involved brain are not obvious macroscopically, or where the surgical activity is close to eloquent areas of central nervous tissue, which are still clinically functional.

The surgeon also needs to know, how well the intra operative tissue diagnosis correlates with the final histological diagnosis. The importance of this correlation arises only in few occasions, such as when a surgeon is in doubt regarding the nature of the lesion and the decision regarding the extent of excision is being made by intra operative tissue diagnosis, while the final diagnosis may be totally different.

In this study we have analyzed the accuracy of intra operative diagnosis, and it's correlation with the permanent paraffin section diagnosis.

OBJECTIVES:

1. To assess the accuracy of intra operative tissue diagnosis using smear and frozen section.

2. To evaluate the disparity between intra operative and final histopathological diagnosis and analyze the reasons for disparity.

3. To assess the accuracy of intraoperative grading of gliomas.

MATERIAL AND METHODS

The present study has been carried out on patients who have undergone routine neurological surgery between the period of 1st July 2003 & 31st December 2003 and where tissue was sent for intra operative diagnosis. The relevant clinical and radiological data were provided to the pathologist whenever requested. All the slides where there was discrepancy between the intra operative and permanent paraffin sections where reviewed. Gliomas were graded based on the WHO (1993) grading system for gliomas. The intra operative histological diagnosis was made either on smear preparation or on frozen section. The smear preparation is where the tissue is squashed with little pressure and a smear is made, where as a frozen section is one, where the tissue is frozen and cut and made into a smear. The definitive histopathological diagnosis is made on paraffin fixed sections. The time taken for Smear technique is 5-6minutes and for Frozen section is 10-15minutes.

Smear preparation:

It is ideal for soft tissues especially in gliomas and metastatic carcinomas where the tissue is soft in consistency and in stereotactic biopsy where the amount of tissue is limited.

Method:

In this method a pinhead sized soft tissue is placed in a clean microscopic slide. The tissue is gently squashed with minimum pressure and drawn towards the edge of the slide, evenly with another clean slide. Then the slide is fixed in 95% ethanol for 30seconds. A drop of stain (toludine blue 0.5-1% in 70% alcohol) is applied for 15seconds. The slide is washed well with tap water. Then the slide is examined. Nuclei will stain Blue in color; Metachromatic granules and Mucin will stain shades of pink. The same fixed smear can be stained by rapid Hematoxylin and eosin stain.

Hematoxylin–Eosin Stain: Two drops of Hematoxylin stain is poured on the fixed smear for one minute. The slide is washed in tap water and gently dipped in 0.05% ammonia water, to blue the nucleus. The slide is washed well and a drop of alcoholic Eosin (0.25-0.5) is added. The slide is washed again and dehydrated in alcohol. The slide is dipped in Xylene and mounted with DPX and examined under microscope.

The result is read as: Nucleus - Blue, Cytoplasm and other organelles – Shades of pink

The smear preparation has its own advantages and limitations.

The main advantages are:

1) Speed: results are available much more quickly than with the cryostat. This can save valuable time when repeated intraoperative samples are needed, for example to guide the surgeon to the correct location, or to give information on the margins of infiltrating tumor.

2) Ease of preparation: Creating and staining smear preparations are easy skills to acquire and results can be of consistently high quality even in relatively inexperienced hands. If so desired the surgeons can smear the tissues themselves, thus ensuring that the areas of interest down the operating microscope are the ones that are examined by the pathologist. For, out -of –hour's cases, most pathologists can quickly prepare and stain the smears without the need to call in a laboratory scientist.

3) Technical simplicity: No specialized equipment is needed and smears can be prepared and stained easily in a small area using only an extraction cabinet. If main pathology

laboratory is some distance away, this makes it easy to set up a diagnostic outstation within the operating suite, to facilitate more direct communication with the surgeon at the time of surgery.

4) Cytological preservation: smears give a high quality of cytological information in comparison with that available from cryostat sections.

5) Small sample size: Large areas of smeared tissue can be examined from a very small sample, thus maximizing the information available when there are technical limitations on the size of the biopsy. This is especially important when stereotactic procedures are being used.

The important limitations of this technique include:

I. It relies on the tissue or the lesion being soft enough to smear out satisfactorily. This is most often the case for central nervous lesions, but by no means always so. Even intrinsic tumors can become too tough to smear if they have undergone desmoplasia and some types of glial tumors including subependymomas, can make very poor smears simply because they are naturally very dense, fibrillary lesions.

II. The currently available experience of interpreting smear preparations is very largely confined to lesions of central nervous tissue and its immediate coverings, with a few notable exceptions such as schwannomas, lymphomas and some intracerebral metastasis. In consequence it is probably better to plan a frozen section from the outset for tumors such as extradural tumors of the skull base and spine.

III. Whilst smear preparations give excellent cytological details, much of the familiar histological architecture of tumor is not apparent. Examples include typical vascular patterns, tumor cell palisades and most type of rosettes.

Frozen section technique:

Terry in 1928 examined thick sections of organs cut by free hand with a sharp knife. This technique is now superseded by frozen section technique (Pearse 1960, Hollands 1962)

Principle: Most sectional methods are based upon the conversion of the tissue in to a uniform consistency suitable for thin sectioning, by altering the initial consistency by freezing or infiltrating with embedding medias such as wax or celloidin.

In frozen section the water contents of the tissue is allowed to freeze in a cooling cabinet at a temperature of -15 degree to –20 degree, so that the tissue attains a thicker consistency and can be cut into thin sections.

The advantages of this technique are:

1. It gives a quick diagnosis.
2. It will demonstrate lipids, which may be destroyed in the routine processing.
3. In immuno histochemistry for demonstration of enzymes.
4. Better penetration of dyes and in fluorescent antibody techniques

The disadvantages of this technique are:

1. Experience and skill necessary for reporting.
2. Permanent sectioning and preservation of slides inadequate as the stain will fade.
3. Thin sectioning is not possible.
4. Serial sectioning and sampling is not possible.

5. Differential diagnosis for metastatic tumors and poorly differentiated tumors is poor.

6. Special staining techniques yield poor quality of staining.

Procedure and Method:

Sampled specimen may be mounted on the stage of the cryostat (a cold chamber with inbuilt microtome. Temperature is at –15degrees to –20degrees in the chuck, using a O.C.T. (optimum cool temperature) compound (gum like material). When the specimen attains the cooling temperature (2-3minutes), it is cut into sections (5-8micron thickness). Pick up the section in clean micro slides attaching the slides to the section. The slide is fixed in 95% alcohol for 30 seconds. The slide is stained with alcoholic Toludine Blue for 10 seconds. The slide is washed and cleaned and mounted. The balance tissue can be subjected to routine paraffin processing for comparison and confirming the frozen diagnosis.

Paraffin processing of tissue samples:

Principle: Tissue bits are converted to higher consistency by impregnating with supporting media (Paraffin wax) and subsequently cutting with microtome to yield uniform and thin sections.(3-5micron)

Procedure: The paraffin section preparation involves fixation, processing, cleaning, and impregnation, blocking, cutting in microtome and staining.

The first step is Fixation. The tissue fixed in 10% buffered formaline for 2-12 hours- depending on the size of the tumor. Tissue can be stored in fixative until processed. Then the tissue is grossly examined and representative blocks are taken and are loaded in the Histokinnete for processing. The next step is processing. This is done in Histokinnete. The tissue is dehydrated by immersing in Ethanol (C2H5-OH). It is immersed in dilute alcohol of strength 50%, 70% and 90% for one hour each. Then in 100% absolute alcohol in three chambers, one hour in first chamber, one hour thirty minutes in second chamber and two hours in third chamber. Then the tissue is cleaned of the alcohol with a clearing agent, which is miscible with paraffin. It is immersed in two chambers of chloroform, first for one hour thirty minutes and the second for two hours. Then the tissue is impregnated with molten paraffin wax (Wax at 58-60degrees). It is immersed in two chambers containing molten paraffin wax for one hour and one and a half each. Up to this stage, the procedure is done in Histokinnete.

The next step is blocking with molten paraffin wax and allowed to cool. The tissue is cut in microtome with thickness of sections 3-5microns. Then the sections are stained. The staining method used is routine Hematoxylin and eosin stain.

Routine Hematoxylin and eosin stain:

The sections are kept in the oven at 60 degrees for 30minutes. The sections are then transferred to xylene to deparaffinize, 2 changes of 5minutes are made. Then the slides are hydrated sequentially with 100%alcohol, 70%alcohol and 50% alcohol. Slides are washed under tap water for 2 minutes. Then the slides are transferred to Hematoxylin stain for 8min and washed in tap water. The slides are differentiated in 1% acid alcohol. (70%alcohol- 100ml, con Hcl 1ml) and washed in tap water. Then the bluing of the slides is done with dilute NH4OH solution. The slides are again washed in tap water for 5minutes. The slides are dipped in absolute alcohol and in alcoholic Eosin (0.5%) for 30seconds. The slides are washed, dehydrated in alcohol and mounted with DPX (Dipthalate xylene)

The result is read as, Nucleus-Blue, Cytoplasmic elements – Shades of pink.

REVIEW OF LITERATURE

Even when there is time to apply routine techniques, pathologist's final report may sometimes have to be a matter of opinion rather than a single unarguable conclusion. The comment made from urgent intraoperative diagnosis is more often likely to fall short of a single confident diagnosis, which can be easily audited against the final paraffin section. Despite its speed and simplicity, the technique of rapid intra operative tissue diagnosis clearly lacks many of the advantages that a paraffin section can confer, and for this reason should always be regarded as an interim measure pending more definitive information. Urgent cryostat histology deprives the pathologist of many of the advantages of routine paraffin methods, including special stains and immuno histochemistry. Moreover the pathologist's assessment of an intra operative tissue diagnosis, stem's not just from interpretation of the cytology or cryostat histology down the microscope, but relies heavily on the subjective appraisal of the clinical and radiological details in each individual case. All these observations need to be borne in mind when attempting to judge the diagnostic accuracy of rapid intra operative preparations. It should be remembered that it is possible to provide useful

The next step is blocking with molten paraffin wax and allowed to cool. The tissue is cut in microtome with thickness of sections 3-5microns. Then the sections are stained. The staining method used is routine Hematoxylin and eosin stain.

Routine Hematoxylin and eosin stain:

The sections are kept in the oven at 60 degrees for 30minutes. The sections are then transferred to xylene to deparaffinize, 2 changes of 5minutes are made. Then the slides are hydrated sequentially with 100%alcohol, 70%alcohol and 50% alcohol. Slides are washed under tap water for 2 minutes. Then the slides are transferred to Hematoxylin stain for 8min and washed in tap water. The slides are differentiated in 1% acid alcohol. (70%alcohol- 100ml, con Hcl 1ml) and washed in tap water. Then the bluing of the slides is done with dilute NH4OH solution. The slides are again washed in tap water for 5minutes. The slides are dipped in absolute alcohol and in alcoholic Eosin (0.5%) for 30seconds. The slides are washed, dehydrated in alcohol and mounted with DPX (Dipthalate xylene)

The result is read as, Nucleus-Blue, Cytoplasmic elements – Shades of pink.

REVIEW OF LITERATURE

Even when there is time to apply routine techniques, pathologist's final report may sometimes have to be a matter of opinion rather than a single unarguable conclusion. The comment made from urgent intraoperative diagnosis is more often likely to fall short of a single confident diagnosis, which can be easily audited against the final paraffin section. Despite its speed and simplicity, the technique of rapid intra operative tissue diagnosis clearly lacks many of the advantages that a paraffin section can confer, and for this reason should always be regarded as an interim measure pending more definitive information. Urgent cryostat histology deprives the pathologist of many of the advantages of routine paraffin methods, including special stains and immuno histochemistry. Moreover the pathologist's assessment of an intra operative tissue diagnosis, stem's not just from interpretation of the cytology or cryostat histology down the microscope, but relies heavily on the subjective appraisal of the clinical and radiological details in each individual case. All these observations need to be borne in mind when attempting to judge the diagnostic accuracy of rapid intra operative preparations. It should be remembered that it is possible to provide useful

information without knowing the precise diagnosis, and from the surgeon's point of view, successful intraoperative comment does not necessarily have to be diagnostic or even totally conclusive.

The frozen section technique is not free of artifacts. Artifact specific to frozen sections include, ice artifact due to slow freezing of the tissue, which allows ice crystals to form. Ice artifact can severely distort the morphology of cells & introduce vacuoles into the tissue section rendering it useless for diagnostic purposes. This form of artifact is avoided by adopting a method of rapid freezing of the tissue, which are not too large to prevent rapid freezing. Other problems specific to frozen sections mainly occur during cutting the sections with the microtome: for example chattering of the blade produces parallel lines in the section & the thickness of the section may be some what variable.

The assessment of the cytological features is also difficult, as many different histological conditions present with similar features.

The cytological appearance of low-grade astrocytoma (WHO grade II) is given below.

1. Smear easily, clumps of cells seen by naked eye.

2. Low to moderate cellularity.

3. Irregular clusters with ill-defined margins, some loosely arranged around blood vessel forming a vague papillary pattern.

4. Variable nuclear appearances with Cytoplasmic glial processes.

5. Individual cells seen in background.

6. Thin-walled blood vessels.

7. No mitoses, vascular proliferation, tumor necrosis or foamy histiocytes.

The differential diagnosis of low grade astrocytoma include:

a) *Normal white matter:* The normal white matter is very soft and, when smeared, it spreads out evenly into a monolayer sheet. The overall cellularity is lower than that of gliomas or gliosis, and the cell processes of normal glia are inconspicuous.

b) *Reactive gliosis*: Astrocytes in reactive gliosis usually have numerous highly branched cytoplasmic processes. The presence of foamy histiocytes usually indicates reactive process. However, they can be encountered in high-grade tumors such as

glioblastomas, but, in such circumstances, neoplastic cells are obviously malignant. Micro cysts seen on frozen section favor gliomas rather than gliosis.

c) *Pilocytic astrocytoma (PA)*: Long hair-like bipolar processes of PAs, although characteristic, may mimic diffuse astrocytoma. The age of patient, location of tumor and radiologic features raise a concern for a PA. The presence of Rosenthal fibers and/or eosinophilic granular bodies suggests low-grade lesions.

d) *Oligodendroglioma*: Oligodendrogliomas usually smear out into a thin film. Cytoplasmic processes are less distinct, and numerous branching capillaries and calcification are characteristic.

e) *Ependymoma*: Perivascular pseudorosettes of ependymomas seen as papillary structures on smear are better organized.

The appearance of Anaplastic Astrocytoma (WHO grade III) on smear preparations include:

1. Smear easily, clumps of cells seen by naked eye.
2. Increased nuclear pleomorphism and cellularity as compared to low grade diffuse astrocytoma.

3. Presence of mitotic figures.

4. No necrosis or vascular proliferation.

The Differential diagnosis of Anaplastic astrocytoma are:

a) *Pilocytic Astrocytoma*

b) *Glioblastoma*

c) *Anaplastic Oligodendroglioma*: Cytoplasmic processes of tumor cells in oligodendroglial neoplasms are less prominent, and the tumor usually contains calcification, as well as capillaries with extensive branching.

d) *Progressive multifocal leukoencephalopathy* (PML): Bizarre astrocytes can be observed in PML, but overall the cellularity is much lower than that of anaplastic astrocytomas or glioblastomas. The presence of foamy histiocytes and enlarged nuclei of oligodendrocyte favors PML.

The cytological appearance 0f Glioblastoma (WHO grade IV) are:

1. Smear easily, clumps of cells seen by naked eye.

2. Resemble anaplastic astrocytoma plus vascular proliferation

and/or tumor necrosis. Nevertheless, it should be noted that necrosis could be seen in many other neoplastic and non-neoplastic conditions; therefore, its presence does not always indicate the glioblastoma.

The differential diagnoses of glioblastoma are:

a) *Anaplastic astrocytoma:* Anaplastic astrocytomas are different from Glioblastomas because of their absence of vascular proliferation and necrosis. It should be emphasized however that normal large vessels should not be misinterpreted for vascular proliferation, and scattered protein-rich fluid should not be mistaken for necrosis. When in doubt, it is safer to call the lesion 'diffuse astrocytoma at least grade III or high grade diffuse astrocytoma' awaiting permanent section.

b) *Anaplastic Oligodendroglioma*

c) *Pleomorphic xanthoastrocytoma (PXA)*: As the name implies, pleomorphic tumor cells are the feature of PXA, but mitoses are rare or absent, as opposed to high-grade lesions. The presence of eosinophilic granular bodies suggests low-grade tumors.

d) *Subependymal giant cell astrocytoma (SGCA)*: Given marked

nuclear pleomorphism of tumor cells in the SGCA, mitotic figures are rare. Clinical features should be taken into account.

e) *Pilocytic astrocytoma (PA)*: PAs may show nuclear pleomorphism and contains vascular proliferation, but the overall cellularity and mitotic index are low. The presence of Rosenthal fibers or eosinophilic granular bodies favors low-grade lesions.

f) *Lymphoma*: Lymphoma cells may be admixed with reactive astrocytes. Care should be taken to determine which part of the lesion is malignant.

g) Poorly differentiated neoplasm: In poorly differentiated areas of glioblastomas, tumor cells may lack cytoplasmic processes; therefore, it is difficult to distinguish from other poorly differentiated tumors. If a better-differentiated area with distinct glial processes is entirely absent, the definite diagnosis should be deferred. Necrosis can be found in abscesses. Numerous neutrophils admixed with reactive astrocytes point to an inflammatory process.

The cytological appearance of Pilocytic Astrocytoma (WHO grade I) include:

1. Smear easily, clumps of cells seen by naked eye

2. Well defined clusters of cells with irregular margin and perivascular arrangement

3. Round or elongated nuclei with bipolar long hair-like processes

4. Rare mitoses and no necrosis

5. Nuclear pleomorphism may be encountered.

6. Rosenthal fibers

7. Eosinophilic granular bodies

8. Vascular proliferation (no prognostic significance)

The Differential diagnoses of Pilocytic astrocytomas are:

a) *Diffuse astrocytoma*

b) *Long-standing reactive gliosis* :In long-standing reactive gliosis as in the brain tissue adjacent to craniopharyngiomas or hamangioblastomas, there may be numerous Rosenthal fibers, which should not be mistaken for the pilocytic astrocytoma.

The cytological appearances of Pleomorphic Xanthoastrocytoma (WHO grade II) are :

1. Smear easily, clumps of cells seen by naked eye
2. Strikingly pleomorphic astrocytes with round, elongated, irregular nuclei
3. Astrocytic processes containing lipid droplets
4. Eosinophilic granular bodies
5. Lymphocytic infiltrates are common.
6. No mitoses, vascular proliferation or necrosis

The Differential diagnoses of Pleomorphic anthoastrocytoma are *Glioblastoma* and *Ganglioglioma*

The cytological appearance of Subependymal giant cell astrocytoma (WHO grade are:

1. Tend to be tough, clumps of cells seen by naked eye
2. Large astrocytes with abundant cytoplasm and cell processes
3. Large neuron-like cells with prominent nucleoli
4. Small astrocytic tumor cells
5. Calcospherites.
6. Absence or few mitoses
7. No vascular proliferation or necrosis

The differential diagnosis of Subependymal giant cell astrocytoma are

a. *Glioblastoma,*

b. *Ganglioglioma*

It should be noted that it is difficult to diagnose SGCA on the basis of smear alone with no clinical and radiological findings.

The cytological features of Oligodendroglioma (WHO grade II) are:

1. Smear easily into thin film, may show a gritty sensation of calcification
2. Monolayer sheets of uniform round tumor cells with speckle chromatin, indistinct nucleoli and inconspicuous cell processes
3. Fine branching capillary network with perivascular aggregates of tumor cells
4. Calcification
5. Absence of mitoses and necrosis

The differential diagnoses of Oligodendroglioma are:

a) *Diffuse astrocytoma*

b) *Pituitary adenoma*: A thin film of smear is also the characteristic feature of pituitary adenomas. However, under the microscope, adenoma cells have a tendency to form sheets and cohesive clusters. Blood vessels in pituitary adenomas are usually thicker and do not show extensive branching.

c) *Central neurocytoma:*It is difficult to distinguish an oligodendroglioma from a central neurocytoma even on the basis of paraffin section stained with H&E. Clinical features, especially the location of the tumor, point to a correct diagnosis.

d) *Dysembryoplastic neuroepithelial tumor (DNT)*

The cytological appearance of Anaplastic Oligodendroglioma (WHO grade III) is:

1. Smear easily, clumps of cells seen by naked eye, and may show a gritty sensation of calcification
2. Clusters of tumor cells with nuclear pleomorphism and scant cytoplasmic processes
3. Fine branching capillary network with perivascular aggregates of tumor cells

4. Calcification

5. Presence of mitoses and vascular proliferation

6. Necrosis can be seen.

The differential diagnoses of anaplastic oligodendroglioma include:

a) *Anaplastic astrocytoma*

b) *Glioblastoma*

The cytological features of Ependymoma (WHO grade II) are:

1. Organized papillae around vascular cores formed by several layers of tumor cells with fibrillary zone between cells and the walls of blood vessels

2. Fibrillary processes at the edge of cell clusters

3. Nuclei are rounder and larger than those of astrocytomas.

4. Ependymal rosettes are rarely seen.

The differential diagnoses of ependymoma are:

a) *Diffuse astrocytoma*

b) *Choroid plexus tumor*: In choroid plexus tumor the

neoplastic cells do not have cell processes.

The cytological appearance of Primitive Neuroectodermal Tumor (WHO grade IV) are:

1. Soft, easy to smear into thin 'blue' film
   ii. Monolayer sheets of oval or carrot-shaped nuclei with finely granular chromatin, indistinct nucleoli, and no discernable cytoplasm
2. Nuclear molding is common.
3. Frequent mitoses
4. Homer Wright rosettes, necrosis, neuronal or astrocytic differentiation may be seen.
5. Varying appearances of blood vessels (thin to hyperplastic)
6. Scattered reactive astrocytes can be encountered.

The Differential diagnoses of Primitive Neuroectodermal Tumor are

a) *Normal granular cell layer of cerebellum*: The normal granular cells are smaller and regularly round, and show no mitotic activity.

b) *Metastatic Carcinoma especially small cell carcinoma (SCC)*: SCCs can be easily confused with the PNET group. Clinical features including the age of patient and the location of tumor should be considered.

c) *Neurocytoma*: Tumor cells in neurocytomas are rounder and more uniform; the chromatin is evenly distributed and more delicate. Mitotic figures are rare.

d) *Glioblastoma*: Abundant glial processes point towards glial neoplasms rather than PNETs.

e) *Lymphoma*: Lymphoma cells usually have prominent nucleoli and discernable cytoplasm.

The cytological appearance of Central Neurocytoma (WHO grade I) are :

1. Soft, easy to smear into thin film.
2. Monolayer sheets of tumor cells with no cohesion, embedding in fine fibrillary background.
3. Uniform round nuclei with diffuse granular chromatin and little cytoplasm.
4. Fine branching blood vessels

5. Calcification

6. Rare mitoses

7. Scattered reactive astrocytes may be found.

The Differential diagnoses of Central Neurocytoma are:

a) *Oligodendroglioma*

b) *Pituitary adenoma* Smears of pituitary adenomas and central neurocytomas are very similar. The location of tumor should be considered. The presence of reactive glia, admixed with tumor cells, makes a diagnosis of adenoma unlikely.

c) *PNET*

The cytological features of Ganglioglioma (WHO grade I-II) are:

1. Tough, clumps of cells seen by naked eye

2. Neoplastic astrocytes showing glial processes

3. Neoplastic neurons with enlarged nuclei (sometime multinucleated), abundant cytoplasm, and abnormal cell processes

4. Low mitotic index

5. Eosinophilic granular bodies

6. Lymphocytic infiltrates are common.

The differential diagnoses of Ganglioglioma are

a) Entrapped *neurons in infiltrating glioma*: Entrapped neurons are morphologically normal. The presence of multinucleated neurons favors a neoplasm.

b) *PXA*: The distinction between a ganglioglioma and a PXA may require immuno histochemistry on paraffin section. Nonetheless, the PXA tend to arise more superficially in the cerebral hemispheres.

c) SGCA: A SGCA may resemble a ganglion cell tumor. The clinical setting of tuberous sclerosis and the intraventricular location point to a correct diagnosis.

d) *DNT*: A mixture of astrocytes, oligodendrocyte-like cells and ganglion cells in DNTs may give an impression of the ganglion cell neoplasm, and it may not be possible to distinguish these two entities of the basis of cytology. The presence of multinucleated neurons prompts a consideration of ganglion cell tumors.

The cytological appearance of Choroid Plexus Papilloma (WHO grade I) is:

1. Easy to smear, papillary structures seen by naked eye.
2. Well-formed papillary fronds consisting of ill-defined vascular cores surrounded by multiple layers of uniform columnar cells, with small basally located round nuclei, fine stippled chromatin and indistinct nucleoli
3. No cytoplasmic processes
4. Calcification and/or psammoma bodies are common.

The differential diagnosis of Choroid Plexus Papilloma are :

a) *Normal choroid plexus*: Smear of normal choroid plexus is much less in cellularity. The epithelial layer however may appear more than a single cell layer on smear because of the thickness of tissue smeared, but it is lower and more regular than that of papilloma. Vascular cores in normal choroid plexus are also more conspicuous.

b) *Metastatic well-differentiated adenocarcinoma*: This possibility should be considered, especially in adult subjects, and the distinction may require paraffin sections. The presence of

mucin material and goblet cells favors adenocarcinoma.

c) *Ependymoma*: Ependymomal cells, in contract to choroid plexus papilloma, have cytoplasmic glial processes.

The cytological appearance of Choroid Plexus Carcinoma (WHO grade III) are :

1. Soft, easy to smear, clumps of cells seen by naked eye
2. Wide range of cytological features depending on the degree of differentiation

The differential diagnoses of Choroid Plexus Carcinoma are:

a) *Metastatic adenocarcinoma*: Metastatic adenocarcinoma should be considered, especially in adult patients, and the definitive diagnosis may require permanent sections.

b) *Medulloepithelioma*: Medulloepitheliomas usually show distinct orderly psudostratified epithelial layers. Neuroglial differentiation may be encountered.

c) *Malignant teratoma*: The search for other germ line components on smear or frozen section should be done to exclude this possibility.

d) *Malignant papillary ependymomas*: The presence of glial cytoplasmic processes suggests a glial origin of the neoplasm.

The cytological features of Meningioma (WHO grade I) are:

1. Soft enough to make a good smear, clumps of cells seen by naked eye
2. Clumps of tumor cells with irregular margins and no relation to blood vessels.
3. Uniform oval nuclei with diffuse chromatin, indistinct nucleoli, occasional intranuclear vacuole and indistinct cytoplasmic borders among tumor cells
4. Scattered tumor cells in the background showing delicate wispy cytoplasm
5. Psammoma bodies
6. Cellular whorls

The differential diagnosis of meningioma is:

a) *Normal arachnoid cell*: Smear of normal arachnoid cells is much less in cellularity.

b) *Schwannoma*: Schwannomas and meningiomas (particularly fibroblastic variant) may be easily confused, especially when a

cerebellopontine lesion is encountered, and there are no well-formed cellular whorls in the latter. However, neoplastic cells in schwannomas usually form very distinct cell clusters (see below) with no, if any, tumor cells scattered in the background. In contrast to meningiomas, scattered tumor cells among cell clusters showing delicate wispy cytoplasm are characteristic.

c) *Astrocytoma*: Cytoplasm of meningioma cells may superficially mimic cell processes of neoplastic astrocytes, but tumor cell processes in meningiomas appear soft looking and are more delicate.

d) *Hemangiopericytoma*.

The cytological features of Hemangiopericytoma (WHO grade II-III) are:

1. Tend to be tough and difficult to make a good smear
2. Relatively uniform round or oval tumor cells with often prominent nucleoli and scanty cytoplasm.
3. Mitotic figures may be seen.
4. Stag horn' blood vessels may be demonstrable on frozen section.

The differential diagnoses of Hemangiopericytoma are:

a. *Meningioma*: The absence of meningothelial features (whorls, intranuclear vacuoles and delicate cytoplasmic processes) makes a diagnosis of meningioma unlikely. However, a definitive diagnosis of Hemangiopericytoma usually requires paraffin section.

The cytological appearance of schwannomas are:

1. Tend to be tough and difficult to make a good smear Very cohesive clusters of tumor cells with clean background
2. Cords of spindle cells separated and entwined about each other forming 'twisted rope' appearance.
3. Uniform spindle cigar-shaped cells with stippled chromatin, no nucleoli and indistinct cytoplasm

The differential diagnosis of schwannomas is Meningioma, *especially Fibroblastic variant*

The cytological features of Hemangioblastoma (WHO grade I) are:

1. Difficult to make a good smear, discrete clumps of cells seen by naked eye

2. Densely vascular tissue with occasional hemorrhages

3. Clumps of medium to large oval or elongated nuclei with indistinct cytoplasm

4. Absence of mitoses

5. Mast Cells (demonstrable on toludine blue)

The cytological features of Pituitary Adenoma are :

1. Soft, easy to smear into a thin film

2. Smooth monolayer sheets of uniform round cells with fine chromatin, indistinct nucleoli and distinct cytoplasm of the same color

3. Occasional nuclear pleomorphism and multinucleate cells.

4. Occasional rosettes, cell clusters and papillary formation

5. Abundant fresh blood

6. Ghost tumor cells, PMNs, infarcted tissue and red cells in apoplexy

The Differential diagnoses of pituitary adenoma are:

a) *Normal adenophypophysis*: The normal anterior pituitary

gland shows less cellularity on smear and consists of polymorphous cell populations, as reflected by the difference in color of the cytoplasmic staining.

b) *Oligodendroglioma*

c) *Central neurocytoma*

d) *Pituitary carcinoma*: Diagnosis of pituitary carcinoma requires the presence of clinical metastasis. Nuclear pleomorphism and some mitotic figures can be seen in pituitary adenomas and should not be interpreted as the evidences of malignant transformation.

The cytological features of lymphoma are:

1. Soft, easy to smear into thin film
2. Tumor cells in blood vessel wall, as well as in the background with monolayer arrangement
3. High nuclear-cytoplasmic ratio, prominent nucleoli with little but discernable cytoplasm
4. Mitoses, hemorrhages and necrosis are common.

The differential diagnosis of lymphoma are: *Glioblastoma*, *Germinoma* , *PNET*

The cytological features of Germinoma are :

1. Soft, easy to smear
2. Two cell populations: a diffuse monolayer sheets of polygonal to spheroidal large tumor cells with vesicular nuclei, prominent nucleoli and ill-defined cytoplasm b. Small lymphocytes
3. The fragile cytoplasm, which spread over the slide, produces a lace-like ('tigroid') background on Diff-Quik (Wright's) stain.
4. Occasional epitheloid granulomas

The of Differential diagnosis of germinoma are:

a) *Lymphoma* Lymphomas show a single cell population. Tigroid background on Diff-Quik stain suggests germinoma.

b) *PNET* Tumor cells in PNET do not have distinct nucleoli. Tigroid background on Diff-Quik stain favors germinoma.

The cytological features of Chordoma are:

1. Tough tissue mixed with soft gelatinous material
2. Prominent extramucinous material showing strong

metachromasia on toludine blue

3. Round uniformed tumor cell nuclei possessing voluminous clear cytoplasm with occasional vacuolation

4. Trabeculae or cord of uniform round cells

The differential diagnoses of Chordoma are:

a) *Chondrosarcoma* The distinction usually requires paraffin sections. However, the location of tumor should be considered.

The cytological features of Craniopharyngioma are: squamous cells with intercellular bridges may be seen. The characteristic 'machine oil' appearance containing cholesterol crystals visualized under polarization is a helpful diagnostic clue.

The cytological features of Epidermoid and Dermoid cysts are: Keratin and/or maturing squamous cells can be encountered in both the cystic conditions. The distinction requires histological examination. In addition to the epidermis in epidermoid cysts, dermoid cysts also contain skin appendages.

The cytological features of Rathke's cleft and colloid cysts are: Both have a similar ciliated cuboidal epithelium. Location of the lesion is very important for a correct diagnosis.

The cytological features of Metastatic lesions to the CNS depend on the primary neoplasm.

If multinucleate *cells* are present the following are to be considered: Glioblastoma, Gemistocytic astrocytoma, Ganglion cell tumors, Pilocytic astrocytoma Pleomorphic Xantho astrocytoma, Subependymal gaint cell astrocytoma, Malignant Teratoma & Choriocarcinoma, Tuberculosis, Progressive multifocal leukoencephalopathy, Fungal infections.

If prominent *capillary blood vessels* are present the following are to be considered: Glioblastoma, Anaplastic astrocytoma, Pilocytic astrocytoma, Hemangioblastoma, Lymphoma, Pituitary adenoma, Myxopapillary ependymoma, Edge of cerebral infarct, Arteriovenous malformations, Radiation damage

If *small round tumor cells* are present the following are to be considered: Oligodendroglioma, Pituitary adenoma, Central neurocytoma, Lymphoma, DNET

If *epithelial cells* are present the following are to be considered:

Metastatic tumors, Choroid plexus tumors, Epidermoid &

Dermoid cyst, Craniopharyngiomas, Pituitary adenoma, Rathke's cleft cyst, Teratoma (Malignant & Mature), Enterogenous cyst

If neuronal *or ganglion cells* are present the following are to be considered: Ganglion cell tumours, Pineocytoma, Infiltrated cerebral grey matter and cerebellar cortex, Cortical dysplasia, Differentiated PNET/Medulloblastoma, Teratoma, Hypothalamic hamartoma

If perivascular *lymphocyte cuffing* is present the following are to be considered: Viral encephalitis, Multiple sclerosis, Lymphoma, Gangion cell tumours, Recent infarction, Glioblastoma, Anaplastic astrocytoma, Pleomorphic Xantho astrocytoma

If *small poorly differentiated tumor cells* are present the following are to be considered: PNET/Medulloblastoma, Ependymoblastoma, Pineoblastoma, Neuroblastoma, Metastatic small cell anaplastic carcinoma, Glioblastoma, Malignant teratoma.

Apart from these cytological features, there are some gross intra operative findings, which will guide the surgeon to think of some possibilities.

If *necrosis* is present the following possibilities to be considered: Glioblastomas, Metastatic tumours, Lymphoma, Anaplastic oligodendroglioma, Anaplastic Ependymoma, Medulloblastoma, PNET, Choroid plexus carcinoma, Malignant Teratoma

If hemorrhage is present the following possibilities to be considered:

Glioblastoma, Metastatic tumours (particularly malignant melanoma), Hemangioblastoma, Anaplastic oligodendroglioma, Anaplastic Ependymoma, Malignant Teratoma & Choriocarcinoma, Intracerebral haematoma, cerebral infarct

If calcification is present the following possibilities to be considered:

Oligodendroglioma, Pilocytic astrocytoma, Astrocytoma, choroid plexus papilloma Subependydoma, Ganglion cell tumours, Subependydmal gaint cell astrocytoma, Central neurocytoma

In spite of these limitations, the experience of using the frozen section diagnosis of central nervous tumours has been very encouraging. A review Groves R, et al (5) of 412 intra operative frozen section examinations performed m has reported a 76%

concordance with paraffin section result when the criteria used was the definitive histological type of the tumor. However this figure rose to 90% when assessment of the malignancy was compared and the authors concluded that the technique could be used with almost complete assurance to distinguish nonneoplastic tissues from true tumours and primary glial lesions from metastatic ones.

Colbassini et al (6) reported a rate of 64% concordance from 80 cases where frozen examination has been employed.

N.D.Kitchen et al (16) reported a diagnostic rate of 69% for intra operative smear preparations where as Kleihues et al achieved a diagnostic rate of 70% of smears in 600 cases. They achieved a diagnostic accuracy of 100% in glial neoplasm 55% in metastatic lesions.

Katrina S.Frilick et al (9) have reported the use of cytological preparations for the intraoperative diagnosis of stereotactically obtained brain biopsies and found an accuracy of 90% of cases (52% complete correlation and 38% partial correlation). In 10% of cases there was no correlation between the intraoperative and the final diagnosis. In their study, the intraoperative diagnosis was most accurate in cases of abscess,

germinoma, lymphoma, metastasis and malignant glial tumour. They had 96% sensitivity in detecting diagnostic specimens, a specificity of 75%, positive predictive value of 98% and a negative predictive value of 66%. They also performed a survey regarding preferred methods for intra operative diagnosis of stereotactically obtained brain biopsy samples. Of the 148 neuropathologists 92 (62%) responded. 23% of respondents choose frozen section examination alone, 13% choose one or more cytological methods alone and the remainder 64% choose a combination of frozen section examination and cytology.

GRADING SYSTEM OF GLIOMAS: Bailey and Cushing initially graded glial neoplasms. Later several grading systems were evolved. Currently, the grading system commonly used is the WHO 1993 grading system.

The criteria used for the grading of astrocytomas in the WHO system (I-IV) are similar to those used in the Anne-mayo scheme. Pilocytic astrocytoma and sub ependymal gaint cell astrocytomas were regarded as grade I. Low-grade astrocytoma and Pleomorphic Xantho astrocytomas were classified under grade II. Anaplastic astrocytoma and glioblastoma were grouped as grade III and IV respectively. The diagnosis of glioblastoma

requires microvascular proliferation and /or necrosis. The essential variance of this system from the other tired systems discussed above is that astrocytomas can be classified as grade IV based on the presence of vascular features and without the feature of necrosis. In most cases of Glioblastoma both microvascular proliferation and necrosis are present. The apparent discordance between the presence of microvascular proliferation and tumor necrosis occurred in only 7.7% tumours in the kim et al and 11.6% in the series of Barker et al. Thus although it represents a major discrepancy in criteria it may not affect the practicality of grading in vast majority of tumours. This concordance among the individual pathologists who use the St Anne Mayo system is reported to be as high as 94% by Kim et al.

The mean survival time for glioblastoma and grade 4 astrocytomas are generally about the same for all grading systems in use today. However the mean survival time for lower grade astrocytomas, those with well-differentiated and anaplastic tumours are more disparate depending on the system in use. It is essential that the pathologist, surgeon and the oncologist jointly determine the grading system for common use at each institution and those criteria for grading are applied consistently so that

survival information can be predicted for the patients.

WHO 1993 Grading system:

Grade I (Pilocytic astrocytoma, Subependydmal gaint cell astrocytoma)

GradeII(Low grade astrocytoma, Pleomorphic Xantho astrocytoma)

Grade III (Anaplastic astrocytoma)

Grade IV (Glioblastoma multiforme)

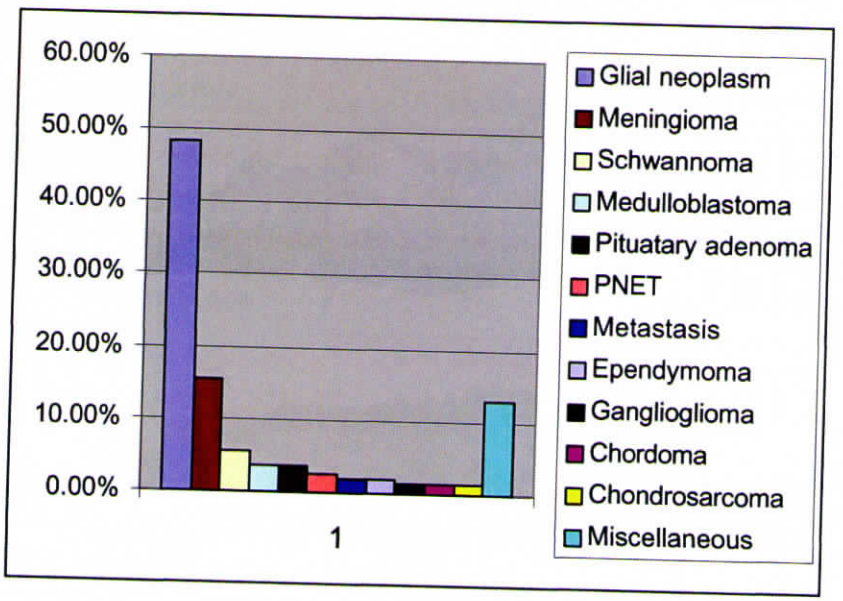
RESULTS

During the period of 1st July 2003 and 31dec2003, a total of 202 cases underwent neurological surgery, where tissue was sent for intraoperative diagnosis. Out of these concordance between intraoperative tissue diagnosis and final paraffin section was achieved in 169 cases (83.17%), partial concordance was achieved in 12 cases (05.94%). There was no concordance in 21 cases (10.89%)

Table no 1 showing break up of the cases in the study

Glial neoplasm	98(48.51%)
Meningioma	31(15.35%)
Schwannoma	11(5.45%)
Medulloblastoma	07(3.47%)
Pituatary adenoma	07(3.47%)
PNET	05(2.48%)
Metastasis	04(1.98%)
Ependymoma	04(1.98%)
Ganglioglioma	03(1.49%)
Chordoma	03(1.49%)
Chondrosarcoma	03(1.49%)
Miscellaneous	26(12.87%)
Total	202(100%)

Chart no 1 showing break up of the cases in the study



ble no. 2 showing concordance between intraoperative and final paraffin section diagnosis

Concordance	No of cases	Percentage
Complete concordance	169	83.17%
Partial concordance	12	05.94%
No concordance	21	10.89%

art no. 2 showing concordance between intraoperative and final paraffin section diagnosis

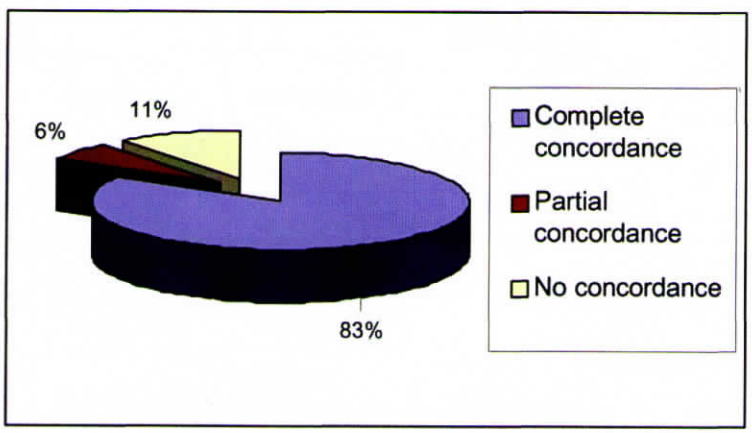


Table no 3 showing the cases where there was no concordance between intraoperative tissue diagnosis and definitive paraffin sections.

Name	Slide no	Frozen section diagnosis	Definitive diagnosis
1.Aboobacker	S (617/03)	Cellular glioma	Metastatic malignant Tumor
2.Lalitha madhavan	S(617/03)	Metastatic carcinoma	Atypical meningioma
3. Ayothi rama raja	S(708/03)	Hemangioblastoma	Schwannoma
4. Farsana	S(777/03)	Cellular neoplasm	Pineoblastoma with pineocytoma
5.Sasi g.	S(848/03)	Neurofibroma	Capillary and cavernous hemagiomatous lesion
6.Shalini p.	S (891/03)	Germ cell tumour	Pnet
7.Rahmath	S (883/03)	Inflammatory	Malignant epitheloid schwannoma
8.Muthiah	S (906/03)	High grade glioma	Primary cns lymphoma
9. Khadeeja	S(923/03)	meningioma	Schwannoma
10. Prema devi	S(934/03)	Ependymoma	Meningioma
11. Jameela	S(947/03)	Chordoma	Angioblastic meningioma
12. venkatesan	S(1007/03)	Glioma, aesthesioneuroblastoma	Schwannoma
13. Bijju	S(1064/03)	Lymphoma vs mets	PNET or malignant undifferentiated rhabdoid tumor
14. Ouseph	S(1146/03)	Not a benign meningioma	Meningioma
15. Ponuswamy	S(1071/03)	Meningioma	Mixed glioma
16. Remlath	S(755/03)	Glioma	Neurocytoma
17. Shantamma	S(828/03)	Pituitary adenoma	Meningioma
18. Rani s	S(839/03)	Germ cell tumour	Astrocytoma II
19. Shashidaran	S(1143/03)	Nerve sheath tumour	Meningioma
20. Thankamani	S(1197/03)	Malignant glioma	Meningioma
21. Sumalata	S 1029/03)	Neurocytoma	Oligodendroglioma

In case no 1, the frozen section was reported as a cellular glioma, where as the final paraffin section showed, hyperchromatic cells with features of pleomorphic cells with mitotic figures and necrosis. The impression was metastasic tumour. The frozen section slides was reviewed and the diagnosis remained the same. This patient had earlier undergone surgery for a testicular seminoma. This information was available at the time of final histopathological diagnosis and was not available at the time of frozen section.

In case no 2, the frozen section diagnosis was metastatic carcinoma where as the final paraffin section showed features of an atypical meningioma, with mitotic figures and invasion into adjacent brain. The frozen section slide reviewed showed that the diagnosis was the same. This patient had presented with vomiting and gait unsteadiness. The radiological diagnosis was cerebellar convexity meningioma or a metastatic lesion. The operative impression was a meningioma.

In case no 3, the frozen section diagnosis was a hemangioblastoma, whereas the final paraffin section revealed a schwannoma. The frozen section slide reviewed showed that the diagnosis was the same. This patient had presented with features

of right C.P. angle lesion. The diagnosis on MRI was either a hemangioblastoma or a schwannoma. The operative impression was an lower cranial nerve schwannoma.

In case no 4, the frozen section diagnosis was a cellular neoplasm, the paraffin section showed neoplastic cells with hyperchromatic nuclei with discrete foci of necrosis and was interpreted as mixed variant of pineoblastoma with pineocytoma. The frozen section slides reviewed showed the same cellular lesion. This 2-½ year old child had presented with raised intra cranial tension. Radiological picture was suggestive of a teratoma or a pineoblastoma. The operative impression was a pineoblastoma.

In case no 5, the frozen section diagnosis was a neurofibroma, the paraffin section showed benign hemangiomatous lesion with capillary and cavernous vascular channels. The frozen section slides reviewed, did not change the diagnosis. This patient has presented with a dorsal compressive myelopathy. MRI was suggestive of a vascular malformation.

In case no 6, the frozen section diagnosis was Germ cell tumour, the paraffin section showed highly cellular and diffusely infiltrating neoplasm with discrete foci of necrosis and was

interpreted as a primitive neuroectodermal tumour. The frozen section slides reviewed, did not change the diagnosis. This patient has presented with perinaud's syndrome. The radiological impression was either a pineoblastoma or a germinoma.

In case no 7, the frozen section diagnosis was inflammatory lesion, the paraffin section showed a neoplastic lesion with diffusely arranged cells, with round to oval nuclei, with a mucoid stroma and foci of necrosis. It was interpreted as malignant epitheloid schwannoma. This patient had presented with headache, vomiting and gait unsteadiness. The radiological diagnosis was a schwannoma. The operative impression was lower cranial nerve schwannoma.

In case no 8, the frozen section diagnosis was high grade glioma, the paraffin sections showed a diffusely scattered neoplasm with sheets of cells with vesicular nucleus, perivascular distribution of cells, presence of necrosis and mitotic figures, perivascular lacy pattern of reticulin fibres. Immunostaining was positive for B cells, T cells and LCA. The diagnosis was Primary CNS lymphoma. The frozen section slides reviewed, did not change the diagnosis. This patient had presented with one episode of generalized tonic, clonic seizures. The radiological impression was a high-grade glioma or a metastasis

In case no 9, the frozen section diagnosis was meningioma, the paraffin sections showed neoplastic cells displayed in interdigitating bundles of spindle cells, nuclei of these cells arranged in distinct periodic rows (Antoni A areas) Imperceptibly merging with these cells are loosely textured and sparsely cellular cells in a pale connective tissue stroma (Antoni B areas). The final impression was schwannoma. The frozen section slides reviewed, did not change the diagnosis. This patient had presented with features of left cerebellopontine lesion. The radiological impression was vestibular schwannoma. The operative impression was also a schwannoma.

In case no 10, the frozen section diagnosis was ependymoma, the paraffin sections showed neoplastic cells arranged in concentric whorls, also seen are psammoma bodies. The diagnosis was a meningioma. The frozen section slides reviewed, did not change the diagnosis. This patient had presented with paraethesias and bladder symptoms. The radiological opinion was a meningioma or a schwannoma.

In case no 11, the frozen section diagnosis was chordoma, the paraffin sections showed angioblastic variant of meningioma. The frozen section slides reviewed, did not change the diagnosis.

This patient was earlier operated for a cavernous sinus meningioma and came with a recurrence. This information was not available at the time of frozen section. The radiological and intraoperative opinion was a meningioma.

In case no 12, the frozen section diagnosis was glioma or a aesthesioneuroblastoma, the paraffin sections showed neoplastic cells in spindle shape with nuclei showed distinct palisadic arrangement. Loosely textured stellate cells are also seen. These were classical features of schwannoma. The frozen section slides reviewed, did not change the diagnosis. This patient presented clinically with a frontal lobe symptoms. The radiological impression was olfactory groove meningioma and intraoperative impression was also a meningioma.

In case no 13, the frozen section diagnosis was lymphoma or a metastasis, where as the final paraffin section showed evidence of diffusely infiltrating cells with hyperchromatic cells with pleomorphic nuclei and mitotic figures and a possibility of PNET with malignant undifferentiated rhabdoid tumour was suggested. The frozen section slides reviewed, did not change the diagnosis. This patient presented with headache, vomiting and right sided weakness. The radiological impression was a glial neoplasm.

In case no 14, the frozen section diagnosis was 'not a benign meningioma, where as the final paraffin section showed evidence of neoplastic cells arranged in abortive whorls. There was also partial effacement of the architecture of the meningioma. Nuclear atypical changes were seen. The impression was atypical meningioma. In this case, probably the atypicality of the lesion was responsible for the diagnosis of not a benign meningioma on frozen section. The radiological and intraoperative impression was a middle cranial fossa floor meningioma.

In case no 15, the frozen section diagnosis was meningioma, where as the final paraffin section showed evidence of neoplastic cells made up of sheets of monomorphic cells. These cells showed central nucleus surrounded by clear cytoplasm. Stellate shaped neoplastic cells are also seen with these cells. The impression was a mixed glioma consisting predominantly oligodendroglial cells. The frozen section slides reviewed, did not change the diagnosis. This patient presented with seizures and had papilloedema. The radiological and operative impression was a cystic glioma.

In case no 16, the frozen section diagnosis was a glioma, where as the final paraffin section showed evidence of neoplastic cells with two cellular components. The first and predominant

component is composed of monomorphic cells having vesicular nucleus surrounded by a clear cytoplasm. The other component was made up of stellate shaped cells scattered on fibrillary stromal matrix. Immunostaining was positive for neuron specific enolase and variable for synaptophysin. The final impression was a neurocytoma. This patient presented with features of raised intracranial pressure. The radiological and operative impression was a neurocytoma or an oligodendroglioma.

In case no 17, the frozen section diagnosis was a pituitary adenoma, where as final paraffin sections showed evidence of neoplastic cells composed of lobulated masses of well differentiated meningothelial cells. The neoplastic cells were arranged in concentric whorls. The impression was a meningothelial variant of meningioma. This patient presented with raised ICP features. The radiological and operative impression was a sphenoid wing meningioma.

In case no 18, the frozen section diagnosis was germ cell tumour, where as final paraffin sections showed evidence of neoplastic cells composed of stellate shaped fibrillary astrocytes. These fibrillary astrocytes are supported on a glial fibrillary stromal matrix. The final impression was a fibrillary astrocytoma

grade II. This patient had presented with headache and vomiting. The radiological impression was a pineocytoma or an astrocytoma.

In case no 19, the frozen section diagnosis was a nerve sheath tumour, where as final paraffin sections showed evidence of neoplastic cells arranged in concentric whorls supported by a vascular connective tissue stroma. Numerous discrete nodular hyaline masses are also seen. The impression was meningioma transitional variant. This patient presented with raised ICP features. The radiological and operative impression was sphenoid wing meningioma.

In case no 20, the frozen section diagnosis was a malignant glioma, where as final paraffin sections showed evidence of tumour consistent with transitional meningioma. This patient presented with lower limb paraethesias and spasticity. The imaging diagnosis was a neurofibroma or a meningioma. Operative impression was a meningioma.

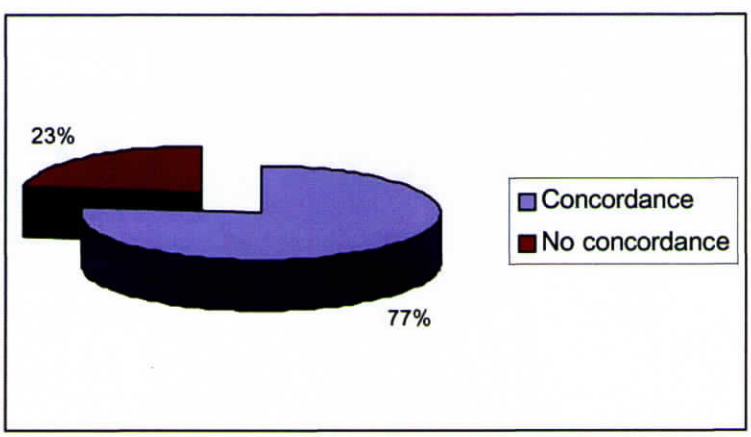
In case no 21, the frozen section diagnosis was a neurocytoma, where as final paraffin sections showed evidence of neoplastic cells consistent with oligodendroglioma. This patient presented with headache, vomiting and had papilloedema. CT scan was suggestive of frontal glioma.

Ieningiomas consisted of maximum number of cases (07) he intra operative tissue diagnosis did not match with the araffin section diagnosis. Out of 31 cases of meningiomas ntraoperative tissue diagnosis was sought, it was diagnosed y as meningioma in 24 (77.42%) cases. In the remaining %) cases it was diagnosed as glioma, metastasis, moma, chordoma, not a meningioma, pituitary adenoma and heath tumour.

Table no 4 : Meningiomas

Total no	**31**
Concordance	24(77.42%)
No concordance	07(22.58%)

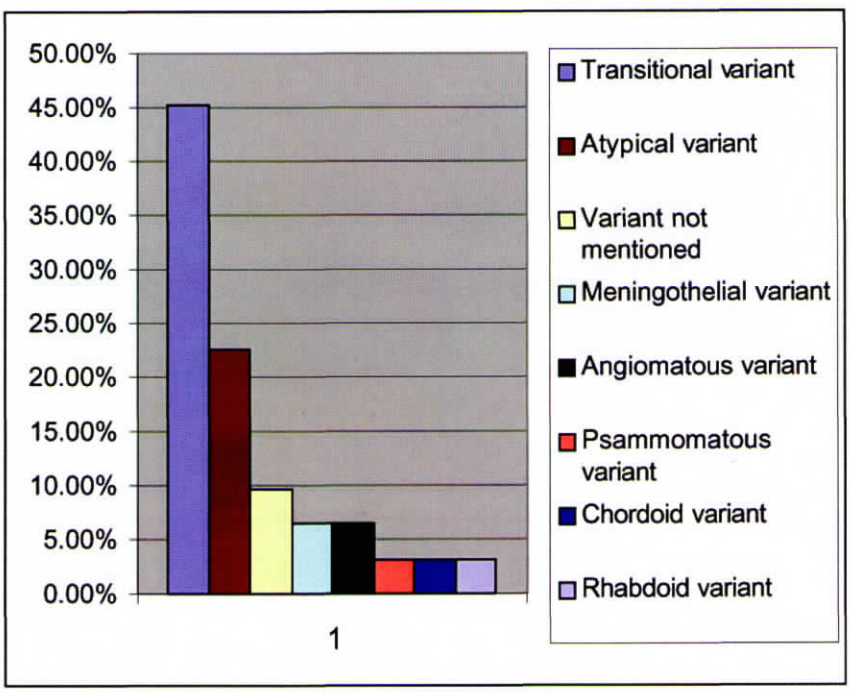
Chart no 3 : Meningiomas



ble no 5 showing the histological subtype in these 31 cases of meningiomas:

Transitional variant	14(45.16%)
Atypical variant	07(22.58%)
Variant not mentioned	03(9.68%)
Meningothelial variant	02(6.45%)
Angiomatous variant	02(6.45%)
Psammomatous variant	01(3.23%)
Chordoid variant	01(3.23%)
Rhabdoid variant	01(3.23%)

Chart no 4 showing the histological subtype in these 31 cases of meningiomas:



Schwannomas consisted the next common group (04 cases
f eleven cases sent for frozen section diagnosis (36.36%)
the intraoperative tissue diagnosis failed to identify
tly. On frozen section, out of these four cases, one was
sed as Hemangioblastoma, one as a Meningioma, one as
matory lesion and the other one as Glioma or an
sioneuroblastoma.

Table no 6: Schwannomas

Total no	11
Concordance	07(63.64%)
No concordance	04(36.36%)

Chart no 5: Schwannomas

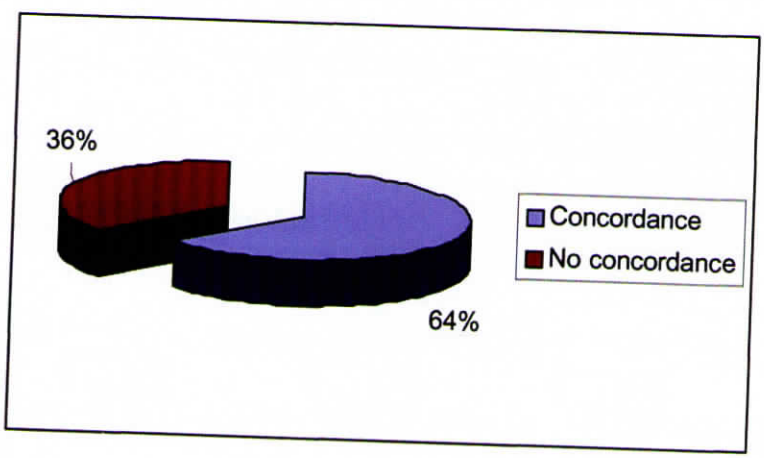


Table no 7 showing the predominat histological subtype of the 11 schwannomas:

Antoni a and antoni b	05(45.5%)
Antoni b predominat	02(18.18)
Malignant epitheloid variant	01(9.09%)
Type not mentioned	03(27.27%)

Out of four metastatic lesions (on paraffin sections), 1(25%) of them was diagnosed as glial neoplasm on frozen section.

Table no 8: Metastatic lesions

Total no	04
Concordance	03(75%)
No concordance	01(25%)

Of these 4 metastatic lesions, one was an adenocarcinoma, one was a squamous cell carcinoma, one was from a testicular carcinoma and the other one was metastatic lesion probably from bronchus or genitourinary tract.

Out of five primitive neuroectodermal tumours, three were diagnosed correctly on the frozen section diagnosis. Among the remaining 2 (40%) cases, one was diagnosed as a germ cell tumor and the other was diagnosed as lymphoma or metastasis.

Table no 9 : Primitive neuro ectodermal tumours

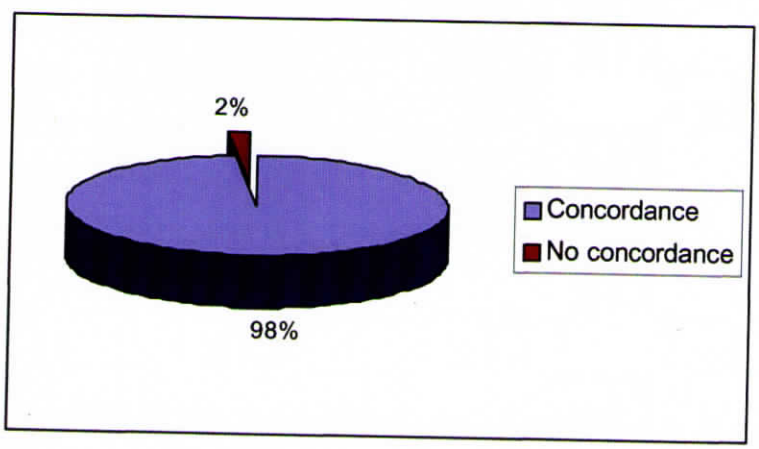
Total no	05
Concordance	03(60%)
No concordance	02(40%)

Out of 202 cases, 98 were glial neoplasms. Out of these glial neoplasms, only two cases were diagnosed as a non gliomatous lesion on frozen section, where as nine non gliomatous lesions were diagnosed as glial neoplasm on frozen section.

Table no 10: Glial neoplasms

Total no	98
Concordance	96(97.96%)
No concordance	02(2.04%)

Chart no 6: Glial neoplasms



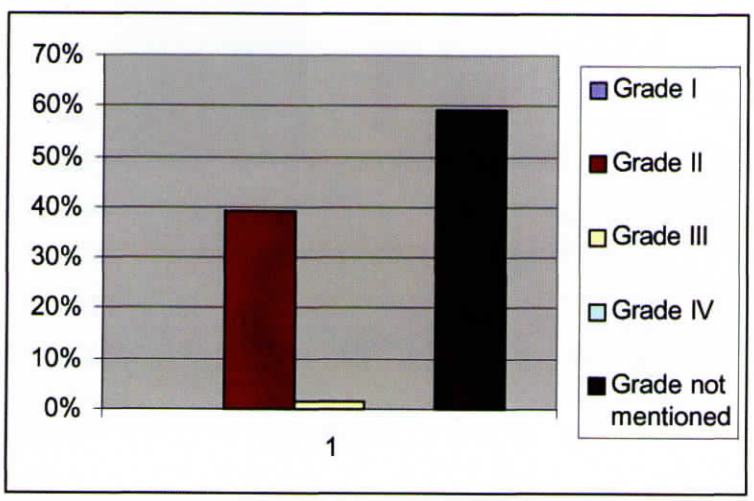
Out of the 98 glial neoplasms, only two were a WHO grade I tumour and they were diagnosed as grade one glioma on frozen section.

There were 59 WHO grade II tumours, of which, on frozen ion, 23 were diagnosed correctly as grade II, in 35 cases grade d not be mentioned and in one case the lesion was overgraded rade III.

ble no 11 showing grading of GRADE II GLIOMAS on frozen section

Grade I	00
Grade II	23(38.98%)
Grade III	01(1.69%)
Grade IV	00
Grade not mentioned	35(59.32)

art no 7 showing grading of GRADE II GLIOMAS on frozen section

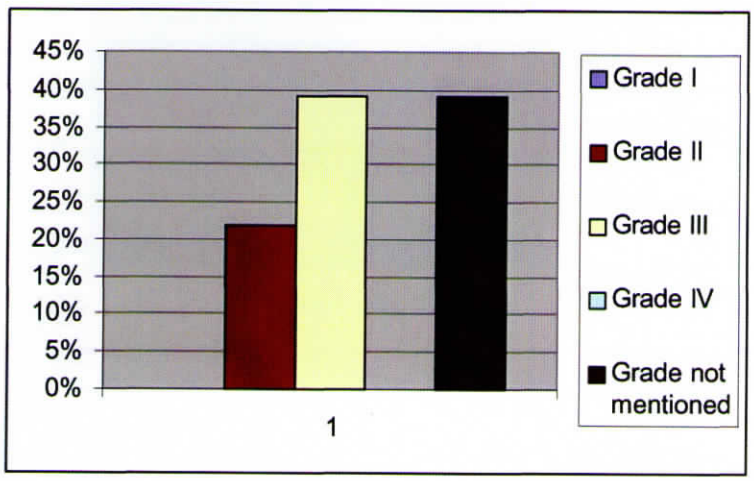


There were 23 WHO grade III cases , of which , on frozen section re diagnosed correctly as grade III, in 14 cases grade was not ioned or graded less, but none of the tumours were over graded.

Table no 12 showing grading of GRADE III GLIOMAS on frozen section.

Grade I	00
Grade II	05(21.74%)
Grade III	09(39.13%)
Grade IV	00
Grade not mentioned	09(39.13%)

Chart no 8 showing grading of GRADE III GLIOMAS on frozen section.



There were 14 grade IV gliomas, of which, on frozen on, 6 cases were diagnosed correctly as grade IV and in the ining 8 cases, they were either less or grade was not ioned. None of the lower grade gliomas were diagnosed on en Section as glioblastomas.

no 13 showing on frozen section of GRADE IV GLIOMAS:

Grade I	00
Grade II	00
Grade III	02(14.29%)
Grade IV	06(42.86%)
Grade not mentioned	06(42.86%)

rt no 9 showing on frozen section of GRADE IV GLIOMAS:

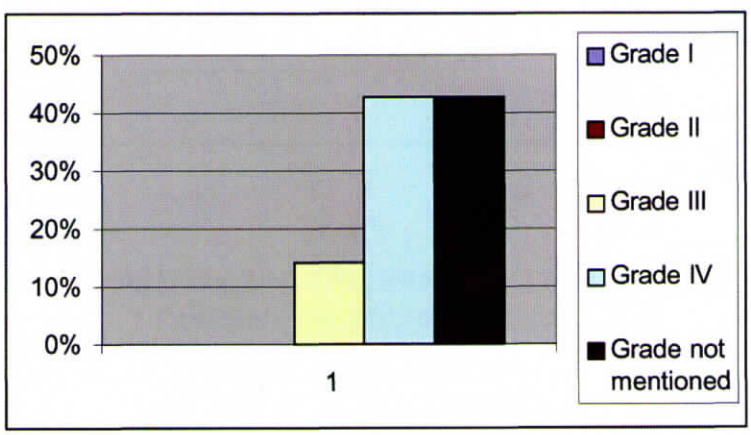
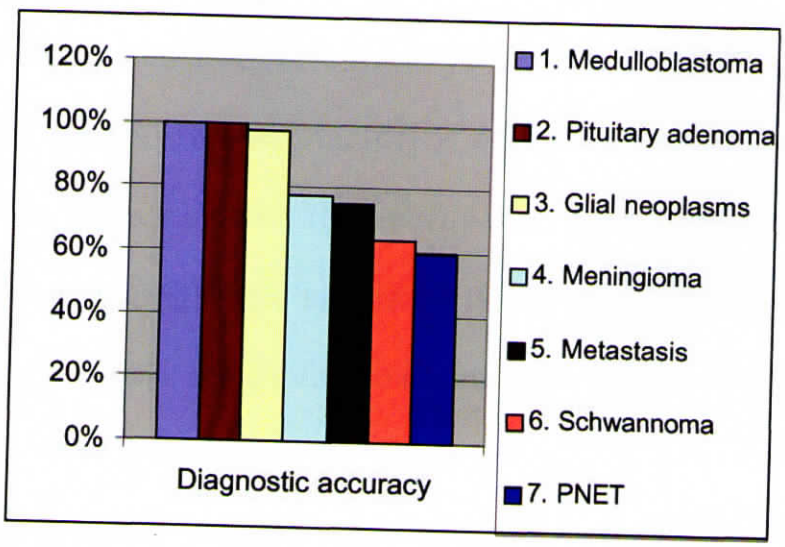


Table no 14 showing the diagnostic accuracy of individual tumours on frozen section:

Tumour	Total no	Diagnostic accuracy
1. Medulloblastoma	07	100%
2. Pituitary adenoma	07	100%
3. Glial neoplasms	98	97.96%
4. Meningioma	31	77.42%
5. Metastasis	04	75.00%
6. Schwannoma	11	63.64%
7. PNET	05	60.00%

Chart no 10 showing the diagnostic accuracy of individual tumours on frozen section:



DISCUSSION

Immediate frozen preparations of tumour samples obtained at surgery is commonplace in neurological surgery. The aim of intra operative pathological diagnosis is two fold. Firstly to confirm that the given specimen represents the pathology and secondly to provide a provisional histological diagnosis which will facilitate management decisions in the intra operative and immediate post operative period.

The use of intraoperative pathological diagnosis has become a routine day-to-day event in the neurosurgical practice. It has a significant bearing on the intraoperative management. For open tumour resections the intraoperative diagnosis may influence the extent of resection. For stereotactic biopsies, the goal is to obtain diagnostic tissue. In vast majority of cases the neurosurgeon's main reason for consulting the neuropathologist intraoperativley is to ensure that a diagnostic specimen is obtained. Because the intra operative diagnosis are often broad and less specific, the results in this study were analyzed in three categories of classification between intraoperative and final diagnosis: complete correlation, partial correlation and no correlation. In this way, partial credit could be given to the neuropathologist for making a tentative

diagnosis such as "malignant neoplasm" or "glioma", which can be clinically useful, despite a lack of precise grading.

Table no 15 showing examples of complete, partial or no correlation of diagnosis.

Level of correlation	Intraop diagnosis	Final diagnosis
Complete correlation	Glioblastoma	Glioblastoma
	Could be primary CNS lymphoma	Primary CNS lymphoma
Partial correlation	Metastasis/inflammatory	Metastasis
	Glioma/lymphoma	Glioma
No correlation	Meningioma	Schwannoma
	Hemangioblastoma	Schwannoma

In this study, out of 202 cases, complete concordance between intraoperative and final paraffin section diagnosis was obtained in 168 cases (83.17%). In 12 cases (5.94%), a partial concordance was obtained. In the remaining 21 cases, (10.89%), there was no concordance between the intraoperative tissue diagnosis and the paraffin section diagnosis. A review (Groves R, et al) (5) of 412 intra operative frozen section examinations performed has reported a 76% concordance with paraffin section result when the criteria used was the definitive histological type of

reported as grade III and in 59.32% grade was not mentioned. None of the grade II gliomas were graded as grade I or grade IV on frozen section.

In grade III gliomas, 21.74% were reported as grade II, 31.13% were reported as grade III and in 39.13% grade was not mentioned. None of the grade III gliomas were reported as grade I or grade IV.

In grade IV gliomas, 14.29% were reported as grade III, 42.86% were reported as grade IV and in 42.86% grade was not mentioned. None of the grade IV gliomas were reported as grade I or grade II.

Katrina S. Frilik et al (9) have also reported similar findings. In their 259 cases of malignant gliomas, they reported a complete correlation in 58% and partial correlation of 35% and no correlation in 7%. They had 84 cases of benign glial neoplasms and obtained a complete correlation of 40%, partial correlation of 48% and no correlation in 12%. J Haapasalo et al (8) have studied the grading of gliomas by ultra rapid Ki-67 immunostaining in 34 patients and compared with MIB I immunostaining on paraffin sections. They had 9 pilocytic astrocytomas, 9 grade II astrocytomas, 4 grade III astrocytomas and 12 grade IV

astrocytomas. The obtained a complete correlation in all but 3 cases.

The diagnostic accuracy of frozen section in identifying medulloblastomas was 100% in our study. Katrina s Frilick et al (9) has also reported 100% accuracy in diagnosing medulloblastomas on smear cytology.

The diagnostic accuracy of frozen section in identifying pituitary adenomas in our study was 100%.

The diagnostic accuracy of frozen section in diagnosing meningiomas was 77.42%. In 22.58% there was no correlation. Of these two were atypical meningiomas. On frozen section, they were diagnosed as metastasis in one case and 'not a meningioma' in another case. Pratima Savargoankar et al (19) in their series of 103 cases have reported most discrepancies were due to failure to identify atypia in meningioma.

Two of the cases were transitional variant of meningioma. On frozen section, one was diagnosed as nerve sheath tumour and the other one as malignant glioma. Deon Louw et al (4) have reported two cases of meningiomas, which were diagnosed as schwannomas. Both these cases resembled schwannomas on

paraffin sections. Only immunohistochemistry and electron microscopic examination could identify them as meningiomas. The S -100 protein is characteristically present in schwannomas and in 15% of meningiomas. Leu 7 is detected in 80% of schwannomas and it is absent in meningiomas. Electron microscopy demonstrating basement membrane encompassing tumour cells is compelling evidence in favour of a diagnosis of schwannoma. The meningioma cells are not usually surrounded by basement membrane. However it has been reported that an amorphous basement membrane like material may be demonstrated between meningothelial cells. Kudo m et al (13) has described reticulin fiber staining of crush preparations for the rapid differentiation of schwannomas and meningiomas. Schwannomas showed extremely numerous, uniformly delicate straight fibres in a streaming or interlacing pattern in cellular areas and less dense, more wavy or curly fibres in degenerative areas. Fibroblastic meningiomas showed a few loose fibres of variable thickness and root like tangle, while meningotheliomatous meningiomas showed no fibres except in areas of fibro vascular stroma. It has also been postulated that, the pluripotent arachnoidal cap cells, the precursor cell of meningioma, exhibits diverse differentiation and variability in histological expression. Thus these lesions may simulate

oligodendroglioma, astrocytoma or schwannoma. Both meningiomas and schwannomas also have similar molecular mechanism of tumorigenesis. Both of these tumours have been associated with loss of regions of long arm of chromosome 22.

In the remaining three cases, one was an angiomatous meningioma, it was a diagnosed as chordoma on frozen section. One was a meningothelial variant, this was diagnosed as a pituitary adenoma. One was psammomatous variant; it was diagnosed as an ependymoma.

In our study the, accuracy of diagnosing schwannomas on frozen section was 63.64%. In 36.36% (4) cases there was no correlation. Two of these cases contained both Antoni A and Atoni B areas. On frozen section, one was diagnosed as a hemangioblastoma and the other as meningioma. One case was a malignant epitheloid schwannoma and had plasma cells and lymphocytic infiltration. It was diagnosed as inflammatory lesion on frozen section. The other case was reported as astrocytoma on frozen section. It had significant AntoniB areas containing loosely textured stellate shaped cells, which is known to mimic glial neoplasm.

The accuracy of diagnosing metastatic lesions on frozen

section was 75% in our study. Two of the metastatic lesions were adenocarcinoma and one was a metastatic lesion with probable origin from bronchus or genitourinary tract. These were identified correctly on frozen section. One lesion from a testicular seminoma was diagnosed as a glial neoplasm on frozen section. N.D.Kitchen et al (16) found 55% correlation between smear preparations and final paraffin sections in metastatic lesions.

In our study, the accuracy of diagnosing primitive neuroectodermal tumours was 60%. In 3 cases it was identified correctly on frozen section. In one case of a pineal region tumour, it was diagnosed as germ cell tumour and in other case it was diagnosed as lymphoma or metastasis.

In our study, two vascular lesions were included. One was identified correctly, in the other case it was diagnosed as a neurofibroma. Katrina S Frilick et al (9) could not obtain any correlation in all their 3 cases of vascular malformations.

Some factors that can help in reducing this error in the intra operative diagnosis include:

1. Making available the clinical and radiological data to the pathologist.

2. Staining the frozen section slides with rapid Hematoxylin and eosin stain instead of toludine blue. However the disadvantage of this technique is it takes longer time.

3. Using immunohistochemical staining in the frozen sections.

4. Experience of the pathologist.

CONCLUSION

1) Intraoperative tissue diagnosis with smear and frozen section is a fairly reliable and useful diagnostic method.

2) Intra operative tissue diagnosis was most accurate in the diagnosis of glial neoplasms, pituitary adenomas and medulloblastomas.

3) Meningiomas and schwannomas were the common lesions where the intraoperative tissue diagnosis did not match with final paraffin section diagnosis.

4) The grading of the glial neoplasms done by the intraoperative tissue diagnosis correlated well with final grading.

BIBLIOGRAPHY:

1. Brainard JA, Prayson RA, Barnett GH: Frozen section evaluation of stereotactic brain biopsies. Diagnostic yield at the stereotactic target position in 188 cases. Arch Pathol Lab Med 1997, 121: 481-484.

2. Burger PC: Use of cytological preparations in the frozen section diagnosis of central nervous system neoplasia. Americal journal of surgical pathology. 1985 May; 9(5) 344-54.

3. Cahill EM, Hidvegi DF: Crush preparations of lesions of the central nervous system. A useful adjunct to the frozen section. Acta Cytol.1985 May-June; 29(3): 279-85.

4. Deon Louw, Garnette Sutherland, William Halliday, John Kaufmann: Meningiomas mimicking cerebral schwannoma. J Neurosurgery 1990 73 : 715-19.

5. Groves R, Hesselvik M: The diagnostic accuracy of the frozen section examination in neurosurgery. Acta neurol Scand 1966; 42: 268-74.

6. H J Colbassani , S Nishio, KM Sweeney, RA Bakay and Y

Takei: CT assisted stereotactic brain biopsy: value of intra operative frozen section diagnosis. Journal of Neurology, Neurosurgery and Psyciatry, 1988, vol 51,332-341.

7. Hume Adams J, Graham DI, Doyle D Brain biopsy The smear technique for neurosurgical biopsies. Chapman & Hall, London, 1981

8. J Haapasalo, A Mennander P Helen, H Haapasalo and J Isola: Ultrarapid Ki-67 immunostaining in frozen section interpretation of gliomas. Journal of clinical pathology 2005; 58: 263-68.

9. Katrina S.Firlik, A.JulioMartinez, L.Dade Lunsford: Use of cytological preparations for the intraoperative diagnosis of stereotactically obtained brain biopsies: a 19-year experience and survey of neuropathologists. J of Neurosurgery 1999, 91:454-458.

10. K Neckelmann et al, Improved histological evaluation of gliomas using tissue Fragments obtained by ultrasonic aspiration: Clinical neuropathology, vol 23-no2/2004 (47-52)

11. Kobayashi S, Haba R, Hirakawa E, Yamadori I, Miki H,

Ohmori M: Cytology and immunohistochemistry of anaplastic meningiomas in squash preperations. A report of two cases. Acta Cytol . 1995 Jan-Feb; 39(1): 118-24.

12. Kobayashi S: Meningioma, neurilemmoma and astrocytoma specimens obtained with the squash method for cytodiagnosis. A cytologic and immunochemical study. Acta Cytol. 1993 Nov-Dec;37(6):913-22.

13. Kudo M, Mikami T, Maeda Y: Reticulin fibre staining of crush preperations for the rapid differentiation between schwannomas and meningiomas: Acta Cytol. 1991 sep-oct; 35 (5): 521-3

14. Mark A Mitller et al, Observer reliability in histological grading of astrocytoma in stereotactic biopsies. J of Neurosurgery 1996, 85:1091-94.

15. Murphy et al, Neuropathological diagnostic accuracy, British journal of Neurosurgery 2002; 16(5): 461- 464.

16. N.D. Kitchen, R. Bradford, and J.E. Mc Laughlin: The value of per operative Smear examination during stereotactic biopsy Acta neurochir (wien) 1993, 121, 196- 198.

17. Nguyen GK, Johnson ES, Mielke BW: Cytology of meningiomas and neurilemmomas in crush preparations. A useful adjunct to frozen section diagnosis. Acta Cytol. 1998 May-June; 32 (3): 362-6.

18. Patt S. Weigel K, Zimmer C, Schreiner C : Experience with the intraoperative frozen section technique for stereotactic brain tumour biopsies. Zentralbl Pathol. 1991; 137(1): 35-40.

19. Pratima Savargaonkar and Peter M farmer: Utility of intra operative consultations for the diagnosis of central nervous lesions. Annals of clinical and laboratory science 2001, 31: 133-139.

20. Regragui A, Amarti Riffi A, Maher M, ElKhamlichi A, Saidi A: Accuracy of intra operative diagnosis in central nervous system tumours: Report of 1315 cases. Neurochirurgie. 2003 May; 49(2-3Pt1): 67-72

21. Takekawa Y, Kinukawa N, Nemoto N, et al: Usefulness of cytology applied simultaneously to frozen section at rapid intraoperative diagnosis of intracranial tumours. Rinsho Byori . 1998 Sep; 46(9): 954-58.

22. Tim H Moss et al, James A.R. Nicoll, James W. ironside: Intra operative diagnosis of CNS Tumours. 1997. Oxford university press, Inc. New York.