CELL PROLIFERATION MARKERS IN HUMAN BRAIN TUMOURS: CORRELATION WITH CLINICAL OUTCOME

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Abstract

The specific aims of this study were to investigate the proliferative potential of three groups of CNS tumours, high grade glioma, meningioma and pituitary adenoma using two Immunohistochemical markers thought to label only proliferating cells, Ki-67 and PC10, an antibody which recognises the proliferating cell nuclear antigen (PCNA). This was carried out entirely in fresh frozen material collected during surgery to ensure that comparison between the two labelling methods were not influenced by formalin fixation and embedding in paraffin wax. Samples from one hundred and twenty three patients were collected and the labelling index determined by immunoperoxidase staining. This group included 61 meningiomas, 32 pituitary adenomas and 24 gliomas. Malignant gliomas had higher labelling indices (LIs) than lower grade tumours although glioblastoma multiforme had lower PCNA LIs than previously reported studies using conventional histologically processed material. Meningiomas tended to have low PCNA LIs, but higher Ki-67 LIs, while pituitary tumours had uniformly low Ki-67 LIs but apparently elevated PCNA LI's. This appeared to be related to the hormone secretions displayed by the tumour. The mean PCNA LIs were higher than mean Ki-67 LIs in prolactinomas, ACTH producing adenomas and silent adenomas. Clinical information was collected on all the patients, including data on tumour recurrence and survival. There did not appear to be a relationship between labelling index, with either antibody, and survival or length of preoperative symptoms or signs for any of the three groups of tumour. Although the use of these proliferation associated markers do not provide additional information about prognosis in patients with these benign or malignant brain tumours, they may provide an important insight to the control of hormone expression by pituitary tumours.
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Dedication

To my Abu jan and Ami jan
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Abbreviations

ACNU  (1,4-amino-2-methyl-5-pyrimidinyl)-methyl-3-(2-chlorethyl)-3-nitrosourea
ALL  Acute lymphoblastic leukaemia
AZQ  Aziridinylbenzoquinone
BCNU  1,3-bis(2 chlorethyl-1-nitrosourea)
BPH  Benign prostatic hypertrophy
BR2  MRC Brain Tumour Working Party, study number 2
BR5  MRC Brain Tumour Working Party, study number 5
BRW  Brown-Roberts-Wells
BTCG  Brain Tumour Cooperative Group
BTSG  Brain Tumour Study Group
BTWP  Brain Tumour Working Party
BUdR  Bromodeoxyuridine
Ca  Cancer
CCNU  1-(2-chlorethyl)-3-cyclohexyl-1-nitrosourea
CLF  Cell loss factor
CNS  Central nervous system
CSF  Cerebrospinal fluid
CT  Computer tomography
DAB  Diaminobenzidine
DBD  Dibromodulcitol
DNA  Deoxyribose nucleic acid
DPα  DNA polymerase alpha
DXT  Deep X-ray therapy
EORTC  European Organisation for Research into the Treatment of Cancer
ER  Oestrogen receptor
FCM  Flow cytometry
FITC  Fluorescein isothiocyanate
GBM  Glioblastoma multiforme
GF  Growth fraction
Gy  Gray
ICP  Intracranial pressure
IgG  Immunoglobulin
LI  Labelling index
MAB  Monoclonal antibody
mg  Milligram
MI  Mitotic index
MNU  MethylNitrosourea
MPA Medroxyprogesterone
MRC Medical Research Council
MRI Magnetic resonance imaging
Na$_2$HPO$_4$ Sodium hydrophosphate
NaOH Sodium hydroxide
NF Neurofibromatosis
NHL Non-Hodgkin's lymphoma
NORs Nucleolar organiser regions
O.C.T Optimum cutting temperature
PBS Phosphate buffered saline
PC Proliferating cell
PCNA Proliferating cell nuclear antigen
PLP Periodate-lysine-formaldehyde
RB Retinoblastoma
RFLPs Restriction fragment length polymorphisms
RS Reed-Sternberg
SLE Systemic lupus erythematosus
SPF S-phase fraction
sq.m Square meter
TB Tuberculosis
Tc Cell cycle time
TGF-β Transforming growth factor beta
TLI $^3$H-thymidine labelling index
TS Tuberous sclerosis
Ts Duration of S phase
VSG Valid study group
WHO World Health Organisation
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Chapter 1

Introduction

1.1. Tumours of the central nervous system

1.1.1. Incidence of the CNS tumours world-wide

Tumours of the central nervous system are not uncommon. Over the last thirty to forty years, a number of large scale studies have been reported which indicate that neoplasm of the central nervous system comprise 8-15% of all the human tumours. One of the largest of these studies in which incidence rates, prevalence rates and death rates of intracranial and intraspinal neoplasms were determined was in the resident population of Rochester over a 10-year period between 1945 and 1954 (Kurland et al., 1958). This study was based on reports from both local hospitals and pathology laboratories and included the vast majority of patients with such neoplasms in the study area. The average annual incidence rate, that is, the number of newly diagnosed cases each year, was 9.2 per 100,000 population. Adjusted rate for all CNS tumours in 21 countries falls within a relatively wide range of 1.1-7.8 per 100,000 population. The rate is 5.0 per 100,000 population in Sweden, 5.6 per 100,000 population in Denmark, 7.8 per 100,000 population in Israel, whilst only 1.1 per 100,000 population in Mexico, 2.2 per 100,000 population in Japan and 2.3 per 100,000 population in Chile. It may not be appropriate to conclude that the rate in Israel is higher than that of other countries because of the different working system at the Central Bureau of Statistics in Israel. In Mexico and Chile the apparent low rate may be due to faulty reporting or poor diagnostic facilities (Goldberg and Kurland, 1961). Another epidemiological survey was conducted by Schoenberg et al. (1976) based on a report from the Connecticut Tumour Registry between 1934 and 1964. From a total of 3210 patients, in approximately 75%, the diagnosis was confirmed pathologically. This study demonstrated for tumours of the brain and meninges an early peak, followed by a taller and sharper peak in the 55 - 65 year age group. Meningiomas were more common in elderly females, with an average incidence of 2.40 per 100,000 population, exactly the same incidence as the South of England study (vide infra).

1.1.1.1. Total incidence of CNS tumours in UK

A survey of cancer in a population of 2 million people in Southern England was carried out by Barker et al. (1976). They included 894 patients aged 15 or over who had been treated at the Wessex Neurological Centre in Southampton between 1965
and 1974 for primary neoplasms of brain and spinal cord. The average annual incidence of all forms of glioma was 3.94 per 100,000 population, with a lower frequency in large urban areas. Grade III and IV astrocytoma were the commonest form of glioma with a peak incidence of 7.53 per 100,000 population in the 50-59 year age group and were more common in males. A study carried out in Carlisle, between 1955 and 1961 by Brewis et al. (1966), indicated that the average annual incidence of all intracranial tumours was 12.4 per 100,000 and average incidence of primary brain tumours was 6.0 per 100,000 population. Another epidemiological survey of 526 patients with primary tumours of the brain and spinal cord in South East Wales indicated the average annual incidence rate for all types of primary CNS tumours to be 5.6 per 100,000 population. Grade III and IV astrocytomas were the most common types of tumour, with a peak incidence of 8.4 per 100,000 in the 55-64 year age group (Cole et al., 1989).

1.1.2. Analysis by histology

1.2.1.1. Glioma

The most common nervous system tumours are those arising from neuroepithelial cells of brain and spinal cord and are known as gliomas. These tumours account for 40-50% of all intracranial tumours. The annual incidence rate of gliomas increases with age and there is a peak incidence of 8.4 per 100,000 population in the 55-64 year age group. The most commonly occurring glioma is the malignant astrocytoma with a peak incidence rate of 7.6 per 100,000 in the same age group. In contrast the low grade astrocytoma is the most common astrocytic tumour of childhood with a peak incidence of 0.9 per 100,000 between the ages of 5 and 14 years. However, medulloblastoma is the commonest malignant intracranial neoplasm of childhood, accounting for up to 25% of all such intracranial tumours (Cole et al., 1989).

1.1.2.2. Meningioma

Meningiomas are common tumours and account for 19% of all intracranial neoplasms. They are generally benign, well circumscribed and slow growing. They have an average annual incidence of 1.23 per 100,000. Meningioma are more common in older people, having a peak of 2.40 per 100,000 population in the 60-69 year age group. There is a marked predominance in females, with an incidence of only 0.64 per 100,000 in males and 1.76 per 100,000 in females (Barker et al., 1976). The incidence of meningiomas in Africa is greater than that reported for the developed countries. This may be because of poorer diagnostic facilities.
Therefore, in some African countries, benign tumours may be the only sort of intracranial tumour to reach a neurosurgeon and it is possible that the true total brain tumour incidence is similar to that elsewhere (Templeton, 1973).

1.1.2.3. Pituitary tumour

Pituitary adenomas constitute about 8% of all the CNS tumours and arise from the cells of the anterior pituitary gland. The annual incidence rate of 1-2 per 100,000 population, reported by Percy et al. (1972) is similar to those reported by Brewis et al. (1966).

1.1.3. Aetiology

1.1.3.1. Development of brain tumours in well characterised genetic diseases of the nervous system

The aetiology of most CNS tumours is unknown although some tumours of the nervous system are associated with well characterised genetic diseases such as Von-Recklinghausen's disease, tuberous sclerosis, retinoblastoma, Li-Fraumeni syndrome and Turcot's syndrome.

(i) Neurofibromatosis

Neurofibromatosis is a classic form of the commonest autosomal dominant disorder involving the nervous system.

Type 1 neurofibromatosis (Von-Recklinghausen's disease)

NF1 is an autosomal dominant disorder ranging in incidence from 1:2000 to 1:3000 of the population. It is characterised by markedly variable clinical features and by a strong predisposition to malignancy. The major clinical defining features of the disease are multiple cafe au lait spots, peripheral neurofibromas and Lisch nodules, which are present in virtually all adults patients with the disease and upon which the diagnosis is based. Less common features include short stature and macrocephaly. Although it has long been established that NF1 is caused by an autosomal dominant gene mutation, yet how this produces its varied phenotypic features is still not understood. The most common tumours of the central nervous system in NF1 are acoustic neuromas, meningiomas and optic nerve gliomas. These are all slowly growing lesions that may be present for years before symptoms become severe enough to warrant medical attention. Glial tumours
occasionally complicate NF1 cases, including astrocytomas and ependymomas which may involve the cerebral hemispheres, posterior fossa or spinal cord. These tumours tend to occur in adults, but may be seen in children. Seizures and mental retardation are seen more commonly in NF1 patients than in the normal population. Localised hypertrophy, which may involve any body segment, is one of the peripheral manifestations (Rubinstein et al., 1981).

**Type 2 neurofibromatosis**

The major defining features of this form of neurofibromatosis are bilateral acoustic neuromas, frequently in association with other CNS tumours (particularly meningiomas) and a few cutaneous manifestations, although Lisch nodules are not seen in this condition. It has been demonstrated that the loss is of genes on chromosome 22 in the tumour tissue, suggesting the existence of a locus on chromosome 22 involved in the development of both sporadic and inherited acoustic neuromas (Seizinger et al., 1986).

(ii) **Tuberous sclerosis (TS)**

Tuberous sclerosis (TS) is a complex genetic disorder characterised by the formation of multiple hamartomas. An estimate of prevalence based on patients studied in the Oxford region yielded a TS rate of 1 in 29,000 for persons under 65 years, 1 in 21,500 for those under 30 years, and 1 in 15,400 for children under 5 years of age (Gomez, 1987). Tuberous sclerosis may affect almost any body organ although it is most often characterised by lesions in the skin, brain, retina, kidneys, heart or lungs. TS most often presents with seizures, mental retardation, intracranial hypertension and focal neurological deficit. Intracranial hypertension may be due to the obstruction of the CSF circulation at the foramina of Monro and by the presence of tumour in the third or fourth ventricles (Gomez, 1988). It is also an autosomal dominant inherited disease, although most cases are new mutations. There have been suggestions that the gene for TS is on chromosome 9, linked to the ABO blood group locus at 9q34 or on chromosome 11 (Fryer et al., 1987).

(iii) **Retinoblastoma (RB)**

Retinoblastoma is a tumour arising multifocally in the retina of one or both eyes, in new-born or young children. RB has an incidence of about 5 per 100,000 children and between quarter and one third of these cases are bilateral. The disease seems to occur with comparable frequency in most geographic regions and ethnic groups. About one third of the cases are thought to be hereditary and the
mode of inheritance superficially appears to be autosomal dominant. However, further molecular studies indicates that inheritance of a tumour suppressor gene on chromosome 13 is important.

(iv) Li-Fraumeni syndrome

Li-Fraumeni syndrome is a dominantly inherited predisposition to sarcomas, breast cancer, brain tumours, leukaemia, lung and laryngeal cancer and adrenal cancer. Most of the patients with this syndrome do get cancer and usually develop multiple tumours before the age of 45 years. It appears that these patients have a constitutional mutation in the p53 gene which is inherited in an autosomal dominant manner. Further mutation occurring in the normal allele give rise to multiple tumours which occur at younger ages than their sporadic counterparts (Lee, 1991).

(v) Turcot's syndrome

Turcot et al. (1959) described a brother and sister with polyposis of the large bowel and tumours of the central nervous system (glioma and medulloblastoma). Further reports which have appeared in the literature since then suggest the presence of a clinical syndrome linking bowel and brain cancers. The clinical symptoms arise most often in the second decade of life, with patients initially developing colonic polyps and malignancy followed by neurological signs of brain tumour and diarrhoea resulting from polyps of the colon (Jarvis et al., 1988). The genetic basis of this disease is still uncertain, although these patients do not seem to have germ line mutation in p53.

1.1.3.2. Environmental factors

(i) Radiation

Ionising radiation has been implicated as a cause of human brain tumours and in experimental animals (Zulch, 1986; Pilkington and Lantos, 1990). Modan et al. (1974) retrospectively followed up for between 12 and 23 years, 11,000 children who received cerebral irradiation for ringworm of the scalp (tinea capitis). These individuals had a significantly higher risk of both malignant and benign head and neck tumour as well as tumours of the brain, the parotid gland and the thyroid. Anderson and Treip (1984) also reported three cases of radiation induced astrocytoma, meningioma and ependymoma after irradiation for CNS malignancy. There is also an increased risk of second intracranial tumour in
patients with pituitary adenoma treated with surgery and radiotherapy (Brada et al. 1992).

(ii) Chemical carcinogens

The discovery of the carcinogenic action of nitroso compounds by Magee and Barnes (1956) and later its introduction into the field of experimental neuro-oncology, mainly through the work of Druckrey and his colleagues (1965), greatly increased the possibilities for the study of the pathogenesis of neural tumours. Polycyclic aromatic hydrocarbons and nitroso compounds are the two most widely used chemical carcinogens for the induction of brain tumours in animals. Experimental brain tumours in animals can also be produced by chemical carcinogens like nitrosoureas. A single dose of N-ethyl N-nitrosourea (ENU) to pregnant rats after the 15th day of gestation results in a high incidence of glial tumours in the offspring. Neurological signs of neoplasia in these animals normally become evident at around 250 days. Such models have proved valuable in studying the early changes which occur in the nervous system before the appearance of a tumour (Pilkington and Lantos, 1990). Moreover, epidemiological studies have suggested a link between occupational exposure to chemicals and an increased incidence of intracranial tumours, the workers in the rubber manufacturing industry (Selikoff, 1982).

1.1.3.3. Biological factors

A variety of both DNA and RNA viruses have been found to induce brain tumours in experimental animals. Two groups of DNA viruses, the adenoviruses and the papovaviruses have been used extensively for neuro-oncological studies. Brain tumours have been induced using human adenovirus 12, simian adenovirus 7 and 20 and the avian adenovirus in laboratory rodents. The yield of tumour using these viruses is between 80 and 93%, but the latency may be in excess of nine months and the tumours produced resemble sarcoma or undifferentiated neuro-ectodermal tumours rather than high grade gliomas. (Bullard and Bigner, 1980). Neonatal animals appear to be the most susceptible, to induction of tumour in this way (Cheville, 1966). However there is very little evidence to support the view that human brain tumours are virus induced (Zulch, 1986).

1.1.3.4. Physical factors

The "misgeneration" theory of the classical pathologists (Zulch, 1986) has suggested that traumatic alteration of neuroepithelial tissue can trigger
autonomous growth during the regenerative process. Analysis of statistical data on brain tumours in a series of brain injured patients has suggested a link between the development of brain tumour and head trauma. Cushing and Eisenhardt (1938) considered head injury to be of importance in the development of meningiomas. Although others have found that brain tumour was not related significantly to the brain trauma (Parker and Kernohan, 1931). However, cerebral contusion may play some part in aggravating the clinical symptoms of a tumour already present.

1.1.4. Nervous system tumour classification

1.4.1.1. Gliomas

The exact pathological diagnosis of a brain tumour has an important effect on its management and prognosis. To achieve this, a common classification system or language has to be developed which can be widely understood by the workers in this field. The current pathological classification of astrocytomas is based on the histogenetic scheme proposed by Bailey and Cushing in 1926. Bailey and Cushing, (1926) studied more than 400 verified gliomas taken at operation and 167 necropsy specimens. In this study, Cushing developed a new approach correlating macroscopic appearance, point of origin, method of growth and spread together with the life history and with the cellular architecture of the tumour. This was based on the resemblance of tumour cells to embryonic cells in different stages of differentiation. This classification was later modified by Bailey (1933) to include other types of glioma, medulloblastoma, spongioblastoma, ependymoma and oligodendroglia. This classification proved to be useful clinically because of the correlation between the histological appearance of the tumour and survival. In an attempt to simplify the diagnosis of gliomas, Kernohan and Sayre (1952) introduced a system of grading the tumours based on the work of Broders (1926) and suggested that microscopic appearance of a tumour could be correlated with prognosis. The World Health Organisation (WHO) has also introduced a classification system in 1979 which has recently been revised. This classification based primarily on the microscopic characteristics of the tumour seen with light microscope (Kleihues et al. 1993) is shown in Table 1.1.
Table 1.1. Histopathological classification of tumours of neuroepithelial tissue based on the revised WHO classification (Kleihues et al., 1993)

A. Astrocytic tumours
1. Astrocytoma
   (a) fibrillary
   (b) protoplasmic
   (c) gemistocytic
2. Anaplastic (malignant) astrocytoma
3. Glioblastoma
   (a) Giant cell glioblastoma
   (b) Gliosarcoma
4. Pilocytic astrocytoma
5. Pleomorphic xanthoastrocytoma
6. Subependymal giant cell astrocytoma (Tuberous sclerosis)

B. Oligodendrogial tumours
1. Oligodendroglioma
2. Anaplastic (malignant) oligodendroglioma

C. Ependymal tumours
1. Ependymoma
   (a) Cellular
   (b) Papillary
   (c) Clear cell
2. Anaplastic (malignant) ependymoma
3. Myxopapillary ependymoma
4. Subependymoma

D. Mixed gliomas
1. Mixed oligo-astrocytoma
2. Anaplastic (malignant) oligo-astrocytoma
3. Others

E. Choroid plexus tumours
1. Choroid plexus papilloma
2. Choroid plexus carcinoma

F. Neuroepithelial tumours of uncertain origin
1. Astroblastoma
2. Polar spongioblastoma
3. Gliomatosis cerebri

G. Neuronal and mixed neuronal-glial tumours
1. Gangliocytoma
2. Dysplastic gangliocytoma of cerebellum (Lhermitte-Duclos)
3. Dysembryoplastic neuroepithelial tumour
4. Ganglioglioma
5. Anaplastic (malignant) ganglioglioma
6. Central neurocytoma
7. Paraganglioma of the filum terminale
8. Olfactory neuroblastoma (Esthesioneuroblastoma)
   (a) Olfactory neuroepithelioma

H. Pineal tumours
1. Pineocytoma
2. Pineoblastoma
3. Mixed pineocytoma / pineoblastoma

I. Embryonal tumours
1. Medulloepithelioma
2. Neuroblastoma

A comparison is shown in Table 1.2 between the Kernohan and WHO grading of the astrocytic tumours.

Table 1.2. A comparison between the Kernohan and WHO grading in astrocytic tumours

<table>
<thead>
<tr>
<th>Description</th>
<th>Kernohan grade</th>
<th>WHO grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile astrocytoma</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Diffuse astrocytoma</td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>II, III</td>
<td>III</td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>IV</td>
<td>V</td>
</tr>
</tbody>
</table>

Recently Daumas-Duport et al. (1988) suggested a simple objective numerical grading for astrocytic tumours based on nuclear atypia, mitosis, endothelial proliferation and necrosis. The application of this grading system was reported in a series of astrocytomas treated with radiation therapy at the Mayo Clinic between the years 1960 and 1969. The attractions of this particular scheme are that it correlates better with survival and uses a small number of objective features to classify the tumours. This method results in a score which is translated into grades
as follows: none of the criteria - grade 1, one criterion - grade 2, two criteria - grade 3 and three or four criteria - grade 4. Kim et al. (1991) also reviewed 251 cases of supratentorial astrocytomas and correlated prognosis in these patients using the Daumas-Duport grading system. The period of survival was found to be significantly associated with histological grade. Similarly, Revesz et al. (1993b), studied reliability of histological diagnosis in 419 gliomas biopsied by image-guided stereotactic technique. Their study has shown that the different grades of astrocytic tumours obtained by using the Daumas-Duport criteria correlated better than Kernohan grading with survival of patients.

1.1.4.2. Meningioma

These are frequently slow growing, encapsulated tumours which, in contrast to glioma, cause displacement of brain tissue without infiltration. They may arise from a variety of sites in the cranium. According to the WHO classification meningiomas have been subdivided based on their microscopic appearance as follows:

Table 1.3. Histopathological classification of meningeal tumours based on the revised WHO classification (Kleihues et al. 1993)

A. Tumours of meningotheilial cells

1. Meningioma

   (i) Meningotheliomatous
   (ii) Fibrous (fibroblastic)
   (iii) Transitional (mixed)
   (iv) Psammomatous
   (v) Angiomatous
   (vi) Microcystic
   (vii) Secretory
   (viii) Clear cell
   (ix) Choroid
   (x) Lymphoplasmacyte-rich
   (xi) Metaplastic variants (xanthomatous, myxoid, osseous, cartilaginous etc.)

2. Atypical meningioma

3. Papillary meningioma
4. Anaplastic (malignant) meningioma

B. Mesenchymal, non-meningothelial tumours

Benign neoplasms

1. Osteocartilaginous tumours
2. Lipoma
3. Fibrous histiocytyoma
4. Others

Malignant neoplasms

5. Haemangiopericytoma
6. Chondrosarcoma
   (i) Mesenchymal chondrosarcoma
7. Malignant fibrous histiocytoma
8. Rhabdomyosarcoma
9. Meningeal sarcomatosis
10. Others

C. Primary melanocytic lesions

1. Diffuse melanosis
2. Melanocytoma
3. Malignant melanoma
   (i) Meningeal melanomatosis

D. Tumours of uncertain histogenesis

1. Haemangioblastoma (Capillary haemangioblastoma)

1.1.4.3. Pituitary adenoma

Early in this century, pituitary tumours had been divided into acidophilic, basophilic and chromophobic adenoma (Cushing, 1912). Acidophilic adenomas were thought to be associated with acromegaly or gigantism, basophilic adenomas were implicated in the development of Cushing's disease, and chromophobic adenomas were regarded as endocrinologically non-functioning tumours. The inadequacy of this classification became obvious as soon as it was shown that acromegaly and Cushing's disease may be accompanied by chromophobic adenomas. This classification lost its further credibility when McCormick and
Halmi (1971) proved that true chromophobic (agranular) pituitary tumours do not exist.

Table 1.4. Tumours of the anterior pituitary classified according to conventional staining.

A. Pituitary adenoma
   1. Acidophil
   2. Basophil (mucoid-cell)
   3. Mixed acidophil-basophil
   4. Chromophobe
B. Pituitary adenocarcinoma

In view of substantial progress in pituitary pathology and due to the use of electron microscope and immunocytochemistry, a new classification has been proposed based on hormone content and cellular origin of adenomas (Kovacs and Horvath, 1986).

Table 1.5. Tumours of the anterior pituitary classified according to immunocytochemistry

A. Endocrinologically active adenoma
   1. Prolactin secreting adenoma or prolactinoma
   2. Growth hormone or somatotropic hormone secreting adenoma
   3. Corticotrophic adenoma
   4. Thyrotropic adenoma
   5. Gonadotrophic adenoma
   6. Mixed secretory
B. Endocrinologically inactive adenoma or silent

1.1.5. Pathophysiology of intracranial tumours

The internal volume of the intracranial space is of the order of 1500 ml and 90% of this is occupied by brain tissue. The brain is composed of 80% of water and 10% of CSF or blood and is largely incompressible. Any space occupying lesion within the skull rapidly takes up the reserve volume available by displacement of CSF, and causes increase in intracranial pressure. Raised ICP produces headache, due to stimulation of sensitive nerves in the dura, vomiting, due to stimulation of the centre in the medulla and visual disturbances, caused by papilloedema of the
optic disc. Untreated raised ICP will eventually cause the brain to herniate through the margins of falx or tentorium causing tentorial herniation or coning. Coning can also take place when the cerebellar tonsil is forced by pressure through the foramen magnum and compresses the medulla. Blockage of the normal CSF pathways by small tumours, particularly at the foramen of Monro, the aqueduct of Sylvius and the outlet foramina of the fourth ventricle may give rise to hydrocephalus producing increased ICP. Acute hydrocephalus may produce very severe headache while chronic hydrocephalus may give rise to headache, vomiting and papilloedema. Intracranial tumours may also cause local brain dysfunction. Any supratentorial tumour, either in the cerebral hemisphere or in the surrounding structures may cause epilepsy. The epilepsy may be focal or generalised, motor or sensory. Failure of brain function, particularly dysphasia, hemiparesis and visual loss are also common presenting features. Infratentorial lesions may exert their local effects on cerebellum, medulla and lower cranial nerves and can cause ataxia, severe vomiting, deafness and difficulty in swallowing.

1.1.5.1. Pathological and clinical factors which predispose towards recurrence in meningioma

Meningiomas are encapsulated intracranial benign tumours which may be permanently cured by surgical removal. However, on occasion, late recurrence does take place, even after what appeared to be satisfactory macroscopic removal, in perhaps 15-20% of cases (Cushing and Eisenhardt, 1938, Simpson, 1957, Adegbite et al. 1983). The development of a recurrence means either that the initial resection was inadequate or that the neoplastic changes were multifocal or that viable tumour was incidentally implanted during an otherwise successful resection (Simpson, 1957). However, the chief causes of recurrence are probably unnoticed invasion of a dural venous sinus or spread across a free dural septum. Infiltration of bone and the presence of multiple primary tumours are other causes of recurrence (Simpson, 1957). Crompton and Gautier-Smith (1970) suggested that the syncytial meningiomas are most likely to recur and fibroblastic ones least so. Mitotic figures, focal necrosis and cerebral infiltration are factors suggestive of eventual recurrence. Angioblastic meningiomas, however, in spite of their frequently containing mitoses and regions of focal necrosis, do not appear to have a tendency to recur if completely removed. Borovich and Doron (1986) advocated wider resection of dura around globular meningiomas in order to reduce the incidence of clinical growth after total macroscopic removal. They also demonstrated the regional multicentric origin of some meningiomas and suggested a hypothesis that many areas of dura carrying meningotheelial cells
seem to be capable of forming tumours, however the reasons for multiplicity are not clear. Other hypotheses include spread along the cerebrospinal fluid (CSF) pathways and venous transmission (Boylan and McCunniff, 1988). Adegbite et al. (1983) in their review of 114 surgically treated patients with intracranial meningiomas found that the sex of patients, anatomical site, histological subtype and post-operative radiotherapy had no statistically significant influence on recurrence. The only factor found to have a significant influence on this was the extent of initial surgery for example total or partial removal. However, in another study, Christensen et al. (1983) found a higher recurrence rate in parasagittal meningiomas and in younger patients. Though Mirimanoff et al. (1985) did not find any difference in the recurrence progression rates according to the patient’s age or sex or the duration of symptoms, yet it was reported at 5 years as 37% for meningiomas partially resected and 7% for lesions totally removed. Boylan and McCunniff (1988) found that despite aggressive surgery recurrence rates of approximately 9% are seen and this may be much higher with the removal of malignant meningiomas.

1.1.6. Clinical features

Primarily the pattern of symptoms resulting from a brain lesion depends on its type and location. The local and generalised effects of an intracranial lesion may combine to give the following symptoms: epilepsy, headache, vomiting, visual loss, hemiparesis, diplopia and change in the level of consciousness. The presenting symptoms and signs of patients are due to raised intracranial pressure, and local or general dysfunction of the brain.

1.1.6.1. Epilepsy

Epilepsy is the initial symptom of cerebral glioma in nearly 40% of cases (McKeran and Thomas, 1980). Epilepsy, particularly epilepsy of late onset, is a common feature of brain tumour as well as of other organic brain disease. Focal epilepsy, temporal lobe attacks or generalised convulsions can all be symptomatic of an intracranial neoplasm. Focal neurological symptoms or signs may provide strong evidence of the presence and probable site of a tumour. These focal signs and symptoms mainly include visual field loss, diplopia, deafness, ataxia, hemisensory loss or hemiparesis and dysphasia (Thomas et al., 1991).
1.1.6.2. Headache

Headache is a common and early symptom to appear in patients with intracranial tumours. The frequency of headache at presentation was found to be 35.2% when investigated at the National Hospital for Neurology and Neurosurgery, London (McKeran and Thomas, 1980). Such headaches usually start early in the morning and gradually becomes severer and longer in duration. It is usually throbbing, bifrontal or occipital in nature and occasionally it can occur at the site of the underlying lesion. Any rise in the abdominal or thoracic pressure may increase the intensity of pain. The headache associated with intracranial tumours is due to the effect of raised intracranial pressure, which produces vascular distension and distortion (McKeran and Thomas, 1980).

1.1.6.3. Vomiting

Any rise in the intracranial pressure is frequently associated with vomiting, which is often worse early in the day or following exertion. It can be associated with nausea but without any abdominal discomfort. Vomiting is generally more frequent and severe when the ventricular system is dilated, particularly with infratentorial tumours and tumours in the fourth ventricle may cause constant vomiting (McKeran and Thomas, 1980).

1.1.6.4. Visual failure

This is a common symptom of raised intracranial pressure and may be the initial presenting symptom in about 5% of patients with intracranial tumours (McKeran and Thomas, 1980). It is due to raised ICP causing papilloedema over a period of time which may result into visual failure and loss of visual acuity. Prolonged raised intracranial pressure may also lead eventually to optic atrophy with complete blindness. Tumours in the pituitary gland may give rise to field defects particularly bitemporal hemianopia.

1.1.6.5. Changes in the level of consciousness and behaviour

Any rise in the intracranial pressure may bring a change in the conscious level, ranging from drowsiness to deep coma. A frontal lesion may bring changes in personality with intellectual failure (Thomas and McKeran, 1990).
1.1.6.6. Other clinical features

Meningiomas arising in the convexity of the cranial vault may produce dysphasia. Outer third of sphenoid wing meningiomas may give rise to facial weakness, hemiparesis or epilepsy. Meningiomas of the olfactory groove may cause anosmia and may lead to ipsilateral optic atrophy with contralateral papilloedema (Foster-Kennedy syndrome). Basal meningiomas which arise from the clivus or foramen magnum may present with cranial nerve palsies. Prolactinomas may cause amenorrhea, galactorrhea and infertility in young females while in elderly male patients, large size prolactinomas may show invasive features with extrasellar extension. Growth hormone producing tumours usually present with acromegaly or, in children, with gigantism. Those tumours with ACTH production are more often associated with Cushing's disease. Non-functioning pituitary adenomas with suprasellar extension may present with bitemporal hemianopia, headache and optic atrophy (Russell and Rubinstein, 1963).

1.1.7. Prognostic factors

The word "prognosis" in a medical context usually means the prediction of duration, course and final outcome in patients with a given disease, based on information about the individual patient combined with a knowledge of how the disease behaves generally. This is of considerable significance in a disease such as which is usually without a definitive curative treatment and fatal. However, it is known that patients with brain cancer have marked variability in the course of this disease. A knowledge of prognostic factors is therefore useful in understanding the natural history of the disease, to predict the expected course for individual patients and especially to select appropriate treatment for individual patients.

1.1.7.1. Age

Age is of considerable prognostic significance irrespective of treatment in patients with brain tumours. This is particularly marked in patients with malignant glioma; for example in a trial conducted by the Brain Tumour Study Group, the median survival time for patients with malignant glioma under the age of 40 years was 36 weeks while for the patients aged 60 years or more it was only 15 weeks. In another study, there was a statistically significant difference between the survival times for patients under the age of 40 years compared with those aged 60 years or more (Walker et al., 1980). Further trials have confirmed that the age of the patient at the time of diagnosis is inversely related to the length of survival and that age is
weakly related with tumour histology, duration of symptoms and performance status (Byar et al. 1983). In another study conducted at the Westminster Hospital, London, by Scott and Gibberd (1980), patients with intracranial tumours were studied between 1961-1976. It was found that the survival time for the patients aged less than 55 was significantly better than those who were older than 55 years.

1.1.7.2. Tumour location

The anatomical location of the tumour within the brain does not seem to be a particularly important prognostic factor although some authors have suggested that location in the thalamus or basal ganglia is associated with a poorer prognosis (Byar et al., 1983). Occasionally, parietal tumours have been reported as exhibiting poorer prognosis, at least in some analyses (Gehan and Walker, 1977). The lack of consistency in reports suggests that it is a weak prognostic sign.

1.1.7.3. Exact histological grading

Because of the difference in classification systems used by different workers, particularly between those in Europe and the United States, it has often been difficult to assess the exact role of histological grade and outcome in patients with malignant glioma (Sheline, 1976). However, Byar et al. (1983), found in studies by Brain Tumor Study Group (BTSG) that the death rate was 1.5 to 2.0 times higher in patients with glioblastoma multiforme compared to those with anaplastic astrocytoma. Burger and Vollmer (1980) have found that the presence of giant cells in malignant gliomas was associated with prolonged survival, while highly cellular neoplasms had a poorer prognosis than less cellular tumours.

1.1.7.4. Performance status

The performance status at the time of initial diagnosis has been found to be an important prognostic factor. Performance status can be measured using the Karnofsky scale which ranges between 0 and 100 (Karnofsky et al., 1948). Walker et al., 1980, have demonstrated progressively higher death rates as the initial Karnofsky performance status falls. Other performance status scores have been proposed by other workers. Gilbert et al. (1981), measured performance using a simple three point scoring system as functional status: full care, partial self-care and non-self care. The WHO clinical performance status scale is also widely being used in neuro-oncology. It divides patients into five groups (0-4), where 0 is the ability to carry out all normal activity without restriction and 4 is completely
disabled and totally confined to bed. This scale is simple and easy to apply to patients with brain tumours even in retrospective analyses, shown in Table 1.6.

Table 1.6. WHO scale of performance status

<table>
<thead>
<tr>
<th>Score</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Able to carry out all normal activity without restriction.</td>
</tr>
<tr>
<td>1</td>
<td>Restricted in physically strenuous activity but ambulatory and able to carry out light work.</td>
</tr>
<tr>
<td>2</td>
<td>Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours.</td>
</tr>
<tr>
<td>3</td>
<td>Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.</td>
</tr>
<tr>
<td>4</td>
<td>Completely disabled; can not carry out any self-care; totally confined to bed or chair.</td>
</tr>
</tbody>
</table>

1.1.7.5. Epilepsy

A history of seizures has been associated with a better prognosis and may be associated with the presence of a brain tumour in any part of the cerebral hemisphere. In malignant glioma this symptom is the commonest initial presenting symptom and is related to a better prognosis. A possible explanation could be that epilepsy may draw attention to the tumour at an early stage (Scott and Gibberd, 1980). The length of history of fits has been studied by MRC Brain Tumour Working Party (BTWP), 1990 and found it to be of prognostic importance.

1.1.7.6. Duration of symptoms

The duration of symptoms is another variable which has been found to have prognostic significance. In one study, patients who had symptoms for longer than six months had a death rate 50% less than patients who had symptoms for less than six months (Walker et al., 1980). In another BTSG study, those patients who presented with a history of symptoms exceeding 25 weeks were divided into four categories and the median survival time following operation increased as the length of symptoms increased. One possible explanation for the prognostic significance of this variable may be that the patients with long duration of symptoms have more slowly growing tumours.
1.1.7.7. Extent of surgical removal

The extent of surgical removal may be of prognostic significance as it could reflect the size, invasiveness and the location of tumour and its proximity to vital structures. Types of surgical resection can be divided into the following: biopsy only, partial resection and radical resection with or without lobectomy. Scott and Gibberd (1980) have shown that patients with partial or complete removal of tumour survived longer than those patients who were not operated on or who had only a biopsy. While some have reported similar results, others have been unable to show a relationship between extent of tumour resection and survival (Gehan and Walker, 1977, MRC BTWP, 1990). Similarly a study at the National Hospital for Neurology and Neurosurgery, London, showed no significant relationship between extent of tumour resection and survival in 653 patients with glioma, of whom approximately each one third had biopsy, partial or extensive resection (McKeran and Thomas, 1980).

1.1.8. Treatment of brain tumours

The advances in neurological localization and the development of aseptic surgery made possible the beginning of modern neurological surgery. In Glasgow, Sir William Macewen successfully removed a meningioma for the first time in 1879, and in 1884, Mr. Rickman Godlee, in association with the neurologist Dr. Hughes Bennet, partially removed a right parietal glioma (Thomas, 1985).

1.1.8.1. Role of surgery in neuro-oncology

Primarily, surgery for any intracranial mass lesion is carried out for the following reasons:

(i) Establishment of pathological diagnosis

Though CT and MR are excellent at localising and implying the nature of many intracranial lesions, nevertheless they do not identify with certainty the pathological diagnosis. Therefore, biopsy is still a required surgical procedure for the management of patients with intracranial mass lesions if only to confirm that the patient indeed does have a brain tumour and what type it is. This is essential not only for an appropriate treatment in each individual case but also to provide a prognosis for the patient.
(ii) Relief of symptoms

The second purpose is to resect as much tumour as is safely possible to relieve symptoms of raised intracranial pressure and neurological dysfunction and in many cases of benign tumour to achieve radical removal and cure.

(iii) Short-term improvement of quality of life

In the presence of severe neurological disability fatal conditions it may not be justifiable for the surgeon to strive to extend survival with an unacceptable quality of life. A severely disabled patient is a stress not only to the patient's family in particular but also to the society in general. However, intracranial decompressive surgery in patients with malignant glioma improve the quality of life in the short term by preventing hemiplegia, dysphasia or blindness (Hitchcock and Sato, 1964).

(iv) Improvement of survival

Total or partial removal of a intracranial tumour may relieve the symptoms of raised intracranial pressure and prolong survival by avoiding the herniation of brain into foramen magnum or tentorial hiatus.

1.1.8.2. Surgical techniques

(i) Burr hole biopsy

Brain tumour biopsy can be obtained usually without much difficulty through a burr hole in skull. These burr holes are usually sufficient for biopsy, cyst drainage or shunt procedure. However, the success rate has been increased and the morbidity reduced by using stereotactic control (vide infra)

(ii) Craniotomy

This is one of the commonest neurosurgical approaches to the removal of many types of intracranial tumours. It involves the removal and replacement of an osteoplastic bone flap in the skull vault and is usually reserved for the removal of relatively large brain tumours.
a. Glioma

Gliomas in the cerebral hemisphere are diffusely invasive and cannot be removed completely. However, they may often be partially removed, depending upon the site and its relation to vital structures in the brain. Histological examination of an operation specimen is necessary in order to establish a definitive diagnosis. A lobectomy, frontal, temporal or occipital or other types of radical surgery, is often associated with craniotomy for gliomas (Thomas et al., 1991). During the last decade new technical methods including "stereotactic craniotomy" have been introduced to perform radical and accurate resection in the cerebral hemisphere of lesions shown on CT or MRI scan, with reduced surgical mortality (Kelly et al., 1982).

b. Meningioma

When these tumours occur in the skull vault or in the falx, they can be excised radically together with surrounding dura in order to prevent later recurrence. Meningiomas are encapsulated so that total removal is relatively easier. However, meningiomas at the skull base present more technical problems because of the limited surgical access. Since these tumours may also involve the surrounding bone and structures, it may not be possible to remove them completely. The introduction of the surgical microscope, together with use of ultrasonic aspirator (CUSA) and different types of surgical laser have made it possible to remove completely some meningiomas where there is limited surgical access.

c. Pituitary adenoma

Large pituitary adenomas, particularly those with irregular, lateral or frontal suprasellar extensions are generally approached by a transfrontal craniotomy (Symon et al., 1981). However, with the introduction of new techniques of approaching these tumours via the sphenoid or ethmoid sinuses, pituitary microadenomas and selected large pituitary tumours with suprasellar extension may also be removed successfully by these methods.

(iii) Stereotactic surgery

Advances in microneurosurgical techniques, modern neuroanaesthesia and an early diagnosis with CT and MRI have made it possible to remove totally benign lesions. However the situation has not changed generally for malignant tumours of CNS. Although most of the primary and secondary intracranial tumours can be
approached surgically and significant portions can be removed, a complete cure by surgery alone for malignant gliomas is not usually possible. The modern surgical management of CNS tumours has been improved by the development of image guided stereotactic surgery. These techniques have made it possible to access most of the tumours surgically with minimal mortality. Classical stereotactic apparatus has been used for years for an accurate localization of paramedian brain structures, for example in the placement of lesions in thalamic region to treat the movement disorders associated with Parkinson's disease. Gliomas in the thalamus and brain stem or affecting the deep midline structures of the brain, as well as those in the parietal or posterior frontal regions of the dominant hemisphere, may not be resected safely without serious neurological deficit. However, it is possible to obtain biopsies from such tumours and drain cysts, using stereotactic surgical methods linked to CT or MRI scans (Thomas et al. 1986, 1988, Thomas and Nouby, 1989). The introduction of modern radiological imaging with CT and MRI, has revolutionised these techniques. Brown-Roberts-Wells (BRW, Radionics Inc) CT-guided stereotactic frame is one example of such a stereotactic system which has been in use for almost a decade. The extensive use of cranial computed tomography (CT) scanning has resulted in the diagnosis of an increased number of small lesions, many of which are in deep parts of the brain. Diagnostic and therapeutic applications of CT-guided stereotactic methods are emerging including biopsy, drainage procedure, stimulation electrode placement and insertion of interstitial sources for radiation therapy (Thomas et al., 1988). A series of CT-directed procedures was performed by Thomas and Nouby (1989) in 300 patients with deep sited, multiple, diffuse, extensive inflammatory lesions, brain stem haematomas and tumours. A positive diagnosis was obtained in 271 cases (92.8%) whilst no diagnosis was obtained in 21 cases (7.2%). Therapeutic results were also obtained in 8 cases (2.7%) of brain stem haematoma aspiration. Thomas and Nouby (1989) suggested that because of the importance of an accurate diagnosis in order to avoid inappropriate therapy, together with the relative safety of the technique, CT-directed stereotactic biopsy should be considered in all patients with deep seated, multiple and diffuse brain lesions. The BRW system has been modified for use with MRI or PET images and Thomas et al. (1986) also described MRI directed biopsy of brain tumour. However, the application of the BRW neurosurgical frame by pins to the skull vault is an invasive procedure, the frame can remain in position for only a limited period of time and cannot be accurately relocated. The Gill-Thomas stereotactic frame is a non-invasive relocatable frame based on the BRW neurosurgical frame and was developed for stereotactic biopsy and multiple imaging at the National Hospital for Neurology and Neurosurgery, London, (Gill and Thomas, 1989). Relocation is achieved via an individually moulded impression of the upper dentition. This
A relocatable frame allows accurate 3-dimensional target localisation by CT, MRI, PET, cerebral angiography and precise isocentric positioning for radiotherapy. The new prototype is manufactured in aluminium alloy and has been adapted successfully at the Royal Marsden Hospital, London for fractionated stereotactic radiotherapy (Graham et al., 1991). In another study at the National Hospital for Neurology and Neurosurgery, the Gill-Thomas locator along with BRW stereotactic system was used for interstitial brachytherapy in 18 patients. In patients with good upper dentition the frame was highly relocatable yielding an accuracy within 0.5 mm on all pre-operative tests. However, in two edentulous patients, the accuracy was slightly less at between 1 and 1.5 mm. The results in patients with partial dentition were similar to those who had a full set of teeth (Sofat et al., 1992). The majority of cerebral lesions revealed by CT scanning or MR imaging require a tissue diagnosis before appropriate therapy can be commenced. Brain biopsy with CT stereotactic techniques have reduced mortality to less than 1% and achieved a diagnostic accuracy rate as high as 96%. A definitive pathological diagnosis can also be achieved with the same system in intrinsic brain stem lesions (Bradford et al., 1987). In a recent study by Kratimenos et al. (1992), a series of 45 brain lesions were approached stereotactically with CT or MRI guidance. Haematoma was preoperatively diagnosed in 10 cases and the procedure was for therapeutic aspiration. Of 35 cases where the diagnosis was uncertain, although intrinsic tumour was suspected, positive results were obtained in 33, while unexpected findings of granuloma, lymphoma, angioma, vasculitis etc. were found in over 10% cases. It has become apparent that magnetic resonance imaging may reveal cerebral lesions which are not clearly visualised by CT scanning. It may be necessary to use a modified stereotactic system, compatible with MRI, in order to get tissue samples from such cases. Bradford et al. (1987) in a study, showed that it has been technically feasible to perform MRI-directed stereotactic biopsy in cases where CT controlled biopsy would not be possible and in the majority of cases to obtain a pathological diagnosis.

(iv) Occipital craniectomy

In the posterior cranial fossa, surgical exposure is generally obtained by craniectomy involving the piecemeal removal of parts of the occipital bone, often together with the arch of the atlas.

1.1.8.3. Post-operative radiotherapy in the management of brain tumours

Malignant gliomas still represents a major therapeutic challenge for post-operative radiation. Radiotherapy significantly increases the median survival of patients
with malignant glioma by 100-150% over surgery alone (Walker et al., 1978). When Jelsma and Bucy (1967), treated glioblastoma multiforme radiation following surgery, they found that the survival time improved in patients who had extensive surgery followed by radiation therapy of 45-50 Gy in fractionated doses. A prospective randomised trial in high grade gliomas conducted by the BTSG (Walker et al, 1978) showed a significant improvement in median survival, from 14 weeks in a group of patients who received only surgery and best conventional care to 35 weeks for those who received in addition radiotherapy. In another study, a total of 474 adult patients with malignant glioma grade were randomised to receive either 45 Gy (in 20 fractions over 4 weeks) or 60 Gy (in 30 fractions over 6 weeks) following operation. Using 2:1 randomisation scheme, 318 patients were allocated the 60 Gy course and 156 the 45 Gy course. The median survival was significantly increased from 9 months in the group receiving 45 Gy to 12 months in the group receiving 60 Gy (Bleehen and Stenning, 1991). Although many meningiomas can be completely excised, nevertheless in certain cases this may not be possible, and adjuvant radiotherapy has been applied in cases of incomplete removal. Meningiomas, however, are of limited radiosensitivity, although inoperable meningiomas may slowly decrease in size after irradiation and recurrence following incomplete excision can be prevented or delayed by post operative radiotherapy. Wara et al. (1975) reviewed 92 patients who had subtotal removal of the tumour, 58 did not receive postoperative irradiation and the recurrence rate in these patients was 74%. Thirty four patients had post-operative radiotherapy and the recurrence rate was reduced to 29%. Carella et al (1982) reviewed 68 patients with meningioma, and reported positive results in the treatment of subtotal excised and inoperable meningiomas. 43 patients underwent operation followed by radiation, 14 patients received irradiation at recurrence after operation, and 11 patients did not have operation and had radiation therapy only as the primary therapy. Carella et al. (1982) believed that radiation therapy has an established role in the treatment of meningiomas. Similarly Barbaro et al. (1987) reporting a study on the efficacy of radiotherapy in partially resected meningiomas showed that 60% of cases recurred after surgery alone, but post operative irradiation reduced this to 32%. These results appear to confirm that radiotherapy is beneficial in the treatment of subtotal resected meningiomas. Radiotherapy has also played an important role in the treatment of pituitary tumours. Pistenmma et al. (1975) in a review of the literature found that the five year recurrence rate in pituitary tumours following surgery alone was 22-71% but this was reduced to 8-21% following postoperative radiotherapy. The better results combining surgery and post operative radiotherapy can be achieved by simple decompression usually by the trans-sphenoidal route followed by radiotherapy (Thomas et al., 1991).
1.1.8.4. Role of chemotherapy in the management of brain tumours

The poor prognosis of malignant gliomas following surgery and radiation therapy has motivated the search for effective chemotherapy. The prognosis of an individual patient with a cerebral glioma depends more on precise tumour type and grade, as well as on the patient's age and his clinical performance score after surgery, than on operative, radiotherapeutic or chemotherapeutic interventions. A standard therapy for malignant glioma includes an operative resection to reduce the bulk of tumour followed by external beam radiation with 55-60 Gy delivered in 25-30 fractions over six weeks. Chemotherapy may be given as an adjuvant therapy following radiation, or alternatively at the time of tumour recurrence. Although nitrosoureas have a significant biological effect on cerebral glioma, this effect is modest, and therefore multiple drug combinations have been attempted in order to improve results. A detailed review of chemotherapy for brain tumours was carried out by Kornblith and Walker, (1988). They found in various BTSG studies, patients with malignant gliomas had undergone a surgical procedure. Radiotherapy was given in the range of 50-60 Gy delivered in 20-25 fractions. Various cytotoxic drugs were also used such as BCNU, methyl-CCNU, ACNU, CCNU, PCNU, cisplatin, diaziquone (AZQ), hexitol derivatives dibromodulcitol (DBD) and its metabolite dianhydrogalactitol (DAG) as well as VM-26 and VP-16 have been used in the treatment of malignant gliomas. In further studies conducted by the BTSG, 467 patients with histologically proved malignant glioma were randomised to receive one of the four treatment regimens: Me-CCNU alone, radiotherapy alone, BCNU plus radiotherapy, or Me-CCNU plus radiotherapy. Radiotherapy used alone or in combination with nitrosourea significantly improved survival in comparison with Me-CCNU alone. The group receiving BCNU plus radiotherapy had the best survival. This study suggested that it is best to use radiotherapy in the post-surgical treatment of malignant glioma (Walker et al., 1980). There appears to be no markedly better effect from combination chemotherapy than from nitrosourea alone, although the combination of procarbazine, CCNU and vincristine may be better as an adjuvant regime for grade III gliomas than is BCNU alone (Levin et al. 1985; 1990). Jeremic et al. (1992) performed a nonrandomized adjuvant chemotherapy programme comparing postradiotherapy PCV with a teniposide plus lomustine combination. One hundred thirty-nine patients were treated: 36 anaplastic astrocytoma and 31 glioblastomas in the PCV arm and 37 anaplastic astrocytomas and 29 glioblastomas in the teniposide plus lomustine arm. Prognostic factors were equally distributed in the two treatment groups. The PCV group had a doubling of the time to tumour progression and the median survival, supporting similar results reported by Levin et al. (1990). In an attempt to increase drug delivery,
Shapiro et al. (1992) have compared intraarterial versus intravenous BCNU, with or without intravenous 5-fluorouracil in patients with malignant gliomas as an adjunct to radiotherapy. The survival rate between the intravenous and intraarterial BCNU patients was not different. However, the evidence from numerous clinical trials confirms that chemotherapy, particularly with nitrosoureas or procarbazine, does have a significant biological effect in glioma, producing modest increases in median survival and in the rate of long term survival.

Section II

1.2. Analysis of cell kinetics

In normal tissue, for example bone marrow and skin, the cell population remains stable as cell loss is balanced against cell division. However in neoplastic tissue this balance is disturbed and there is a progressive and uncontrolled increase in cell division. Cell kinetics studies have been made extensively in the last 30 years in an attempt to understand the growth of tumour and to improve therapy.

1.2.1. The cell cycle

The cell cycle is the term used to describe the period between one mitosis and the next. For most of the cell cycle little can be seen morphologically which yields clues to the molecular events leading to cell division. The cell is said to be in interphase. However, morphological events which occur during start of mitosis in M phase have been described in considerable detail. Briefly, during prophase, individual chromosomes condense and are seen, after staining to be separate but intertwined entities. During metaphase, the nuclear membrane is lost and 46 chromosomes line up in an arrangement termed the metaphase plate, and each chromosome can be seen to consist of two identical chromatids. Anaphase follows, where the chromatids separate and the spindle mechanism moves the chromatids apart to each end of the cycle. This is followed by telophase where the chromatids become less condensed and primarily daughter nuclei and the cytoplasm divides. Each new cell than enters interphase. The cell cycle has been divided into four phases: \( G_1 \), the period between mitosis and the onset of DNA synthesis; \( S \), the period of DNA synthesis; \( G_2 \), the period between completion of DNA synthesis and mitosis and \( M \), the period of mitosis. Although phases of the cell cycle vary to some extent, the greatest variation occurs in the duration of \( G_1 \), which may be short in rapidly dividing tissues or so long that the cell appear to have altogether
ceased progressing through the division of cycle. Cells in such a quiescent G₀ state are said to be in G₀ phase (Lajtha, 1963).

1.2.1.1. Cell cycle time \( (T_c) \)

Cell cycle time is the period of time required for a proliferating cell to progress from one mitotic division to the next. Not all the cells within a given tumour have the same cycle time. A wide range exists, and cell cycle times are usually expressed as an average in a particular tumour (Hoshino and Wilson, 1975). A typical cell cycle takes 24 hours to complete, although this may be as short as 8 hours or as long as a year in adult animals, with most of the variability being in the length of the G₀ phase. Cells in different tissues, in different species and at different stages of embryonic development have cell cycles that vary greatly in duration. The growth of a tissue is not just a function of the number of mitoses or the length of cell cycle time. Other factors such as tumour doubling time and cell loss are also important. In carcinoma of stomach, the calculated cell cycle time \( (T_c) \) has been shown to be 72 hours and in carcinoma of bronchus, has been calculated as 196-260 hours (Baserga, 1981). The cell cycle time for glioma has been shown fairly constant as 48 hours by Hoshino et al. (1981). If one knows the growth fraction (GF) and mitotic index (MI), cell cycle time can be obtained (Hoshino et al., 1975).

\[
T_c = \frac{t \log 2}{\log (1 + MI/GF)}
\]

Where \( t \) is the interval between the administration of mitostatic agent and the time of tumour excision.

1.2.1.2. Growth fraction

The growth fraction (GF), comprises the proliferating cells within a tumour cell population. Growth fraction is the ratio of proliferating cells to the non-proliferating cells. The mathematical definition for growth fraction is shown as follows:

\[
GF = \frac{\text{Number of proliferating cells}}{\text{Total number of cells in population}}
\]
1.2.1.3. Potential doubling time ($T_{pot}$)

The rate of cell production within a tumour can be determined by two methods, either a stathmokinetic technique based on the rate of accumulation of cells in metaphase after the administration of a mitotic arrest agent like cholchicine, or by measuring both the proportion of cells in S phase ($L_I$) and its duration ($T_s$) (Steel, 1977). $T_{pot}$ is defined as:

$$T_{pot} = \lambda \frac{T_s}{L_I}$$

The constant ($\lambda$) generally lies between 0.7 and 1.0 as discussed by Steel (1977). The potential doubling times of human tumours are invariably much shorter than volume doubling times, ranging from values of a few days in lymphomas to a month or more in breast tumours and in some sarcomas.

1.2.1.4. Cell loss

Cell loss is a common feature of all tissues whether neoplastic or normal. In normal tissue, mature cells complete their function and die in a specific time. However, in neoplastic tissues cells may also die and be removed from the tumour through nutritional deficiency, vascular disruption and overcrowding of the cells. Cell loss in tumour is recognised by the presence of individual dead cells, massive or focal necrosis and exfoliation of cells from the tumour mass, although quantitative estimation of the rate of loss is difficult to produce. Steel (1977) defined the cell loss factor, CLF, as:

$$CLF = \frac{1 - T_o}{T_{pot}}$$

Where, $T_o$ is the theoretical doubling time and $T_{pot}$ is the tumour doubling time. An increase in tumour size is usually accompanied by greater cell loss and a lower growth fraction. Therefore CLF is dependent on the size and nature of tumour (Steel, 1977). CLF represents the rate of cell loss as a fraction of the rate at which cells are added to the total population by mitosis. Apoptosis is a mode of cell death in which single cells are deleted in the midst of living tissue. It is characterised by a set of structural changes with shrinkage in cell volume, loss of contact with the neighbours and loss of specialised surface elements such as microvilli and cell-cell junctions. The most outstanding internal structural changes, however occurs in the nucleus. Chromatin condenses into dense granular
caps under the nuclear membrane. Apoptosis is thought to be a significant component of cell loss from many tumour types (Wyllie, 1992).

1.2.2. Therapeutic consideration of cell kinetics

Frequent movements between cycling and non-cycling populations are observed in glioblastoma. Partial removal might stimulate cells in the non-proliferating pool to move into the proliferating pool and result in more rapid filling of the tumour site. In contrast the most important cell kinetic characteristic of a well differentiated glioma may be the absence of traffic from the non cycling pool to the cycling pool, therefore maximum tumour bulk can be removed as there is less contraindication to the maximum removal of tumour tissue (Hoshino, 1984).

Hoshino and Wilson (1975) were of the opinion that cycle or phase dependent drugs should not be used to treat a low-growth fraction tumour because they may not reduce the tumour size to any beneficial extent. If cells in the unaffected non-proliferating pool remain in that stage after an effective cycle-specific agent destroyed all proliferating cells, the tumour size would not subsequently increase. However, the cells from the non proliferating pool actually would replace the killed cells, restoring and even increasing the number of cells in the proliferating pool (Hoshino and Wilson, 1975).

1.2.3. Growth kinetics of brain tumours

The brain is a unique organ from the growth kinetic point of view, since neurones become incapable of cell division shortly after birth and although there is some background turnover of glial cells, by and large normal brain contains very few proliferating cells. This is clearly in contrast to neoplastic tissues occurring in the brain which usually contain many proliferating cells (Hoshino and Wilson, 1975). Therefore immunohistochemical detection of proliferating cells may be a valuable indicator in therapy in prognosis or in determining therapy. Although conventional paraffin wax histological techniques are invaluable in providing information about the type of tumour, with the exception of identifying mitotic cells, they do not provide any information about the proliferative capacities of a particular tumour.

1.2.3.1. Count of mitotic figures

Simple count of mitotic figures in tissue sections was one of the earliest kinetic techniques to determine the mitotic index (MI). Crompton and Gautier-Smith, (1970) used MI to predict recurrence of meningioma. It appears to be a direct
indicator of cell production and has been used as a guide to proliferative activity. The percentage of mitotic index reflects the rate of proliferation, although this indication may not be exact and true in its magnitude as any fault in the fixative procedure may give a faulty result, and in addition, the variation in duration of observation of the process may also affect the count (Steel, 1977). Mitoses are not always seen in even highly malignant lesions or recurrent tumours (Burger et al., 1986). The mitotic index was defined by Steel, (1977) as:

\[ \text{Mitotic Index} = \text{mitotic rate} \times \text{duration of mitosis} \]

1.2.3.2. Detection of cells in S phase

(i) Radiolabelled thymidine

Thymidine labelling is a simple, rapid and relatively more accurate method of labelling the DNA of proliferating cells. When a population of proliferating cells is exposed to radioactively labelled thymidine, those cells engaged in DNA synthesis incorporate the exogenous thymidine into their own DNA. Two radioactive labels have been widely used for these types of study, tritium (\(^{3}\)H) and Carbon 14 (\(^{14}\)C). Both these isotopes can be separately identified on a autoradiogram. The \(\beta\) particle from \(^{3}\)H has a mean energy of 5.7 KeV and can travel no more than 1 \(\mu\) in tissue and emulsion, while the beta particle from \(^{14}\)C, with a mean energy of 50 KeV, can travel 10 \(\mu\). Consequently, the cell that has incorporated \(^{3}\)H thymidine has grains that are sharply localised over the nucleus, whereas the cell that has incorporated \(^{14}\)C thymidine has grains that surround as well as overlie the nucleus (Hoshino et al., 1972). Two approaches can be used to label tissues either in vitro by incubating freshly excised tumour tissue in a medium containing tritiated thymidine, which is incorporated into its DNA or in vivo where isotopes are administered systemically an hour or two before neurosurgical debulking. The first in vivo study on human glioma was reported by Johnson et al. (1960) and since then this has been followed in a number of studies by different groups. Hoshino and Wilson (1979), injected 30 patients who had malignant brain tumours with \(^{3}\)H-thymidine either intra-venously or intra-arterially shortly before surgery; mitostatic agents were administered in selected patients to facilitate combined stathmokinetic analysis. They presented results obtained from autoradiography studies of 24 glioma samples. The mean LI was highest in glioblastomas, and lowest in well differentiated astrocytomas. In a later study, Hoshino (1984), showed the average LI obtained with the use of \(^{3}\)H-thymidine was very high in medulloblastoma and low in well differentiated astrocytomas. Labelled thymidine studies are less used in practice today because
they are time consuming, laborious, with potential radioactive hazards to patients and cannot be used routinely.

(ii) Bromodeoxyuridine / iododeoxyuridine

5-Bromodeoxyuridine (BUDR) and 5-Iododeoxyuridine are analogues of thymidine that are incorporated into the DNA of proliferating cells. Gratzner (1982) developed a monoclonal antibody that could identify bromodeoxyuridine in tissue sections. Anti BUDR monoclonal antibody can be detected by direct conjugation of fluorescein iso thiocyanate (FITC) to the antibody, by indirect conjugation of FITC using an FITC-tagged secondary antibody, or by immunoperoxidase methods. Several studies in vivo and in vitro have been conducted. Hoshino et al. (1985) studied cell kinetics in situ of various brain tumours using BUDR. 18 patients were given a 1 hour intravenous infusion of BUDR, at a dose of 150-200 mg/m². Surgical samples were obtained after the infusion. Stained using monoclonal antibodies against BUDR and the other portion disaggregated to a single cell suspension and analysed by flow cytometry. They found the fraction of S phase cells in the tissue section was similar to both the percentage of BUDR labelled nuclei and the S-phase fraction determined by flow cytometry. Wilson et al. (1988) also measured cell kinetics in vivo using BUDR. Patients with solid brain tumours were injected with 500 mg of BUDR, intravenously, several hours prior to biopsy or surgical excision, and LI, T₁, and T₂₅ were calculated. The results showed that both the LI and T₁ varied greatly between tumours. However, within this study of 26 patients, tumours of the same grade of malignancy tended to have similar T₁ values. Wilson (1991), studied another large series of 600 patients with various brain tumours using in vivo administration of BUDR and calculated T₂₅, LI and T₂₅ for these tumours. In another study by Hoshino et al. (1989), 182 patients with intracranial gliomas received 200 mg/m² of BUDR, intravenously at the time of operation. The tumour specimens were stained for BUDR and LI were calculated. These workers found labelling indices were highest in patients with glioblastoma multiforme (GBM) and lowest in those with moderately differentiated astrocytoma.
I.2.3.3. Detection of proliferating cells in tissue sections using antibodies against proliferation associated antigens

(i) Monoclonal antibody Ki-67

(a) Introduction

Ki-67 is a mouse, IgG class 1, monoclonal antibody which detects a nuclear antigen which is thought to be present in all proliferating cells. The exact nature of this antigen is unknown although it appears to be present in $G_0$, $S$, $G_2$, and $M$ phases but is absent in $G_0$ phase of the cell cycle (Gerdes et al., 1983). Recently, it has been suggested that the antigen is a type II DNA topoisomerase or DNA polymerase delta (Verheijen et al., 1989) or that the Ki-67 antigen is a nonhistone protein with the controlling gene located on chromosome 10 (Gerdes et al., 1991, Schonk et al., 1989). The existence of such an antigen had been suggested from studies using conventional rabbit antiserum raised against the Hodgkin's disease derived cell line L428 (Stein et al. 1981). Ki-67 was produced during studies aimed at the production of monoclonal antibodies specific to Reed-Sternberg cells using Hodgkin's lymphoma cell lines. While many of the antibodies generated in this way were reactive with nuclear structures unrelated to proliferation, Ki-67 seemed to bind only to the nuclei of proliferating cells. Immunostaining of cells undergoing mitosis revealed that the antigen was present on chromosomes in all phases of mitosis and in the nuclei of proliferating cells in interphase. The immunoreactivity of Ki-67 is widespread in normal cell populations including cells of the germinal centres of cortical follicles, cortical thymocytes, neck cells of gastrointestinal mucosa, undifferentiated spermatogonia and cells of a number of human cell lines. However, the antibody does not react with resting cells such as lymphocytes, monocytes, Paneth's cells of gastrointestinal mucosa, hepatocytes, renal cells, mature sperm cells and normal brain cells. The antigen recognised by Ki-67 can be induced in peripheral blood lymphocytes following stimulation with phytohaemagglutinin and disappears from HL-60 cells stimulated by phorbol esters to differentiate into mature macrophages (Gerdes et al., 1983). The antigen which is recognised by Ki-67 appears to be sensitive to aldehyde fixation or embedding in paraffin wax and has to be used with frozen sections. Despite this shortcoming, a large number of workers have used Ki-67 labelling indices in a wide variety of human tumours. There have, however, been only limited attempts to correlate LI data with clinical outcome (Burger et al., 1986).
(b) Use in glioma

The use of Ki-67 in gliomas was first reported by Burger et al. (1986) in a series of 40 primary and secondary brain tumours. They found the highest numbers of stained nuclei were found in metastatic carcinomas with a mean LI of 33.6%. The percentage of stained cells in gliomas correlated with histological grade, with a mean LI of 1.1% in pilocytic astrocytomas and a mean LI of 6.2% in glioblastoma multiforme. They found a direct relationship between the number of stained neoplastic nuclei and frequency of mitoses. In another study, Giangaspero et al. (1987) in a small series of 22 brain neoplasms, were able to demonstrate a relationship between Ki-67 and the histological grade of malignancy. Anaplastic astrocytoma, glioblastoma multiforme and medulloblastoma all had large numbers of positive cells ranging from 10%-40%, 40%-50% and 40%-60% respectively. In glioblastoma, the positive cells were usually small and anaplastic while large bizarre cells with abundant cytoplasm were often negative. However, the distribution of positive nuclei through the section was highly variable in high grade neoplasms. There was often an irregular distribution of positive showing areas with abundant positive nuclei alternating with others with low positivity or even an absence of stained cells. Zuber et al. (1988) examined fifty one gliomas tumours using Ki-67. Again, they were able to demonstrate a relationship between the degree of histological anaplasia and Ki-67 labelling index. The mean labelling indices for low grade astrocytoma was 1.0%, 3.5% for anaplastic astrocytoma and 11.1% for glioblastomas. The percentage of proliferating cells with Ki-67 should theoretically be higher than those obtained with 3 H-thymidine or BUdR because these reagents recognise only S-phase, whereas in the Ki-67 labelling pattern all the phases of the cycle (Zuber et al., 1988). Patsouris et al. (1988) found that the mean Ki-67 LI in human brain tumours varied from 0.2% in a meningioma to 9.1% in a glioblastoma. The percentage of Ki-67 labelled nuclei was positively correlated with the grade of malignancy. Recurrent gliomas tended also to have higher LIs than tumours at diagnosis, sometimes without an apparent change in the degree of histological anaplasia (Roggendorf et al. 1987, Patsouris et al. 1988). In another study Deckert et al. (1989) examined a large series of 133 human brain tumours. Pilocytic astrocytoma, grade I, (n = 4), showed mean LI < 1% while astrocytoma, grade II, (n = 16), showed mean LI of 1.4%, and two recurrent astrocytomas, grade II had a mean LI of 1.7%. Anaplastic astrocytoma, grade III (n = 5), had a mean LI of 8.6%, whereas one recurrent anaplastic astrocytoma had LI of 10%. Two recurrent highly anaplastic astrocytoma, grade IV showed a mean LI of 48.5%. An interesting finding shown by Deckert et al. (1989), was the difference in mean values between WHO grade II astrocytoma as 1.4% and 4.2% in oligodendroglioma. They also showed in their study that the average labelling
indices of all types of grade III gliomas exceeded those obtained from glioblastomas. Raghavan et al. (1990) have attempted a more objective way of distinguishing between astrocytoma, anaplastic astrocytoma and glioblastoma multiforme with use of the monoclonal antibody Ki-67. The aim of this study was to study cell proliferation patterns in astrocytic tumours more precisely to delineate between histological grades and to correlate the Ki-67 staining patterns with the clinical course in each group of tumours. They found significant differences between the labelling indices for astrocytoma, anaplastic astrocytoma and glioblastoma multiforme, since mean Ki-67 LIs were 0.5% in astrocytomas, 4.1% in anaplastic astrocytomas and 6.4% in glioblastoma multiforme. There were also qualitative differences observed between the Ki-67 staining patterns in these three types of tumour. In astrocytomas, labelled nuclei were widely scattered and stained diffusely brown or in a stippled pattern and no clusters of labelled nuclei were seen in these tumours. In anaplastic astrocytomas, however, the distribution of staining was patchy and irregular, with small clusters of stained nuclei in the viable areas. The nuclei of glioblastoma cells were more uniformly stained than in anaplastic astrocytoma. Interestingly, they also found high levels of Ki-67 positive cells at the infiltrating edge of glioblastomas. This question of regional heterogeneity in the distribution of Ki-67 positive cells has been addressed by Parkins et al. (1991), who assessed cell proliferation in six grade IV astrocytomas using a stereotactic biopsy procedure. Multiple samples were taken along a stereotactic trajectory through the tumour, and serial sections were prepared for conventional histology or stained with Ki-67. They found considerable heterogeneity in cell proliferation within individual tumours and that this correlated well with histological appearance. Ostertag et al. (1987) also used Ki-67 in 52 stereotactic brain tumour biopsies and also found Ki-67 index related closely to the histological grade and that the highest fraction of positive cells with a mean of 17.4% occurred in paediatric brain stem gliomas.

Table 1.7. Results from different groups working with Ki-67 in low and high grade astrocytomas

<table>
<thead>
<tr>
<th>Group</th>
<th>Low grade astrocytoma (mean LI%)</th>
<th>GBM (mean LI%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burger et al., 1986</td>
<td>1.1</td>
<td>6.17</td>
</tr>
<tr>
<td>Shibata et al., 1988</td>
<td>0.9</td>
<td>9.8</td>
</tr>
<tr>
<td>Deckert et al., 1989</td>
<td>&lt;1.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Raghavan et al., 1990</td>
<td>0.5</td>
<td>6.4</td>
</tr>
</tbody>
</table>
The studies conducted by various groups with the use of Ki-67 in low and high grade malignant astrocytomas show a common feature of low labelling indices in low grade tumours and relatively higher scores in high grade tumours. The different results from these workers may be due to loss of Ki-67 antigen before cryosection of tumour samples. Patsouris et al. (1988) in their study froze the tumour sample material for 10 minutes after its removal. In contrast, Giangaspero et al. (1987) reported a time span of 30 seconds to three minutes before freezing. The Ki-67 antigen expression may also decrease during prolonged storage of the cryosections at inadequate temperatures (Patsouris et al., 1988). The sampling problem may contribute significantly to intra-tumour proliferative variation (Deckert et al., 1989). This sampling problem has also been recognised in cell kinetic studies using \(^{3}\text{H}\)-thymidine and BUdR and similar variations with labelling indices ranging from less than 1% to about 20% were obtained in glioblastoma multiforme (Hoshino et al. 1980, 1985).

(c) Use in meningioma

Although many meningiomas can be completely removed, sub total resection may lead to recurrence of the tumour. The evaluation of the proliferative potential of these tumours is of interest because it may help to determine the likelihood of recurrence of individual tumours. Roggendorf et al. (1987) used Ki-67 in 30 meningiomas of different histological subtypes. Meningiotheliomatous, fibrous and angioblastic tumours without atypical histological findings, had a mean LI of less than 1%. In contrast, atypical, recurrent and anaplastic meningiomas had LIs up to 20%. Transitional meningioma showed a wide range of LI. The antibody may therefore be of special value for determining the outcome of transitional meningiomas which show no sign of increased proliferation using conventional histology (Roggendorf et al., 1987). Similar results have been reported by Shibata et al. (1988), who found that the mean LI of meningiotheliomatous meningioma was 1.0% but was as high as 5.0% in recurrent and anaplastic meningiomas. Deckert et al. (1989) showed a mean Ki-67 LI score of about 1.0% for the primary benign endotheliomatous, fibromatous and angioblastic meningiomas, whereas recurrent meningiomas as well as primary anaplastic and transitional meningiomas showed considerably higher fractions of Ki-67 positive cells with maximal values up to 20%. Tsanaclis et al. (1991) also showed a low mean LI of 1.2% and a higher LI of 2.9% in cases of recurrent meningioma using Ki-67.
(d) Use in pituitary adenoma

Although pituitary adenomas are histologically benign tumours, surgical experience shows that they may display features more commonly associated with malignant tumours such as the capacity of invading the surrounding tissues. Invasion may be microscopic or produce large tumour masses. Conventional cytological characteristics (hypercellularity, variation in nuclear size, shape and chromatin content and presence of binucleated or multinucleated cells) are not reliable in assessing the growth of such tumours. Nagashima et al. (1986) have measured the S-phase fraction of 21 samples from pituitary adenomas following BUdR administration before operation. The mean LI was less than 0.1% in nine cases, 0.1% to 0.5% in seven, and greater than 0.5%, in the remaining seven patients. Three patients had received bromocriptine preoperatively (2.5 mg/day by mouth) and in all these cases the S-phase fraction was less than 0.2% suggesting that this drug may inhibit DNA synthesis in these cells. Cell kinetic studies using a variety of techniques have demonstrated low LIs in most of these tumours, which is in accord with their histological appearances. A study was conducted with Ki-67 in 31 pituitary adenomas by Landolt et al. (1987), in order to determine the growth fraction of the various endocrine types of pituitary adenomas. The percentage of nuclei that were immunoreactive to Ki-67 ranged in all adenomas from 0.1% to 3.7%. Low values with a range from 0.1% to 1.0% were found in endocrine-inactive adenomas. Significantly higher mean value of 1.35%, with an astonishingly small variation was found in adenomas causing acromegaly, whereas prolactinomas demonstrated the greatest variation in values. Invasive adenomas have been shown to have a significantly larger growth fraction than non-invasive adenomas (Landolt et al., 1987).

(e) Use in paediatric brain tumours

Shibata and Burger (1987), used Ki-67 to demonstrate immunohistochemically the proliferating cells in 16 tumours of the nervous system in children and these findings were compared with those in 44 adult tumours. They found the percentage of stained tumour cells in children was in general agreement with the histological grade, ranging from 0.2% in schwannoma to 12.4% in a juvenile type of glioblastoma. In a medulloblastoma, the fraction of labelled nuclei was 10.2%. In malignant gliomas of children, the percentage of stained cells did not differ from that in adult tumours. However some cases demonstrated unusually high numbers of positive cells associated with higher cellularity than in adult tumours. In another study by Deckert et al. (1989), there were 9 medulloblastomas, 2 cerebral childhood primitive neuroectodermal tumours (PNET) and 3
ependymomas included in the study and mean LI of 17.9%, 13.7% and 1.3% respectively, were found, while Giangaspero et al. (1987) studied three medulloblastomas with a mean Ki-67 LI of 43%. However, in the Deckert et al. (1989) study, nine medulloblastomas had a mean Ki-67 LI of only 17.9%. This indicates that even in the morphologically relatively homogeneous tumour type of the medulloblastoma, significant differences in the proliferative behaviour of individual tumours can be present.

(f) Use in breast tumours

Cell proliferation has been assessed in invasive breast carcinomas by Franklin (1987) using Ki-67. He found a high number of Ki-67 positive cells in tumours with high mitotic rates, high nuclear grade and high histological grade in premenopausal women. Tumours with low Ki-67 labelling rate tended to have high oestrogen receptor content, while tumours with high Ki-67 labelling rates were found to be oestrogen receptor negative. There appears to be an inverse relationship between the Ki-67 labelling indices and oestrogen receptor ER status. This was apparent whether both variables were determined immunohistologically or the ER status analysed by biochemical methods (Gerdes et al. 1987a, Vollmer et al. 1989). Bilous et al. (1991) also compared Ki-67 antibody reactivity and other pathological variables in breast carcinoma. Frozen sections from 75 specimens of breast carcinoma were immunostained with Ki-67 using an immunoperoxidase technique. The percentage of Ki-67 score was then compared with other variables. A positive correlation was seen between the Ki-67 score and mitotic rate and negative correlation was found between the Ki-67 score and oestrogen receptor status. Walker and Dearing (1992) investigated the role of transforming growth factor beta (TGF-β) in breast carcinomas using Ki-67 and an antiserum against TGF-β 1. They examined 27 ductal carcinomas in situ and 54 invasive carcinomas, employing formalin-fixed, paraffin-embedded material and found no correlation between detection of TGF-β 1 and Ki-67 labelling index.

(g) Use in bone tumours

Ki-67 has been used to clarify the role of macrophages in giant cell tumours of bone and malignant fibrous histiocytomas (Roessner et al., 1987). The results of these studies support the concept that macrophages are a reactive population in bone tumours, whereas the fibroblast-like mesenchymal cells are the proliferating tumour cells.
(h) Use in colorectal tumours

Ki-67 has also been used to identify proliferative cells in sections of colorectal tumours. Hoang et al. (1989) found that there were more proliferating cells in carcinomas than adenomas, although the difference did not reach statistical significance, while in each tumour, heterogeneity in the distribution of positively stained cells was noted. The superficial areas of both carcinomas and adenomas contained a greater number of proliferating cells than deeper areas. This may have a clinical application in the selection of patients with colorectal cancers who might get benefit from radiotherapy and chemotherapy, particularly those with unresectable or locally recurrent tumours.

(i) Use in lymphoma

The number of positive cells in Hodgkin's lymphoma always exceeds 50% (Gerdes et al., 1987b). Furthermore, mononucleated Hodgkin's cells as well as multinucleated Reed-Sternberg cells showed a similar Ki-67 labelling index indicating that both tumour cell types are involved in this malignancy. Ki-67 has been used to assess the growth fraction of non-Hodgkin's lymphomas (Weiss et al., 1987). They found the lowest number of positive cells in small lymphocytic lymphomas, chronic lymphocytic leukaemia and intermediate lymphocytic lymphoma. Diffuse large cell lymphomas and lymphoblastic lymphomas contained higher numbers of stained cells. The largest number of positive cells were seen in small non-cleared cell lymphomas. These workers were of the opinion that Ki-67 has significant utility in providing an estimate of the proliferation rate of non-Hodgkin's lymphomas (NHL). There appears to be a strong relationship between Ki-67 LI and histological grading (Schrape et al., 1987). Schwartz et al. (1989) also performed an immunoperoxidase technique on non-Hodgkin's lymphomas using Ki-67 in order to correlate proliferation rates with tumour grade and type and later compared Ki-67 staining with S-phase content as determined by flow cytometry.

(j) Use in skin tumour

Eckert et al. (1988) studied cutaneous atypical fibroxanthoma using immunohistochemistry with Ki-67. All atypical fibroxanthomas were positive for vimentin and negative for epithelial markers. Monocytic lineage-specific determinants could be demonstrated in varying amounts in cells suggestive of being reactive. In contrast, proliferating Ki-67 positive cells did not express monocyte/macrophage related antigens in atypical fibroxanthoma and malignant
fibrous histocytoma. Smolle et al. (1989) compared previous studies using tritiated thymidine labelling and used the stereological evaluation of Ki-67 staining in melanocytic skin tumours to provide a reliable estimate of proliferative activity, which might be of prognostic importance and found a positive correlation of GF and histopathologically assessed prognostic variables such as tumour thickness, mitotic rate and prognostic index.

(k) Use in urological tumours

Fontana et al. (1987) used Ki-67 staining to distinguish a bladder carcinoma and a subgroup of tumours with proliferative characteristics. They also obtained important results in the study of prostate cancers in hormonal therapy, as they could demonstrate the disappearance of the activity in patients responding clinically to the treatment. Due et al. (1988) demonstrated the proliferative activity and growth pattern of 20 seminomas with Ki-67. The growth fraction of seminomas was 50-80%, in all the tumours regardless of size. There was a slight tendency towards higher growth fraction in less differentiated tumours but no correlation was found with lymphocytic infiltration. Immunohistochemical staining using Ki-67 was performed by Sai et al. (1991) in 30 patients with benign prostatic hypertrophy (BPH), prostatic transitional cell carcinoma and prostatic invasion from bladder cancer. Specimens of BPH had no positive cells while the number of cells positive for Ki-67 ranged from 2.5% to 10.2% (mean 5.9%) in prostatic adenocarcinoma and from 11.9% to 24.3% (mean 18.1%) in prostatic transitional cell carcinoma. Poorly differentiated adenocarcinoma showed a higher growth fraction (mean 6.9%) than well differentiated tumours (mean 5.8%). Nevertheless these workers could not find any correlation with age or clinical status.

(ii) Proliferating cell nuclear antigen (PCNA / cyclin)

(a) Introduction

Proliferating cell nuclear antigen (PCNA) is a 36-kd nuclear protein, associated with proliferating cells. It appears to be the DNA polymerase delta accessory protein and it is essential for cellular DNA synthesis and is also required for in vitro replication of simian virus 40 (SV 40) DNA, where it acts to co-ordinate leading and lagging strand synthesis at the replication fork. 3% of patients suffering from systemic lupus erythematosus (SLE) have antibody against PCNA and it was through the use of sera from these patients that this protein was first identified (Miyachi et al., 1978). It is thought that PCNA is closely involved in the
proliferation of both neoplastic and normal cells (Hall et al., 1990). One striking observation concerning PCNA is its presence in the nucleolus. Nucleolar staining of PCNA is most extensive during the late G, and early S phases. It has also been shown that PCNA is identical to cyclin, one of the proteins known to be synthesised in large amounts during the S-phase of the cell cycle (Mathews et al., 1984). Recently, monoclonal antibodies to PCNA have been increasingly used as tools to obtain valuable information concerning the proliferative characteristics of various types of cancers. One of these antibodies, PC10 against PCNA has been developed. Monoclonal antibodies were generated to genetically engineered rat PCNA using conventional methods. Of the eleven clones generated with anti PCNA specifically, six were found to react with formalin fixed histological material, and one clone designated PC10 was chosen for further detailed study because of its having the highest avidity in an enzyme-linked immunosorbent assay (Waseem and Lane, 1990). Ogata et al. (1987), reported the production of two mouse monoclonal antibodies 19A2 and 19F4 to PCNA/cyclin. Although the anti-PCNA/cyclin monoclonal antibody 19A2 is now commercially available, there are few studies that have investigated its use in human tissue sections.

(b) Uses

PC10 has been used to detect proliferating cells in a wide range of tumours. When nucleoplasmic staining for PCNA was observed in cultured human glioma cells by Tabuchi et al. (1987), they found positive immunoperoxidase staining for PCNA exclusively in the nuclei of approximately 37% of cultured human glioma cells undergoing exponential growth, whereas only 14% of subcutaneous tumours cells from inoculated mice stained positive for nuclear PCNA. In a retrospective study of 42 cases of haemangiopericytoma by Yu et al. (1991) using immunohistochemical staining with PC10, the percentage of tumour cells with positive staining for PCNA was found to correlate well with histological grading. Clinical follow up data showed no deaths in 11 cases with a low proportion of positive cells whereas out of 14 cases with high positive cell count 7 patients died, 2 had metastasis and 2 patients showed multiple recurrence of tumour. In a study where it was applied to 93 gastric carcinomas significant intra-tumoural variation in staining was observed. When the tumours with PCNA indices above and below the median level of 41% were analysed for survival, it was found that those tumours with a higher LI index tended to have a worse prognosis (Jain et al., 1991). In another immunohistochemical study of melanocytic tumours using the antibody 19A2, on tissues routinely processed with formalin fixation and paraffin embedded, it was found that PCNA/cyclin positive tumour cells increased in number and staining intensity according to the following progression: common
melanocytic naevi, dysplastic naevi, primary melanomas and metastatic melanomas. In non-Hodgkin's lymphoma, a linear relation between the number of Ki-67 and PCNA positive cells has been demonstrated (Hall et al., 1990). The prognostic value of immunoperoxidase staining for PCNA has been examined in a series of 140 non-Hodgkin's lymphoma patients with median follow-up of nine years. Those patients with lymphomas containing more than 50% of cells positive for PCNA had poorer survival than those patients whose tumours had less than 50.0% of positive cells (Klemi et al., 1992). In a comparative study, Coltrera and Gown (1990) used three proliferative markers 19A2 to PCNA/cyclin, Ki-67 and BUdR against cell lines chosen to represent proliferation rates from high to low. Comparison of the overlapping distributions of detectable PCNA/cyclin expression and BUdR incorporated demonstrated substantial qualitative and quantitative differences between the different cell lines. They concluded that PCNA/cyclin expression detected by monoclonal antibody differs from different cell subpopulation in different cell types relative to those incorporating BUdR or expressing the target antigen for Ki-67. This may have implications for a clinical study for mixed cell population using these antibodies. In another recent retrospective study, 55 astrocytic tumours were stained with PC10. All the cases were graded according to Daumas-Duport grading and PCNA LIs were correlated with the histological grades. Mean PCNA LIs was found to be 4.1% for grade II, 8.1% for grade III and 26.1% for grade IV tumours (Revesz et al., 1993a).

(iii) DNA polymerase α

DNA polymerase α (DPA) is one of the enzymes important in eukaryotic cell DNA replication and appears to be present throughout the cell cycle except in G₀ phase. Immunostaining with monoclonal antibodies against DPA has been proposed as an alternative technique for identifying proliferating cells in tissue sections and cell culture (Bensch et al., 1982). These workers also demonstrated intranuclear distribution of the enzyme in human cells by immunohistochemical techniques with monoclonal antibodies against the human enzyme. The proliferative potential of brain-tumour cells has been analysed in vitro and in situ using antibodies (CL-22-2-42B) against DPA and Ki-67 by Kunishio et al. (1990). In their in vitro study, two cultured human glioma cell lines (KH and OK) were stained using antibodies against DPA, Ki-67, PCNA and BUdR. During exponential growth of the cells, the percentage of DPA positive cells ranged from 72.0% to 77.1%, the Ki-67 positive cells ranged from 43.4% to 59.4%, PCNA positive cells 30.9% to 41.4% and the BUdR labelling index ranged from 28.6% to 39.3%. However, in situ, 60 human brain tumours comprising of various benign and malignant tumours and two samples of normal brain were examined with
MAB against DPA and Ki-67. They did not find any Immunostaining in normal human brain with either tumour marker. However, in brain tumours, both antibodies against DPA and Ki-67 scores could be correlated with the histological grade of malignancy. DPA score was higher than Ki-67 score in the same tumour specimen. Later the numbers of DPA positive cells were compared with the Ki-67 scores and PCNA/cyclin score in situ. Both the polymerase alpha score and the Ki-67 score in cultured human glioma cells were lower and it appeared that neither score could express the growth fraction completely in cultured human glioma cells. These workers were able to demonstrate a similar relationship between the DPA and Ki-67 positive cells as showed by Kunishio et al. (1990) which correlated with the histological grade.

(iv) Nucleolar organiser regions (NORs)

Nucleolar organiser regions (NORs) are loops of DNA which transcribe ribosomal RNA and are located on acrocentric chromosomes. NORs are associated with certain proteins known as argyrophilic nucleolar organiser regions (AgNORs). In 1975, it was discovered that AgNORs could be stained using a simple silver staining method (Quinn and Wright, 1990). Nucleolar organiser regions have been shown to reflect the cellular proliferation (Crocker and Nar, 1987). This technique, with later modifications, can be applied to paraffin-embedded tissue sections. AgNORs are visualised as black dots within the nucleolus. In a study by Hara et al. (1991), sixteen cases of malignant brain tumour were investigated by using silver colloid method and the results compared with those obtained using Ki-67. The Ki-67 labelling index and the mean number of NORs in malignant brain tumours were linearly related. This silver colloid staining method can be applied to both conventional formalin-fixed and paraffin-embedded sections and therefore the silver colloid staining method probably has an advantage over Ki-67, BUdR or ³H-thymidine labelling methods (Hara et al., 1991).

1.2.3.4. Flow cytometry

Flow cytometry is an automated technique which quantifies cellular DNA content and analyses cell cycle distribution. A single cell suspension, prepared from a sample of fresh tissue or from frozen or paraffin-embedded tissues is stained with DNA-specific fluorescent dyes. The suspension then flows through a laser light source, and passes a stationary fluorescence detector, at a rate of about 5000 cells per second. The light scattered by the cells is registered by the detectors and converted into electronic signals which are analysed by the computer. The computer produces a DNA histogram, from which a number of measurements can
be derived. The cellular DNA content, or ploidy status can be determined. Flow cytometry has the advantage of speed and measuring the cells rapidly. In a large number of individual cells, typically 10,000-100,000 cells or nuclei can be scanned in a few minutes. Multiple fluorescent labels can be measured simultaneously on individual cells. There are, however, problems associated with solid tissues, particularly the need to make the tissue into a suspension of single cells or nuclei, which in some solid tumours may be very difficult without causing unacceptable cellular damage (Camplejohn and Macartney, 1992).

1.2.3.5. Comparison between various labelling methods

A number of studies have been carried out to compare the labelling index produced by each of the different kinetic methods. The relationship between the labelling index determined by $^3$H-thymidine and Ki-67 has been examined by Veneroni et al. (1988). Both $^3$H-thymidine LI and the number of Ki-67 positive cells were higher in high grade tumours. However, the ratio between the labelling indices on individual tumours was not constant. It sharply increased with decreasing $^3$H-thymidine LI values and was much higher in low grade tumour than high grade tumour. With regard to brain tumours, Hoshino (1984) and Hoshino et al. (1986) have compared the S-phase fraction of a number of different types of tumours using either $^3$H-thymidine or BUdR.

### Table 1.8. S phase labelling indices determined by $^3$H-thymidine and BUdR

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Number of cases</th>
<th>$^3$H-thymidine LI</th>
<th>BUdR LI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioblastoma multiforme</td>
<td>13 - 7</td>
<td>9.3% ± 1.0%</td>
<td>10.0% ± 0.8%</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>7 - 11</td>
<td>4.0% ± 0.8%</td>
<td>8.9% ± 2.4%</td>
</tr>
<tr>
<td>Low grade glioma</td>
<td>3 - 4</td>
<td>&lt;1.0%</td>
<td>&lt;1.0%</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>4 - 3</td>
<td>12.0% ± 1.3%</td>
<td>15.2% ± 1.6%</td>
</tr>
</tbody>
</table>

This table demonstrates that mean LI determined by $^3$H-thymidine labelling method in 13 cases of glioblastoma multiforme is similar to mean LI determined by BUdR labelling method in 7 tumour samples from glioblastoma multiforme and similar results have been obtained in medulloblastomas and low grade gliomas. The data in this table convincing indicates that higher-grade gliomas have higher labelling indices, as a group, than low grade gliomas and both methods show increase LI with increase anaplasia. The relationship between Ki-67 LI and that determined by BUdR was studied by Sasaki et al. (1988) in patients with various human malignant tumours including malignant meningioma. They
found a higher Ki-67 LI of 6.3% as compared to BUdR LI of 2.4% in malignant meningioma and in other malignant tumours. Nishizaki et al. (1989) found that labelling indices of Ki-67 and BUdR were parallel to the degree of histological malignancy. They found Ki-67 labelling indices higher than BUdR labelling indices and on the average the former was 1.7 time higher than the latter. Wilson et al. (1988) determined the proliferative potential of a variety of different types of human solid tumours in vivo, using bromodeoxyuridine incorporation and flow cytometry. BUdR was given intravenously prior to operation, and the labelling index, duration of S-phase (T₀) and thus the potential doubling time (Tₚₒ) was determined following biopsy or surgical resection. There was a wide variation in both the LI and T₀ between different types of tumours, however tumours of the same histology had similar T₀ values. For example, melanomas had a short T₀ value of 8.8 hr, while the longest T₀ values (24 hr) were found in tumours of lung and rectum. Gasparini et al. (1991) examined the relationship between Ki-67 LI and S phase fraction determined by flow cytometry in 122 breast carcinomas, with a median Ki-67 LI of 7.5% and 90% of the tumours expressed the same result. DNA flow cytometry analysis revealed that 69 (57%) tumours were aneuploid and 53 were diploid and Ki-67 LIs were significantly higher in aneuploid as compared to diploid carcinomas. The proliferative potential of bone marrow leukaemic cells in bone marrow can be determined by both FC analysis and Ki-67 or PCNA LI (Ito et al., 1992). The Ki-67 LI varied considerably from patient to patient with a mean 18.8% and differed significantly between acute lymphoblastic leukaemia (ALL) and acute non-lymphoblastic leukaemia (ANLL). In patients with (ALL), there was a positive correlation between Ki-67 LI and S-phase fraction determined by DNA flow cytometry analysis, although there was no such correlation in patients with ANLL. The PCNA LI, however, was considerably higher than the Ki-67 LI (mean 90.3%), and there was less variation between individual patients than seen with Ki-67.

Table 1.9. Advantages and disadvantages of different labelling methods

<table>
<thead>
<tr>
<th>Labelling method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>³H-thymidine</td>
<td>Accurate marker of S-phase cells</td>
<td>Requires fresh tissue</td>
</tr>
<tr>
<td></td>
<td>Known intracellular target</td>
<td>Slow, time consuming and laborious</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not readily available in routine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>laboratory services</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethical considerations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inconvenience to patient</td>
</tr>
<tr>
<td><strong>BUdR</strong></td>
<td><strong>Ki-67 labelling</strong></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>Accurate marker of S phase cells</td>
<td>Can be performed rapidly within the time frame of conventional frozen sections and relatively easy to perform</td>
<td></td>
</tr>
<tr>
<td>Known intracellular target</td>
<td>Does not require autoradiography</td>
<td></td>
</tr>
<tr>
<td>Can be detected in tissue section</td>
<td>Non-invasive technique</td>
<td></td>
</tr>
<tr>
<td>No radiation hazard</td>
<td>No radiation hazard</td>
<td></td>
</tr>
</tbody>
</table>

Determine only S phase cells
Potential radiation hazards

May cause myelosuppression
cytocidal teratogenic effects with prolonged administration of high doses
Very rapidly degraded by the liver, more than 90% of a single bolus of BUdR is debrominated within 20 minutes therefore the actual amount incorporated into the cells is very low
Not suitable for routine surgical pathology and also in photosensitive patients

Prolonged storage of tissue samples may damage the antigen detected

Ki-67 antigen sensitive to aldehyde
Can not be used on paraffin wax archival material
The exact nature of the antigen still remains unknown
Limited to human studies only
<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCNA labelling</td>
<td>Can be used in formalin-fixed paraffin sections and frozen sections at the same time</td>
<td>PCNA can be detected by PC10 in some non-proliferating cells</td>
</tr>
<tr>
<td></td>
<td>No use of radioactive isotopes</td>
<td>Immunoreactivity of PC10 is reduced or lost by prolonged formalin fixation or heating tissue sections</td>
</tr>
<tr>
<td></td>
<td>Staining can be used as a routine pathology procedure in laboratory</td>
<td>Poor staining may be observed in frozen sections</td>
</tr>
<tr>
<td></td>
<td>The cellular and tissue architecture is maintained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-invasive</td>
<td></td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Rapid, accurate and reproducible method</td>
<td>Expensive equipment</td>
</tr>
<tr>
<td></td>
<td>Can be applied to fresh, frozen and paraffin-embedded tissue</td>
<td>Does not maintain cellular and tissue architecture</td>
</tr>
<tr>
<td></td>
<td>Easy retrospective analysis of archival material</td>
<td>Cell suspensions often get heavily contaminated with normal cells</td>
</tr>
</tbody>
</table>

1.2.4. Aims and objectives

Brain tumours occupy a unique anatomical localization. They are surrounded by tissues which have limited capacity for proliferation, rarely metastasise out of the CNS and have limited space to expand within an essentially rigid box, the skull. Death is usually the result of raised intracranial pressure produced by a combination of tumour growth and peritumoural oedema. Thus tumour size and growth rate are likely to be the major factors influencing survival in patients with brain tumours. A major obstacle in this area has been the limited number of studies which have been conducted on large numbers of brain tumours of particular histological types. Although several studies have been published using Ki-67 or PCNA, these have often been conducted on relatively small number of
cases, often not representing a wide spectrum of different histological types and with little or no clinical follow up. This study examines two proliferation associated tumour markers, Ki-67 and PCNA in large groups of tumours of the same histological types, together with follow up in order to establish any relationship between the expression of proliferation associated markers and the clinical outcome in several different histological types of brain tumour. A large number of meningiomas have been studied with a view that these tumours being mostly benign in their nature are usually considered innocent whereas in reality they can be malignant. Moreover most of the groups are working with malignant tumours and benign tumours are being neglected. The specific aims of this project were as follows:

1. To investigate the proliferative rate and proliferative potential of various tumours of CNS.

2. To examine whether cell kinetic measurements using monoclonal antibodies would be useful in determining the outcome of the therapy or prognosis in patients with these tumours.

3. Since Ki-67/PCNA index provides information about the proliferation rate of human tumour to determine whether it may be useful in selecting those patients with invasive disease for early radical radiotherapy.
CHAPTER 2

Materials and methods

2.1. Patients

2.1.1. Patient selection

All the patients in this study were admitted to, and operated on, at the National Hospitals for Neurology and Neurosurgery, Queen Square and Maida Vale or at the Royal Free Hospital, London. All patients were operated on by Professor D.G.T. Thomas, Professor L. Symon, Mr. N. Grant, Mr. R. Bradford, Mr. R. Hayward or Mr. M. Powell. These patients were taken into the study at random. All patients were provisionally diagnosed preoperatively with an intracranial lesion and in most of the cases postoperatively diagnosis matched preoperative provisional diagnosis. Each patient was given a number, which contained an abbreviation of diagnosis and a numerical number. However, in glioma patients the date of operation was included with the abbreviation code.

2.1.2. Collection of initial clinical data

A three page protocol was designed to collect the relevant personal and medical information of all patients. The first half of the first page contained all the personal and hospital information, whereas the rest of the protocol contained the clinical information, e.g. age, sex, diagnosis, date of operation, site and side of the lesion, CT and MRI radiological reports, performance status, signs and symptoms along with duration of symptoms and clinical follow up notes (Appendix V and VI). All the above information was gathered by the author from the patients notes. The whole of the clinical and laboratory data can be found in Appendix I.

2.1.3. Pathological reports

The initial pathological report based on a smear report by the neuropathologist was noted down and later a detailed pathological report was taken from the patient's notes or from the results in the appropriate Department of Neuropathology. Details of each pathological report with the protocol for clinical information can be found in Appendix V. These pathological reports were from the Department of Neuropathology at the National Hospitals for Neurology and Neurosurgery, Queen Square and Malda Vale, London. Some pathological reports are from the Department of Pathology at the Royal Free Hospital, London. Later
the neuropathologist at the National Hospital for Neurology and Neurosurgery, London, reviewed all the pathological reports and grouped the patients histologically, under the guidelines of WHO classification for the tumours of the central nervous system.

2.1.4. Follow up data

123 patients suffering from various types of CNS tumours were studied over a period of 3 years. Seven patients were from overseas and could not be followed up in spite of intensive efforts, and therefore they were excluded from the study. Some of the home patients were also part of the BR5 study by Medical Research Council. A second two page protocol was designed to follow up the clinical progress made by these patients after the surgery (Appendix VI). Much of the relevant information could be collected from the patients clinical follow up notes but additional information when required was obtained either by contact with the patient's general practitioner or by direct contact with the patient and/or their next of kin. The laboratory findings, which contained Ki-67 LI and PCNA LI, were also gathered on this protocol along with relevant clinical information including final diagnosis, pre and postoperative and last follow up performance status. The WHO five point-scale was used to assess the clinical performances in patients with meningioma and glioma. However, patients with pituitary adenoma were assessed preoperatively as symptomatic or asymptomatic and postoperatively as improved, remaining the same or becoming worse. All patients who survived were followed up until 1993. The last follow up period was taken as the date in 1993 at which they attended hospital or their general practitioners. The survival period of those patients who died during the study was taken as the period of follow up. In all tumour recurrences the period between the first operation and the second operation or reappearances of tumour on CT / MRI scan, was taken as relapse free interval. In all the dead patients, the date of death was gathered either from the patients notes, general practitioner, patient's next of kin or in a few cases from Registrar General's office at St. Catherine's House, London.

2.2. Methods

2.2.1. Sample collection

A Dewar vessel containing liquid nitrogen was used for collection of tumour samples. Cryovials were marked with case number and the date of collection, which was also the date for operation. The tumour samples were received in a sterile petri dish, 60x15 mm in size, supplied by Falcon (Becton and Dickinson
Ltd). Some larger tumour samples were cut into small pieces with a sterilized No.10 surgical blade and were put into cryovials using sterilized forceps. The caps of cryovials were tightened and cryovials were placed immediately into the Dewar vessel. The time between receiving a tumour sample and freezing it in liquid nitrogen took between 30 and 60 seconds. When tumour samples were taken to the laboratory, they were transferred to a liquid nitrogen refrigerator for long term storage.

2.2.2. Preparation of glass slides

Two mls of 40% formalin solution was added to 98 mls of PBS (phosphate buffer saline) which gave a 2% formalin solution, to which was added 1% gelatin solution. Slides were placed in a glass slide rack and the rack dipped into a glass staining bath. The slides were then placed in a tray overnight to dry at room temperature.

2.2.3. Preparation of block for frozen sections

A small square shaped piece of cardboard 4 x 4 mm in size was cut for each tumour sample and labelled with the case number. A little OCT (Optimum cutting temperature) microscopy compound material from BDH Ltd, Poole, U.K, was placed on the cardboard which was then placed in a petri dish floating on liquid nitrogen which solidified the OCT compound gradually into a white solid base. The tumour samples were removed from cryovials and placed over the white frozen solid base. More OCT compound was poured over the sample. Samples were placed again in the floating petri dish and were gradually frozen. Some OCT compound was also placed on a metallic block (chuck) and the sample was placed on this. The chuck was dipped into the liquid nitrogen sparing the tissue sample till the OCT compound under the cardboard was solidified.

2.2.4. Microtome and cryosection

A rotary retracting type microtome, model OTSAS 5030 with 20° degree microtome steel knife, model B1009 DR, manufactured by Bright Instrument Company Ltd., Huntingdon, U.K, was used for cryosections. The blade of the microtome was first cleaned with acetone and the appropriate adjustment of the blade was made. The temperature of the microtome was kept at -20°C. Cryosections were obtained with a thickness of 6-8 μm. 6 μm thick slices were usually taken for an average size tumour sample whereas 8 μm of thickness were preferred in small size tumour samples, such as pituitary adenomas. The first few
slices from the top of each sample were discarded and then three or more slices were taken on each gelatinised glass slide and in total three glass slides for each tumour sample were prepared. Torn frozen sections were discarded. The slides were kept in 5-slide mailers, small plastic boxes, grooved to hold 76 x 26 mm microscope slides and kept in a -20°C freezer. The remainder of the block of tumour samples was kept in a small airtight plastic bag, marked with the case number in a -20°C freezer. Cryosections were obtained usually 24 hours after tumour collection and immunoperoxidase staining was done after another 24 hours of the cryosectioning.

2.2.5. Immunoperoxidase staining for Ki-67

A sheet of paper towel was placed in a plastic tray and soaked with water to provide humid conditions. The glass slides with cryostat sections were taken out from the -20 °C freezer and placed in plastic trays for 5 minutes in order to bring the temperature of glass slides to room temperature. However, a frequent alteration in the surrounding temperature was avoided and tissue sections were always kept cold while cutting the cryosections or making a tumour sample block. Similarly, the cryosections were not allowed to dry out at room temperature for more than 10 minutes to avoid loss of antigen. Cryostat sections were encircled with Dako pen, Dakopatts, Denmark, which contains wax and thus prevents the spread of solutions from the sections. Cold acetone at 4°C was taken in a glass staining bath and the swing handle rack containing the glass slides was suspended into it for 10 minutes. Meanwhile the primary antibody solution was prepared. The Ki-67 monoclonal mouse anti-human proliferating cells antibody (DAKO-PC, subclass IgG1 kappa) with a code no. M722 was supplied by Dakopatts, Denmark and kept in a refrigerator working at a temperature between 4 °C and 8 °C. A solution was prepared with DAKO-PC in PBS (phosphate buffer saline) in a dilution of 1:10 in sterile conditions. The PBS solution was obtained by adding 20 tablets of PBS to 2000 ml of distilled water. Once the sections were fixed in acetone they were air dried for 1-2 minutes and then the glass slides were given a PBS wash for 5 minutes. The glass slides were placed in a plastic tray and excess water in and around the tissue sections was removed by careful suction. The DAKO-PC solution was mixed well in a whirlimixer. The DAKO-PC solution in a dilution of 1:10 was applied to the tissue sections using small bulb graduated pastettes, manufactured by Alpha Lab. Ltd. Hampshire, U.K. for 45-60 minutes at room temperature under humid conditions. It was attempted to make one large bubble within the circle drawn by the Dako pen. The plastic tray containing the glass slides was covered by another plastic tray. Meanwhile another solution for secondary antibody was made in advance by diluting peroxidase-conjugated
rabbit anti-mouse immunoglobulin with PBS in a dilution of 1:20. This is a mouse immunoglobulin, mainly IgG, isolated from normal mouse serum and supplied by Dakopatts, Denmark. This solution was also mixed in a whirlimixer. After 45-60 minutes of incubation primary antibody was over, slides were washed in PBS for 5 minutes. A new paper towel was placed in the plastic tray and glass slides were placed in it. Excess PBS in and around was removed from the sections. The secondary antibody was applied using small bulb graduated pastettes over the cryostat sections for 1 hour and the plastic tray was covered with another tray. Meanwhile fresh DAB substrate was prepared. In 100 ml of PBS solution, 60 mg of 3-3 diaminobenzidine tetrahydrochloride, prepared by Sigma Chemical Company, St. Louis, USA, was added and warmed a little and filtered using sterile Flowpore filters, size 0.22 μm, supplied by ICN Biomedicals Ltd, Irvine, Scotland, in order to avoid brown pigment particles being deposited on the cryosections. The DAB solution was kept cool and 3μl of hydrogen peroxide was added to it in order to activate the substrate. However, this activator was added just before the application of substrate to the cryostat sections. The pH of DAB solution was maintained between 7.8-8.0 using 1N NaOH solution. This helped to prevent undesirable background activity. Following incubation with the second antibody slides were again washed with PBS for 5 minutes. The excess water was removed from the tissue sections. The glass slides were placed in a tray on a new water soaked tissue towel and freshly prepared DAB substrate was applied for 10 minutes. Brown discolouration was observed in cryostat sections. Another PBS wash was given to the glass slides for 5 minutes and excessive water was removed from tissue sections. The tissue sections were counterstained with Mayer's haematoxylin solution, supplied by Sigma Ltd, Poole, U.K, for 5 minutes as counter staining for shorter periods did not produce sufficient staining contrast. Another PBS wash was given for 5 minutes to the glass slides and they then were dried with tissue paper. Cryosections were dehydrated in 70%, 80%, 90% and 100% alcohol, supplied by Hayman Ltd., Witham, U.K. for three minutes and then placed in xylene, supplied by BDH Company, Poole, U.K., for one minute. Finally cryosections were mounted with DPX, supplied by BDH Company, Poole, U.K.

2.2.5.1. Use of periodate-lysine formaldehyde (PLP)

This fixative was used instead of acetone, in two tumour samples of meningioma for Ki-67 staining. This fixative (PLP), provides acceptable morphologic preservation, excellent mRNA retention and allows acceptable immunochemistry.
The fixative was used on the day of preparation.

Stock buffered lysine 720 ml
4% paraformaldehyde 120 ml
Sodium periodate 1.92 g

The results indicate that it produces a lower labelling index than acetone and it was not used further.

2.2.5.2. Control sections

The control sections, derived from the human bladder carcinoma xenograft, RT112 (kindly supplied by Dr. C.S. Parkins at the Institute of Cancer Research, Sutton, Surrey, U.K.) were used as controls during the staining procedure. A recurrent anaplastic meningioma from the cases in study with a high Ki-67 LI was also used as a control. The detail of these quality control experiments are given in Table 3.2.

2.2.6. Staining method for proliferating cell nuclear antigen (PCNA)

PC10, IgG2a, subclass was used in cryostat sections for the immunocytochemical analysis of proliferation in a series of meningiomas, pituitary adenomas and grade IV malignant gliomas. PC10 was kindly supplied by Professor D. P. Lane, from the University of Dundee, Scotland. The immunoperoxidase staining method followed for PC10 was basically the same as that for Ki-67 staining with minor changes. Cryostat sections of the same thickness of 6μm-8μm were cut and preparations were made in the same style as before for the Ki-67 staining. The cryostat sections were fixed in 4% formal saline at room temperature for 2 minutes followed by ethanol for 10 minutes. 4% formal saline solution was prepared by adding 10 ml of formalin in 90 ml of PBS solution. It was followed by a PBS wash for 5 minutes. After the removal of excess water from the sections, PC10 antibody solution at a dilution of 1:10 in PBS, was applied to cryosections for 45-60 minutes at room temperature in humid conditions. Sections were washed in PBS for 5 minutes to remove the antibody. Excess water was removed around the cryostat sections and peroxidase-conjugated rabbit anti-rat immunoglobulin, mainly IgG, code No. P162 (Dakopatts, Denmark) in a dilution of 1:20 in PBS, was applied to the cryosections for 30 minutes at room temperature in humid conditions. Another PBS wash was given for 5 minutes and excess water was removed from around the sections. Freshly prepared and filtered activated DAB solution was then applied for 10 minutes at room temperature. After a further PBS wash, the
sections were counterstained with Mayer's haematoxylin for 10 minutes at room temperature. This was followed by another PBS wash. The cryosections were dehydrated in 70%, 80%, 90% and 100% alcohol and then cleared in xylene for one minute. The cryostat sections were mounted in DPX.

2.2.7. Selection of area for counting

In each cryosection, the two areas mostly densely populated with positive cells were selected under magnification of x200 using an Axioskop routine microscope (Carl Ziess, Oberkochen, Germany). Positive cells at the edge of the tumour section and in the capillary endothelium were avoided in the count and the areas with maximum positive cells, good background contrast and least artifacts were selected for counting and photography.

2.3.8. Film protocol

A three page film protocol was designed in order to take photographs (Appendix IV). Ilford PAN F ISO 50/18° (black and white) film was used in a MC 80 microscope camera on Axioskop microscope (Carl Zeiss, Oberkochen, Germany). Pre-selected areas with large numbers of positive cells were reconfirmed under high magnification (x 400) and two photographs were taken under x 200 magnification.

2.2.9. Counting method

Two photographs containing large numbers of positive cells were fixed on a sheet of white A4 paper and placed inside clear plastic envelope. Using a light box, positively stained cells, black in case of black and white film and dark brown in case of coloured film, were counted. They were marked as red dots and were counted separately for each photograph. Negative cells, which were less dense when photographed with black and white film and light pink colour on coloured film, were marked with black dots on the photographs. Usually 500-1000 cells were counted in both the photographs for each tumour sample. The tumour cells within the necrotic tissue and vascular endothelium were not counted. In each tumour sample, the percentage of positive cells was calculated for each photograph by dividing the count for positive cells by the count for negative cells and a mean for both photographs was calculated.
CHAPTER III

Results

3.1. Characteristics of the patients studied

The total number of patients studied was 123. The histological diagnoses of these tumours are shown in Table 3.1. The guidelines of the World Health Organization's latest histological classification of tumours of the central nervous system were followed. There were 61 meningiomas, 32 pituitary adenomas, 14 grade IV astrocytomas, 1 grade III astrocytoma, 2 grade II astrocytomas, 1 grade I astrocytoma, 2 mixed oligoastrocytomas with one each of grade III and grade IV, 3 oligodendrogliomas, an ependymoma, a capillary haemangioblastoma, a cerebral lymphoma, a PNET and 3 metastatic tumours with renal adenocarcinoma, malignant melanoma and ovarian tumour as primary lesions. Two patients, one with a diagnosis of an epidermoid cyst and another with malignant glioma grade IV, were excluded from the study because the tumour samples received consisted of only necrotic tissue. There were 58 male patients with a mean age of 50.2 years and 65 female patients with a mean age of 52.9 years. The distribution of patients according to diagnosis, age and sex is shown in Figure 3.1. Males had higher incidence of glioma in the age groups 30-39 years and 50-59 years whereas, male and female patients had an equal incidence in age group 20-29 years and 60-69 years. Female patients had higher incidence of meningioma in the age groups of 40-49 years and 60-69 years than men. Similarly, female patients showed high incidence of pituitary adenoma in 20-29 years and 40-49 year old age groups. Male and female patients showed equal incidence in the age group of 20-29 years, 30-39 years and 50-59 years for meningiomas and 30-39 years, 50-59 years and 60-69 years of age groups for pituitary adenomas.

3.1.1. Quality control

In order to assess any variation between different staining runs and to maintain the quality of staining RT 112, a human bladder carcinoma xenograft and M, a recurrent anaplastic meningioma, both with high proliferative labelling indices were used as controls. The mean LIs for RT 112 and M, were determined as 49.3% and 13.8% respectively and the LI for each determination is shown in Table 3.2.
Table 3.1. Histopathological characteristics of tumours in the study

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meningioma</strong></td>
<td>61</td>
</tr>
<tr>
<td>Meningotheliomatous</td>
<td>44</td>
</tr>
<tr>
<td>Fibroblastic</td>
<td>6</td>
</tr>
<tr>
<td>Transitional</td>
<td>2</td>
</tr>
<tr>
<td>Angiomatous</td>
<td>1</td>
</tr>
<tr>
<td>Psammomatous</td>
<td>1</td>
</tr>
<tr>
<td>Xanthomatous</td>
<td>1</td>
</tr>
<tr>
<td>Atypical</td>
<td>5</td>
</tr>
<tr>
<td>Anaplastic</td>
<td>1</td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>32</td>
</tr>
<tr>
<td>Chromophobe</td>
<td>26</td>
</tr>
<tr>
<td>Acidophil</td>
<td>4</td>
</tr>
<tr>
<td>Basophil</td>
<td>2</td>
</tr>
<tr>
<td><strong>Glioma of different type &amp; grade</strong></td>
<td>24</td>
</tr>
<tr>
<td>Glioblastoma multiforme / Malignant astrocytoma, grade IV</td>
<td>14</td>
</tr>
<tr>
<td>Malignant astrocytoma grade III</td>
<td>2</td>
</tr>
<tr>
<td>Astrocytoma grade II</td>
<td>2</td>
</tr>
<tr>
<td>Malignant oligodendroglioma / oligoastrocytoma, grade III</td>
<td>4</td>
</tr>
<tr>
<td>Oligodendroglioma, grade II</td>
<td>3</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>1</td>
</tr>
<tr>
<td><strong>PNET</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Primary cerebral lymphoma</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Capillary haemangioblastoma</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Metastatic tumours</strong></td>
<td>3</td>
</tr>
<tr>
<td>Renal adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>1</td>
</tr>
<tr>
<td>Ovarian</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>123</td>
</tr>
</tbody>
</table>
Fig. 3.1. Distribution of patients according to diagnosis, age & sex

Glioma

Meningioma

Pituitary adenoma
Table 3.2. LI% in Controls

<table>
<thead>
<tr>
<th>No.</th>
<th>Tumour</th>
<th>Ki-67 LI%</th>
<th>Mean LI%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>RT 112</td>
<td>54.16</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>RT 112</td>
<td>38.88</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>RT 112</td>
<td>54.45</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>RT 112</td>
<td>31.25</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>RT 112</td>
<td>52.54</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>RT 112</td>
<td>60.0</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>RT 112</td>
<td>51.0</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>RT 112</td>
<td>50.01</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>RT 112</td>
<td>49.21</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>RT 112</td>
<td>52.30</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>RT 112</td>
<td>52.0</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>RT 112</td>
<td>45.52</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>M7.1</td>
<td>29.15</td>
<td>13.79</td>
</tr>
<tr>
<td>14.</td>
<td>M7.2</td>
<td>9.50</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>M7.3</td>
<td>8.87</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>M7.4</td>
<td>9.47</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>M7.5</td>
<td>8.57</td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>M7.6</td>
<td>27.54</td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td>M7.7</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>M7.8</td>
<td>9.02</td>
<td></td>
</tr>
</tbody>
</table>

RT 112, human bladder carcinoma xenograft (n = 12). M7, anaplastic recurrent meningioma (n = 8)
3.2. Meningioma

3.2.1. Characteristics of the patients

There were 34 female and 27 male patients with meningioma. The mean age of female patients was 55.5 years, with a range of 29-77 years, and mean age for male patients was 58.9 years, with a range of 23-82 years.

3.2.2. Characteristics of the tumours

The total number of meningiomas studied was 61. There were 44 meningotheliomatous meningiomas, 6 fibroblastic, 5 atypical, 2 transitional and 1 each of the psammomatous, anaplastic, angiomatous and xanthomatous types as shown in Table 3.1. Twenty nine patients had a meningioma on the left side of cerebral hemisphere and the same number had their lesion on the right side and three patients had midline lesions. Thirteen patients had their tumour located in the frontal lobe, 12 had meningioma arising from sphenoid wing, 6 patients had their tumour located in the posterior fossa, 3 of which were located at the cerebello-pontine (CP) angle, 6 patients had meningioma located in the parietal lobe, 5 had parasagittal lesions, 3 patients had tumour in the occipital lobe, 2 patients had suprasellar and tentorial lesions and 2 had lesions in the foramen magnum. One patient each had a lesion in temporal lobe, lateral ventricle and falx attached to superior sagittal sinus. Seven patients had lesions in more than one lobe; three had lesions in the fronto-temporal region, 2 in the fronto-parietal, 1 in the parieto-occipital and 1 in bi-parietal lobes. Fifty two patients with meningioma had a craniotomy, 6 had undergone craniectomy and one each had craniotomy with lobectomy, CT-guided craniotomy and craniocervical exploration. Thirty four patients had total excision of lesion and 7 had a macroscopic removal. However, 19 patients had subtotal removal and 1 patient had only partial removal of the meningioma.

3.2.3. Clinical outcome

The clinical outcome of 61 patients suffering from meningioma was assessed. The mean duration of symptoms was 20.6 months. Five overseas patients were lost to follow up and therefore the mean follow up period of the remaining 56 patients was 30.5 months (range 1 - 53 months). Thirty two patients (52.4%) had a history of epilepsy at time of hospital admission, as shown in Figures 3.4 and 3.5. Performance status was assessed using the WHO 5-point grading scheme. Preoperatively, 17 patients (27.9%) were in grade 0; 22 (36%) in grade 1; 17
Figure 3.2. Ki-67LI in Meningiomas based on histology

![Graph](image)

Tumour subtypes

- AN = Anaplastic
- AB = Angioblastic
- AP = Atypical
- MM = Meningeal
- TS = Transitional
- PS = Psammomatous
- XM = Xanthomatous

Fig. 3.3. Mean Ki-67 LI in Meningiomas based on histology
Figure 3.4. Epilepsy in patients studied

- Glioma: n = 1 (epileptic), n = 24
- Pituitary adenoma: n = 2 (epileptic), n = 32
- Meningioma: n = 32 (epileptic), n = 61

Figure 3.5. Proportion of patients with epilepsy

- Glioma
- Pituitary adenoma
- Meningioma

Epilepsy %
(27.9%) in grade 2; 5 (8.2%) in grade 3 and none of the patient were in grade 4. However, in the immediate postoperative phase or at the time of discharge from hospital, 27 (44.3%) patients were in grade 0; 16 (26.1%) in grade 1; 13 (21.3%) in grade 2; 5 (8.2%) in grade 3 and no patients were in grade 4. However, when the patients were assessed clinically at the time of their last follow up, 39 (63.9%) patients were in grade 0; 6 (9.7%) in grade 1; 3 (4.8%) in grade 2 and no patients in either grades 3 or 4. Eight patients (13.1%) died during the follow up period and 5 (8.2%) patients were lost to follow up as shown in Figure 3.6. Eight patients received postoperative radiotherapy and 9 patients (14.7%) had tumour recurrence, shown in Figures 3.14 and 3.15. The mean relapse free interval in these patients (RFI) was 9.5 months.

3.2.4. Ki-67 labelling index

The mean Ki-67 LI for all meningiomas was 2.8% with a range of 0.12-29.1%. Twelve meningiomas (32.8%) had Ki-67 LIs less than 1%, 17 (51.6%) had LI between 1-5% and only 4 (15.4%) had LIs more than 5%, as shown in Figure 3.7. The mean Ki-67 LI for 34 female patients was 1.8% and the mean Ki-67 LI for 27 male patients was 4.05%, shown in Figure 3.8. The mean LI of 9 (14.7%) recurrent meningiomas was 5.4% with a maximum LI of 29.1%. Mean Ki-67 LI for 44 meningotheliomatous meningioma was 2.3%, 6 fibroblastic meningioma 2.5%, 5 atypical meningioma 3.2%, 2 transitional meningioma 1.83% and single cases of angioblastic, psammomatous, xanthomatous and anaplastic meningioma had LIs of 1%, 0.93%, 0.91% and 29.1% respectively (Figures 3.2 and 3.3.).

3.2.5. PCNA labelling index

The mean PCNA LI for 33 meningiomas was 1.4%, with a range of 0.3-4.2%. Thirteen patients (39.4%) had PCNA LI less than 1% and 20 patients (60.6%) had LI between 1% and 5%, as shown in Figure 3.9. Mean PCNA LI for 20 female patients was 1.4% and eleven male patients had LI of 1.5%. The mean LI for 4 recurrent meningiomas was 1.1%. Twenty three meningotheliomatous meningiomas had mean PCNA LI of 1.5%, 5 fibroblastic meningioma had mean LI of 1.4% and one each of angioblastic, atypical, transitional, xanthomatous and psammomatous meningioma had LIs of 1.9%, 3.9%, 1.2%, 0.9% and 0.8% respectively, as shown in Figure 3.11.
Fig. 3. WHO performance score in meningioma patients
Fig. 3. Frequency distribution in 24 gliomas stained with Ki-67

A. 20% < 1% 1-5% > 5% 60%

Frequency distribution in 61 meningiomas stained with Ki-67

B. 15.52% < 1% 1-5% > 5% 51.72%

Frequency distribution in 32 pituitary adenomas stained with Ki-67

C. 3.12% < 1% 1-5% > 5% 65.62%
Figure 3.8. Mean Ki-67 LI distribution according to sex
Fig. 3.9. Frequency distribution in 33 meningiomas stained with Ki-67

A.

Frequency distribution in 33 meningiomas stained with FCNA

B.
Figure 3.10: Comparison of Ki-67 and PCNA LI in meningioma (n = 33)

Figure 3.11: Comparison of mean Ki-67 and PCNA LI in meningioma based on histology

F=ArTtypical,FB=Fibroblastic,MM=Meningotheliomatous,TS=Transitional,AB=Angioblastic,PS=Psammomatous,XM=Xanthomatous
3.2.6. Comparison of Ki-67 LI and PCNA LI

Mean Ki-67 LI for 33 meningiomas of different histological subtypes was 2.3%, with a range of 0.4%-10.8% as compared with mean PCNA LI of 1.4%, with a range of 0.3-4.2% in the same tumours, shown in Figures 3.10 and 3.11. Atypical meningioma showed the maximum Ki-67 LI of 8.4% and PCNA LI of 3.9%. Twenty three meningotheliomatous meningioma had a mean Ki-67 LI of 2.1% and a mean PCNA LI of 1.4%, 5 fibroblastic meningiomas showed a mean Ki-67 LI of 2.4% and PCNA LI of 1.4%, one each of transitional, angioblastic, psammomatous and xanthomatous type of meningioma showed Ki-67 LIs of 1.9%, 1%, 0.9%, 0.9% and PCNA LI of 1.2%, 1.9%, 0.9%, 0.9% respectively, shown in Figure 3.11. Correlation between Ki-67 LI and PCNA LI in meningiomas found to be statistically significant (r = 0.701, p = <0.001, Figure 3.12).

3.2.7. Comparison of Ki-67 LI and clinical characteristics and outcome in patients with meningioma

There were 12 meningiomas with Ki-67 LIs less than 1% as compared with thirteen meningiomas with PCNA LI less than 1%, 17 had Ki-67 LIs between 1 and 5% as compared with twenty meningiomas with PCNA LI in the same range. There were 4 meningiomas with Ki-67 LIs more than 5% and none had PCNA LIs greater than 5% (Figure 3.9). No significant correlation was found between age and Ki-67 LI (r = 0.174, p = 0.180). Similarly, no significant correlation could be established between Ki-67 LI and preoperative performance score, postoperative performance score and performance score at the last follow up (r = 0.123, p = 0.426; r = 0.109, p = 0.483; r = -0.038, p = 0.826, respectively). A comparison was made between Ki-67 LIs of atypical meningioma, fibroblastic and meningotheliomatous meningioma using the Mann Whitney U test but it was not possible to demonstrate a difference in the LIs of these different groups of tumour.

3.2.8. Comparison of PCNA LI and clinical characteristics and outcome in patients with meningioma

PCNA LI did not correlate with pre- and postoperative performance score, duration of symptoms and follow up period (r = -0.190, p = 0.386; r = -0.265, p = 0.222; r = -0.120, p = 0.585; r = 0.331, p = 0.154, respectively) in a group of 33 meningiomas.
Fig. 3. Correlation between Ki-67 & PCNA LIs in meningioma showing line of regression

\[ r = 0.701, \ p < 0.001 \]
3.3. Pituitary adenoma

3.3.1. Characteristics of the patients

There were 15 male patients and 17 female patients with pituitary adenoma. The mean age of male patients was 54 years, with a range of 24 - 79 years and mean age group of female patients was 51.6 years, with a range of 22 - 79 years.

3.3.2. Characteristics of the tumours

Twenty five patients had tumour confined to the pituitary fossa and 7 patients had suprasellar extension of the tumour. Twenty eight patients had transphenoidal hypophysectomy and 4 patients had transfrontal craniotomy. In twenty one patients the tumour was removed totally and in eleven patients it was only possible to resect the tumour subtotally.

3.3.3. Clinical outcome

Mean duration of symptoms for all patients with pituitary adenoma was 36.4 months. Two patients (6.2%) had history of epilepsy at diagnosis (Figures 3.4. and 3.5). Two overseas patients were lost to follow up and the mean follow up period for the remaining 30 patients was 36.9 months. Preoperatively, 31 patients were symptomatic and 1 patient was asymptomatic. 27 patients (84.4%) improved clinically after operation, 4 (12.5%) remained the same and 1 patient (3.1%) was clinically worse (Figure 3.13). Two patients (6.2%) died during the follow up period and the mean survival period was 14.5 months. Twelve patients received postoperative radiotherapy. Only 1 patient (3.1%) had tumour recurrence with a relapse free interval of 8 months (Figures 3.14 and 3.15).

3.3.4. Ki-67 labelling index

The mean Ki-67 LI for all pituitary adenomas was 1.8%, with a range 0.1 - 9.7%. Twenty one pituitary adenomas (65.6%) had LIs less than 1%, 10 (31.2%) had LIs between 1% and 5% and only 1 (3.1%) had LI more than 5% (Figure 3.7). The mean Ki-67 LI for 15 male patients was 1.4%, whereas 17 female patients had mean Ki-67 LI of 1.1% (Figure 3.8). One patient (3.1%) with Ki-67 LI of 1.6%, showed tumour recurrence. Four acidophil adenomas had mean Ki-67 of 3.4%, 2 basophil had mean LI of 1.6% and 26 chromophobe adenomas showed a mean LI of 0.9% (Figures 3.16 and 3.17). All 32 pituitary adenomas were also classified on the basis of hormone immunocytochemistry. Five GH producing adenomas had mean Ki-
Figure 3. Clinical performance in pituitary adenoma patients

WHO performance scale
Figure 3.14 Recurrence in tumour studied

- Glioma: n = 12 (recur), n = 24
- Pituitary adenoma: n = 1 (recur), n = 32
- Meningioma: n = 9 (recur), n = 61

Figure 3.15 Recurrence percentage in tumours studied

- Pituitary adenoma: 3.1%
- Meningioma: 14.7%
- Glioma: 50%
Figure 3.16. Ki-67 LI in pituitary adenomas based on histology

![Graph showing Ki-67 LI in pituitary adenomas based on histology.]

Figure 3.17. Mean Ki-67 LI in pituitary adenomas based on histology

![Bar chart showing mean Ki-67 LI for different cell types.]

- Chromophobe: n = 26
- Basophil: n = 2
- Acidophil: n = 4

KI-67 LI%
Figure 3.18. Ki-67 LI in pituitary adenoma based on immunocytochemistry.

Figure 3.19. Mean Ki-67 LI pituitary adenoma based on immunocytochemistry.
67 LI of 3.15%, four glycoprotein producing adenomas showed a mean LI of 0.42%, four mixed adenomas had mean LI of 1.06%, four prolactinomas had a mean LI of 1.3% and seven silent adenomas showed a mean Ki-67 LI of 0.7% (Figures 3.18 and 3.19).

3.3.5. PCNA labelling index

The mean PCNA LI for 14 pituitary adenomas was 5.9%, with a range of 1.04 - 20.7%. Ten tumours (71.4%) had LIs between 1 and 5% and 4 (28.6%) had LIs greater than 5% (Figure 3.20). The mean PCNA LI for four acidophil adenomas was 2.9%, the single case of basophilic adenoma had a LI of 20.7% and 9 chromophobe adenomas had a mean PCNA LI of 5.7%. Four GH producing adenomas had mean LI of 3.2%, four prolactinomas had mean LI of 3.7% and 2 each of ACTH producing adenomas and silent adenomas showed mean LI of 12.2% and 13.7%, respectively, shown in Table 3.3.

3.3.6. Comparison of Ki-67 LI and PCNA LI

A comparison if Ki-67 and PCNA LIs for a group of 14 pituitary adenomas is shown in Figure 3.21. There were 6 adenomas with Ki-67 LIs less than 1%, but none of the adenomas had PCNA LI less than 1%, 7 tumours had Ki-67 LIs between 1 and 5% as compared to ten tumours with PCNA LIs between 1 and 5%. There was only one adenoma with Ki-67 LI higher than 5%, while there were 4 adenomas with PCNA LIs higher than 5% (Figure 3.20). When these tumours were divided into different histological groups, four acidophil adenomas had a mean Ki-67 LI of 3.4% and mean PCNA LI of 2.9%. The single basophilic adenoma had Ki-67 LI of 0.9% and a PCNA LI of 20.7%. In nine cases of chromophobe adenoma, the mean Ki-67 LI was 1.1% as compared with a mean PCNA LI of 5.5%, as shown in Table 3.4 and Figure 3.22. However, no significant correlation was found in chromophobe adenomas between Ki-67 LI and PCNA LI (r = -0.284, p = 0.458). A comparison was also made between Ki-67 LI and PCNA LI in tumours classified on the basis of their hormone secretion. The 4 prolactinomas had mean Ki-67 LI and PCNA LI of 1.3% and 3.7%, respectively. Two ACTH producing adenomas had mean Ki-67 LI of 0.6% as compared to a mean PCNA LI of 12.2% and 2 silent adenomas had mean Ki-67 LI of 1.1% as compared to a PCNA LI of 13.7% (Table 3.3, Figure 3.23). In GH producing adenomas and prolactinomas, no significant correlation was found between Ki-67 and PCNA LI (r = -0.976, p = 0.139).
Fig. 3.20. Frequency distribution in 14 pituitary adenomas stained with Ki-67

A.

Frequency distribution in 14 pituitary adenomas stained with PCNA

B.
Table 3.3. Ki-67 LI and PCNA LI in pituitary adenoma based on immunocytochemistry

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Ki-67 LI</th>
<th>PCNA LI</th>
<th>Tumour type</th>
<th>Ki-67 LI%</th>
<th>PCNA LI%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Mean LI%)</td>
<td></td>
<td></td>
<td>(Mean LI%)</td>
<td></td>
</tr>
<tr>
<td>P24</td>
<td>56</td>
<td>M</td>
<td>1.29</td>
<td>19.9</td>
<td>Silent</td>
<td>1.1</td>
<td>13.7</td>
</tr>
<tr>
<td>P26</td>
<td>33</td>
<td>M</td>
<td>0.98</td>
<td>7.48</td>
<td>Silent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P28</td>
<td>63</td>
<td>F</td>
<td>2.55</td>
<td>1.25</td>
<td>Prolactin +</td>
<td>1.3</td>
<td>3.7</td>
</tr>
<tr>
<td>P5</td>
<td>29</td>
<td>F</td>
<td>0.62</td>
<td>1.44</td>
<td>Prolactin +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P18</td>
<td>49</td>
<td>F</td>
<td>0.48</td>
<td>4.77</td>
<td>Prolactin +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P27</td>
<td>33</td>
<td>M</td>
<td>1.41</td>
<td>7.8</td>
<td>Prolactin +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P3</td>
<td>53</td>
<td>M</td>
<td>0.30</td>
<td>3.50</td>
<td>ACTH +</td>
<td>0.6</td>
<td>12.2</td>
</tr>
<tr>
<td>P31</td>
<td>59</td>
<td>F</td>
<td>0.96</td>
<td>10.6</td>
<td>ACTH +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P34</td>
<td>64</td>
<td>M</td>
<td>1.12</td>
<td>4.41</td>
<td>G.H +</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>P25</td>
<td>45</td>
<td>M</td>
<td>1.06</td>
<td>3.18</td>
<td>G.H +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P22</td>
<td>57</td>
<td>M</td>
<td>0.87</td>
<td>2.6</td>
<td>G.H +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P35</td>
<td>25</td>
<td>M</td>
<td>9.74</td>
<td>3.2</td>
<td>G.H +</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Silent (n = 2), Prolactin producing (n = 4), ACTH producing (n = 2), G.H producing (n = 4).
Figure 3. Comparison of Ki-67 LI and PCNA LI in pituitary adenomas

Fig. 3.22. Comparison of Ki-67 LI and PCNA LI in pituitary adenoma based on histology
Table 3.4. KI-67 LI and PCNA LI in pituitary adenoma based on histology

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Ki-67 LI%</th>
<th>PCNA LI%</th>
<th>Tumour type</th>
<th>Ki-67 % (mean)</th>
<th>PCNA%</th>
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<tr>
<td>P30</td>
<td>66</td>
<td>F</td>
<td>1.72</td>
<td>1.07</td>
<td>Acidophil</td>
<td>3.41</td>
<td>2.96</td>
</tr>
<tr>
<td>P34</td>
<td>64</td>
<td>M</td>
<td>1.12</td>
<td>4.41</td>
<td>Acidophil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P25</td>
<td>45</td>
<td>M</td>
<td>1.0</td>
<td>3.18</td>
<td>Acidophil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P35</td>
<td>25</td>
<td>M</td>
<td>9.74</td>
<td>3.20</td>
<td>Acidophil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P31</td>
<td>59</td>
<td>F</td>
<td>0.96</td>
<td>20.68</td>
<td>Basophil</td>
<td>0.96</td>
<td>20.68</td>
</tr>
<tr>
<td>P28</td>
<td>59</td>
<td>F</td>
<td>2.55</td>
<td>1.25</td>
<td>Chromophobe</td>
<td>1.11</td>
<td>5.46</td>
</tr>
<tr>
<td>P5</td>
<td>59</td>
<td>F</td>
<td>0.62</td>
<td>1.44</td>
<td>Chromophobe</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P18</td>
<td>49</td>
<td>F</td>
<td>0.48</td>
<td>4.77</td>
<td>Chromophobe</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P3</td>
<td>53</td>
<td>M</td>
<td>0.30</td>
<td>3.79</td>
<td>Chromophobe</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P27</td>
<td>33</td>
<td>M</td>
<td>1.41</td>
<td>7.26</td>
<td>Chromophobe</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P24</td>
<td>56</td>
<td>M</td>
<td>1.29</td>
<td>19.97</td>
<td>Chromophobe</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P26</td>
<td>33</td>
<td>M</td>
<td>0.98</td>
<td>7.48</td>
<td>Chromophobe</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P22</td>
<td>57</td>
<td>M</td>
<td>0.87</td>
<td>2.0</td>
<td>Chromophobe</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P29</td>
<td>26</td>
<td>F</td>
<td>1.56</td>
<td>1.24</td>
<td>Chromophobe</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Acidophil (n = 4), Basophil (n = 1), Chromophobe (n = 9)
Figure 3.23. Mean Ki-67 and PCNA LI\%s in pituitary adenoma based on hormone secretion.

- **SILENT**: n = 2
- **ACTH**: n = 2
- **PROLACTIN**: n = 4
- **GH**: n = 4

PCNA LI\% and Ki-67 LI\%
Figure 3.24. Correlation between age and Ki-67 LI in pituitary adenoma

\[(r = 0.369, \ p = 0.038)\]
3.3.7. Comparison of Ki-67 LI and clinical characteristics and outcome in patients with pituitary adenoma

Younger patients with pituitary adenomas tended to have higher LIs than older ones ($r = -0.369$, $p = 0.038$, Figure 3.24). However, Ki-67 LI did not correlated with duration of symptoms ($r = -0.063$, $p = 0.730$). Ki-67 LI however did correlate with follow up period ($r = -0.393$, $p = 0.032$, Figure 3.25).

3.3.8. Comparison of PCNA LI and clinical characteristics and outcome in patients with pituitary adenoma

The PCNA LIs of fourteen pituitary adenomas was correlated with the clinical characteristics and outcome. No significant correlation was found between PCNA LI and duration of symptoms or follow up ($r = -0.170$, $p = 0.540$; $r = -0.058$, $p = 0.843$). Similarly, no significant correlation was found in chromophobe adenomas between PCNA LI and duration of symptoms or length of follow up ($r = 0.230$, $p = 0.258$; $r = 0.109$, $p = 0.611$).

3.4. Glioma

3.4.1. Characteristics of the patients

There were 13 male patients and 11 female patients with gliomas of different types and grades in this study. The mean age for male patients was 46 years, with a range of 28-65 years and mean age for female patients was 55.4 years, with a range of 28-71 years.

3.4.2. Characteristics of the tumours

Fourteen patients had tumours in the left cerebral hemisphere, while 8 patients had tumours in the right and 2 patients had midline lesions. Seven patients had tumours located in the frontal lobe, 3 patients had tumour in the parietal lobe, 2 in the temporal lobe and one each in the occipital lobe, the third ventricle, the fourth ventricle and the posterior fossa. Eight patients had tumour in more than one lobe, two patients had lesions in the temporo-parietal and occipto-parietal lobes and one patient each had a lesion in the fronto-temporal, the fronto-parietal, the bifrontal and the parieto-occipital lobes. Eighteen patients had craniotomy, 4 had craniotomy with lobectomy and one each had a craniectomy and CT-directed craniotomy. Ten patients had macroscopic excision of tumour and 14 patients had subtotal resection of the lesion.
3.4.3. Clinical outcome

The mean duration of symptoms for these 24 patients with glioma was 12 months and 11 of these patients (45.8%) had a history of epilepsy (Figures 3.4. and 3.5). Four overseas patients were lost to clinical follow up and the mean follow up period for the remaining 20 patients was 17 months. Thirteen patients (54.2%) died during the follow up period and the median survival period for these patients was 9.6 months. Preoperatively, 3 (12.5%) patients were in grade 0; 8 (33.3%) in grade 1; 5 (20.7%) in grade 2; 8 (33.3%) in grade 3 and none of the patients were in grade 4 of the WHO performance grading scheme. However, postoperatively, 5 (20.7%) patients were in grade 0; 10 (41.7%) in grade 1; 5 (20.7%) in grade 2; 2 (8.3%) in grade 3 and 2 (8.3%) in grade 4. However, clinical assessment at the time of last follow up showed 6 (25.7%) patients were in grade 0; 1 (4.1%) in grade 1 and none of the patients were in grades 3 or 4. Thirteen (54.2%) patients died during the follow up period and 4 (16.7%) patients were lost to follow up, as shown in Figure 3.26. Nineteen patients received postoperative radiotherapy and 7 had chemotherapy. Eleven patients (45.8%) had tumour recurrence with mean relapse free interval of 12.3 months (Figures 3.14 and 3.15).

3.4.4. Ki-67 labelling index

The mean Ki-67 LI for all the gliomas in the study was 4.5% with a range of 0.62 - 33.3%. Five patients (20%) had Ki-67 LI less than 1%, 14 (60%) had LIs between 1 and 5%, 5 patients (20%) had LIs greater than 5% (Figure 3.7). Thirteen male patients had a mean Ki-67 LI of 2.3%, while 11 female patients had mean LI of 7.07% (Figure 3.8). Three recurrent gliomas showed a mean LI of 3.8%, with maximum LI of 5.1%. However, 12 (50%) patients whose tumours recurred after operation had a mean Ki-67 LI of 4.4%. The mean Ki-67 LI for 14 patients with grade IV malignant glioma was 3.05%, the mean LI for 6 malignant astrocytomas grade III/oligodendroglioma/mixed oligoastrocytoma grade III was 8.5% and mean Ki-67 LI for three astrocytomas grade II/oligodendroglioma grade II was 4.03%. The single case of ependymoma had Ki-67 LI of 0.81% (Figures 3.27 and 3.28).

3.4.5. PCNA labelling index

The mean PCNA LI of 15 gliomas of different type and grade was 2.7% (Figure 3.29). The mean PCNA LI for 10 grade IV malignant astrocytomas was 2.4%, 2
Figure 3.25. Correlation between Ki-67 LI and Follow-up period in pituitary adenoma patients.

\( r = -0.393, \ p = 0.032 \)
Fig. 3.26. WHO 5-point performance score in glioma patients (n = 24)
Figure 3.27. Ki-67 LI in gliomas based on histology

Figure 3.28. Mean Ki-67 LI in glioma based on histology
Figure 3.2.9. Mean Ki-67 LI & PCNA LI in tumours studied

Glioma (n = 15) with Ki-67 and (n = 15) with PCNA

Meningioma (n = 33) with Ki-67 and (n = 33) with PCNA

Pituitary adenoma (n = 14) with Ki-67 and (n = 14) with PCNA
Fig. 3.30. Frequency distribution in 15 gliomas stained with Ki-67

A.

Frequency distribution in 15 gliomas stained with PCNA

B.
Figure 3.31 Ki-67 LI% & PCNA LI% in glioma based on histology

Figure 3.32 Mean Ki-67 LI and PCNA LI in glioma based on histology
Figure 3.33: Correlation between Ki-67 LI and PCNA LI in grade IV glioma

\( r = 0.583, p = 0.077 \)
malignant mixed oligoastrocytoma had mean LI of 6.6% and three low grade astrocytoma or oligodendrogliomas had a mean value of 1.1%.

3.4.6. Comparison of Ki-67 LI and PCNA LI

The mean Ki-67 LI for 15 gliomas of different type and grade was 3.8%, with a range of 0.68% - 17.05% while the mean PCNA LI for the same gliomas was 2.7%, with a range of 0.49 - 7.8% (Figure 3.29). There were 2 gliomas with Ki-67 LIs less than 1% as compared with 6 gliomas, with PCNA LIs less than 1%, 10 gliomas had Ki-67 between 1% and 5% as compared with 4 gliomas with PCNA LI in the same range. There were 3 gliomas with Ki-67 LI greater than 5%, as compared with 5 gliomas with PCNA LI greater than 5% (Figure 3.30). Comparison was also made between different histological types and grade of glioma. There were 10 grade IV malignant astrocytomas with a mean Ki-67 LI of 3.5% as compared to a mean PCNA LI of 2.4%. Two malignant astrocytoma grade III had a mean Ki-67 LI of 5.2% as compared to a mean PCNA LI of 6.6% and similarly 3 astrocytoma/oligodendroglioma grade II had mean Ki-67 LI of 4.03% as compared with mean PCNA LI of 1.1% (Figures 3.31 and 3.32). In ten grade IV malignant astrocytomas there was a significant correlation between Ki-67 LI and PCNA LI (r = 0.583, p = 0.077, Figure 3.33).

3.4.7. Comparison of Ki-67 LI and clinical characteristics and outcome in patients with glioma

Age was not found to be significant correlated with Ki-67 LI in gliomas(r = 0.120, p = 0.584). There was no significant correlation between Ki-67 LI and PCNA LI, preoperative performance score, postoperative performance score, performance score at the last follow up, duration of symptoms and follow up period (r = 0.381, p = 0.161; r = 0.045, p = 0.835; r = -0.022, p = 0.919; r = -0.046, p = 0.833; r = -0.198, p = 0.389 respectively). Similarly, no significant statistical correlation was found between Ki-67 LI in 14 high grade malignant astrocytomas and pre and postoperative performance score, duration of symptoms and follow up period (r = -0.014, p = 0.962; r = 0.099, p = 0.737; r = -0.151, p = 0.605; r = -0.288, p = 0.340 respectively).

3.4.8. Comparison of PCNA LI and clinical characteristics of patients including outcome

Age was not significantly correlated with PCNA LI in patients with glioma (r = -0.000, p = 0.999). There was no correlation between PCNA LI, pre and
postoperative performance score, duration of symptoms and follow up period ($r = -0.267, p = 0.336; r = -0.200, p = 0.475; r = -0.100, p = 0.724; r = -0.257, p = 0.355; r = -0.004, p = 0.988$, respectively). Similarly, no significant correlation was found between PCNA LI of 14 malignant astrocytomas and pre and postoperative performance score, duration of symptoms and follow up period ($r = 0.198, p = 0.584; r = 0.393, p = 0.262; r = -0.296, p = 0.407; r = -0.417, p = 0.230$, respectively).

3.4. Survival analysis

Data from these patients was analyzed using Cox’s proportional hazard model. Patients with small numbers of other types of brain lesions were not considered for survival analysis. Variable such as Ki-67 LI, PCNA LI, duration of symptoms, pre and postoperative performance status thought to be related to survival, were tested for their significance using this modelling technique. The variables that were independently related to outcome, age, tumour group and pre and postoperative performance status, remained so after adjusting for each other. For a one year increase in age, the risk of death was increased by 9% with a 95% confidence interval of 1.03 - 1.15. A patient was nearly 26 times more likely to die with a glioma than one that was of a meningioma with 95 % of confidence interval of 7.11 - 92.14. Lastly, the reduced performance status of a patient increased their risk of death. However, it is important to recognise that the very wide confidence intervals for some variables were probably a reflection of the relatively small numbers of patients in this study.

3.5. Other tumours

3.5.1. Primary cerebral lymphoma

There was one female patient, aged 55 years with primary cerebral lymphoma. The lesion was located in the posterior fossa, on the right side. The patient had a posterior fossa craniectomy and there was a subtotal resection of the tumour. Duration of symptoms was only of 1 month. WHO pre- and postoperative performance scores were the same at 2. There was a history of epilepsy at diagnosis. Although the patient was given postoperative radiotherapy, the tumour recurred and the patient died 11 months after operation. The Ki-67 LI was as 0.9%.
3.5.2. Capillary haemangioblastoma

A single female patient, aged 35 years presented with capillary haemangioblastoma with 5 months duration of symptoms. The tumour was located in the posterior fossa on the left side. The patient had a craniectomy and there was total macroscopic excision of the lesion. WHO preoperative performance score was 3, however the patient improved postoperatively with a score of 2 and then 1 at the last follow up. The patient was followed up for 39 months and the tumour had a Ki-67 LI of 0.2%.

3.5.3. PNET (Primitive neuroectodermal tumour)

There was one male patient, aged 36 years with a PNET. The tumour was located in the fourth ventricle. The patient had a craniectomy and a subtotal resection of the tumour. The patient had a history of epilepsy at diagnosis. The duration of symptoms was only 4 months. The WHO preoperative performance score was 3 and it remained the same, postoperatively. The patient died 2 months after surgery following postoperative radiotherapy. The Ki-67 LI and PCNA LI were 2.2% and 1.4%, respectively.

3.5.4. Metastasis

There were 3 patients with metastatic tumours in the brain. The primary tumours, were renal adenocarcinoma, malignant melanoma and ovarian cancer respectively. Secondaries were located in the occipital lobe, parieto-occipital lobes and the temporal lobe. Two patients had lesions on the left side and 1 patient had a lesion on the right side. All the 3 patients had craniotomies and 1 patient had a lobectomy. One of the patients had history of epilepsy at diagnosis. The mean duration of symptoms was 24.3 months. Two patients each had WHO preoperative performance scores of 2, 1 patient had a score of 3 and the scores remained the same postoperatively. 1 patient was lost to follow up and the remaining 2 patients died during the follow up period. The mean survival period for these patients was 18 months. Two patients had tumour recurrence with a mean relapse free interval of 11.5 months. Two patients had postoperative radiotherapy and one patient had chemotherapy. Mean Ki-67 LI was 10.9%, with a range of 1 - 17.3%. However, 2 patients with tumour recurrence showed a high mean Ki-67 LI of 15.9%. These tumours were not included into the statistical analysis because of their small number.
CHAPTER IV

General Discussion

One of the major goals of the pathological study of an excised tumour is to estimate its proliferative potential. This is particularly so in human tumours, where patients and their relatives require some estimate of prognosis and some indication of the necessity for, and usefulness of, adjuvant therapy. There has been the hope that quantification of this fundamental biological process will provide pathologists and clinicians with more effective management for patients with neoplasia. Despite very considerable efforts using a variety of methodologies, this hope has not been fulfilled. There are several reasons for this failure, not least among these is the complexity of the process being studied. It is difficult to believe that a single parameter such as proliferative potential could provide the clinician with data that would alone give a definitive therapeutic answer. Another reason which may be of considerable importance in hindering appropriate studies being performed is that many of the methods for assessing cellular proliferation are somewhat difficult, requiring specialized facilities and expertise. In addition, some methods such as flow cytometry require disruption of the material with possible loss of potential valuable information inherent in proliferating and non-proliferating cells. Such information on the proliferative activity of tumours is particularly important for many lesions of the central nervous system, especially in glioma, where total resection is often not possible and in addition, the form of postoperative adjuvant therapy may depend on the histological grading. At present, this histological classification is carried out with the help of past knowledge and experience. Important features like cytologic nuclear appearance, vascular proliferation, necrosis and presence or absence of mitoses are assessed. However various methods are also being applied to tumour material to assess more accurately the cell kinetics of excised tumour and try to predict the biological behaviour of an individual tumour. In this study Ki-67 monoclonal antibody and an antibody against an epitope of proliferating cell nuclear antigen (PCNA), in frozen sections were used as markers of tumour proliferative activity in a variety of 123 brain tumours of different histologies. Ki-67 (Gerdes et al., 1983), was used in 61 meningiomas, 32 pituitary adenomas and 24 gliomas of different types and grades and in small numbers of other brain tumours and metastases. The potential usefulness of this antibody in defining the growth fraction of cells in human neoplasms has been indicated by many studies. In this technique the positive nuclei are labelled and counted as percentage of the total nuclei. Ki-67 has been shown to be rapid, reproducible, readily available, safe, sensitive and simple method to detect specifically the growth fraction of benign and malignant cell
populations. It can, however, only be used on frozen sections and is sensitive to tissue drying and fixation. Because of this potential limitation of Ki-67, another proliferative marker known as proliferative cell nuclear antigen (PCNA) was also used. PCNA is a 36 kD protein which acts as auxiliary protein to DNA polymerase d and was first described by Miyachi et al. (1978). Although previously PC10 monoclonal antibody has been used exclusively in paraffin-embedded tissues, the present author has used it in frozen sections of 33 meningiomas, 14 pituitary adenomas and 15 gliomas.

4.1. Use of Ki-67 and PCNA in meningioma

The biological behaviour of meningiomas is difficult to predict as many meningiomas with potentially aggressive behaviour may be indistinguishable histologically from more slowly growing ones. In this study, 61 and 33 meningiomas of various subtypes were examined using Ki-67 and PC10 respectively. In previous studies of meningiomas using Ki-67, the labelling index has always been low. In one study, Shibata et al. (1988) showed the mean Ki-67 value for 17 meningiomas as 1.4% and 5% in recurrent and anaplastic meningiomas, respectively. They found the highest Ki-67 LIs in the haemangiopericytic variant and in anaplastic meningiomas. Siegers et al. (1989) also found substantially lower number of positive cells in most of the meningiomas as compared to malignant glioma, anaplastic meningioma and metastatic carcinoma. Fifty two meningiomas were included in the study with LIs ranging from 0.09% - 3.78%. In a study by Deckert et al. (1989), the mean Ki-67 LI for 17 meningiomas was 1.1% with a range between zero and 5%. These results were similar to the results obtained by Roggendorf et al. (1987). Deckert et al. (1989) found Ki-67 LIs of about 1% in most primary benign endotheliomatous, fibromatous and angioblastic meningiomas, whereas recurrent tumours and primary anaplastic and transitional meningiomas showed a higher fraction of Ki-67 positive cells with values as high as 20%. In addition, they found individual cases of fibromatous and endotheliomatous meningiomas with a Ki-67 LI approaching 10% and postulated a more rapid growth and a greater risk for recurrence in these tumours. In the present study, the experience with Ki-67 in meningiomas was similar to that reported by Shibata et al. (1988), Siegers et al. (1989) and Deckert et al. (1989). The mean Ki-67 LI was 2.8% with a range of 0.11-29.1%. There were 44 (72.1%) meningotheliomatous meningiomas and these had a mean LI of 2.3%. Six fibroblastic meningiomas, had a higher mean LI of 3.8%. Five atypical meningiomas also had a higher LIs (3.2%) and one anaplastic meningioma, which had recurred showed the highest LI of 29.2%. One xanthomatous meningioma had the lowest LI in the series of 0.91%.
At the present time Ki-67 is used only on frozen sections. Many workers have also found technical problems in its sensitivity to tissue drying and fixation. In view of these potential limitations in its use the present author has investigated the use of an alternative marker, PCNA, the over-expression of which is thought to be linked with cell proliferation. This is the first report of the use of PC10 in an immunoperoxidase method on frozen sections of brain tumour. However, haemangiopericytomas were studied retrospectively using PC10 by Yu et al. (1991) who found that though the percentage of tumour cells with positive staining with PC10 correlated well with histological grading but not with clinical outcome. The present author studied 33 meningiomas using PC10 in frozen section and then compared the LIs obtained with Ki-67 with those of obtained using PCNA in the same tumour sample and correlated these results with clinical outcome.

4.1.1. Heterogeneity in meningioma

Heterogeneity in the distribution of positive cells is found in both benign and malignant tumours. In this study it was clear that there was a wide range of positive cells scattered at different sites within a single section. However, there was an attempt to select the area on each section with maximum positive cells. Siegers et al. (1989) had also observed the difference in number of Ki-67 positive cells between randomly chosen fields in the same tumour and evaluated the possibility of a heterogeneous distribution of Ki-67 positive cells in meningiomas. The distribution of Ki-67 positive cells has been reported to be heterogeneous in high grade tumours by most of the authors and a more homogeneous pattern is usually described in low grade neoplasm.

4.1.2. Comparison between Ki-67 LI and PCNA LI

In the present study, the mean Ki-67 LI was greater than mean PCNA LI in meningioma. 36.4% of meningiomas had Ki-67 LI less than 1%, as compared with 39.4% of meningiomas stained with PCNA. However, while 12.1% (4 cases) of meningiomas labelled with Ki-67, had LIs greater than 5%, no meningioma had PCNA LI greater than 5%. The cumulative frequency distribution in meningioma varies between Ki-67 and PCNA LIs, shown in Figure 4.1. Fifty percent of meningiomas with Ki-67 LI had the same pattern of cumulative frequency distribution as compared with only 20% of meningiomas stained with PCNA. When a comparison was made between the two LIs in meningiomas, a positive correlation was found. The difference in the two LIs was plotted against the mean of both LIs, as shown in Figure 4.2. A fan shaped distribution skewed towards the
positive side was observed. The mean differences between the two LIs showed a line of regression and indicating that high Ki-67 LIs were associated with high PCNA LIs in meningioma. However, Louis et al. (1991), noted a high PCNA LI (4% - 65%) as compared with Ki-67 LI (≤1.6%) in a comparative study between Ki-67 and PCNA in schwannomas and suggested that this particular PCNA antibody may react with an epitope present in normal Schwann cells. They used the 19F4 mouse monoclonal antibody against PCNA while the present author used PC10 antibody. In the present study, comparison between Ki-67 LI and PCNA LI in various histological subtypes in meningiomas showed that mean Ki-67 LIs were higher for atypical, fibroblastic, meningotheliomatous, transitional types and psammomatous types, whereas mean PCNA LI was higher only in angioblastic type and both the LIs were similar in the xanthomatous type of meningioma.

4.1.3. Clinical parameters in meningioma

In this study, the mean age of the 34 female patients was 55.5 years as compared to a mean age of 27 male patients of 58.9 years. These figures are very similar to those reported by Roggendorf et al. (1987) in which there were 15 female patients with meningioma with a mean age of 59.6 years and 13 males with a mean age of 57.5 years. The mean duration of symptoms before surgery in 61 meningioma patients was 20.6 months with a range of 1 month to 264 months, as compared to 12.04 months (range 1-120) for patients with glioma and 36.3 months (range 4 - 180) for patients with pituitary adenoma. The mean relapse free interval (RFI) following surgery was longer in the meningioma patients than in those with glioma, but less than in the pituitary adenoma group. Pre-operatively, 17 patients (27.9%) with meningioma had a WHO performance score of grade 0, 22 patients (36%) were in grade 1, 17 (27.9%) were in grade 2, 5 (8.2%) patients were in grade 3 and there were no patients with grade 4. There was a slight change in these performance scores in the immediate postoperative period or at the time of discharge from hospital. The number of patients in grade 0 increased to 27 (44.3%), 16 (26.1%) patients were grade 1, 13 (21.3%) were grade 2, 5 (8.2%) patients were grade 3 and there was no patients in grade 4. There were no deaths in meningioma patients in the immediate postoperative period. During follow up WHO performance scores continued to improve. Unfortunately, 5 patients could not be traced after discharge from hospital (most of these patients were from abroad and neither they nor their medical advisor could be contacted). Of the remaining 56 patients in whom the information was available, the number of patients in grade 0 had risen to 39 (63.9%). 6 patients were in grade 1 (9.7%), 3 patient (4.8%) were in grade 2 and there were no patients in grade 3 and 4. In this period 8 (13.1%) patients died. The survival time ranged from 1 month to 40 months, with a mean of 13.6 months. In
comparison, 54.1% of glioma patients and only 6.2% of patients with pituitary adenoma died in the same period of follow up. Although recurrence in meningioma must be dependent upon the extent of the initial surgical resection, the biological behaviour of the tumour studied should not be ignored. The author found the recurrence rate in this study to be 14.7% (9 patients). This incidence is in accordance with that of the other authors, for example, Simpson (1957). In this group of patients, the relapse free interval ranged from 6 to 40 months, with a mean of 18.2 months. As will be seen below, the recurrence rate was much higher in patients with glioma (50%) and significantly less in patients with pituitary adenomas (3.1%). In meningioma patients who survived and in whom there was no recurrence, there was clearly an overall clinical improvement in their neurological status postoperatively and this improvement continued throughout their followup.

The mean Ki-67 LI was higher in recurrent meningiomas compared to primary meningiomas which had not recurred. However, the mean PCNA LI of three recurrent meningiomas was lower than the mean PCNA LI of primary meningioma. In this series more patients with meningioma (52%) had epilepsy at the time of diagnosis than glioma patients (46%) or patients with pituitary adenoma (6.2%). Epilepsy is frequently one of the earliest manifestation of meningioma (as well as other intracranial neoplasms) and thus may lead also to early diagnosis of tumour and treatment with the hope of a both improved prognosis in term of life expectancy and prevention of neurological disability. Similarly, it could be postulated that the rapidity of onset of symptoms might be an indication of the aggressiveness of the tumour.

4.1.4. Ki-67 LI / PCNA LI and clinical parameters

The proliferative and clinical data from 61 meningiomas were analyzed and compared to studies by Roggendorf et al. (1987) and Shibata et al. (1988) both of whom examined smaller groups of tumours than the present author. In the present author’s study, the mean Ki-67 LI for all meningiomas was 2.8%. However, Ki-67 LI for recurrent meningioma was higher (5.4%) data which is in agreement with Shibata et al. (1988). There was no significant correlation between Ki-67 LI in meningioma and clinical outcome. Similarly, no correlation was found between Ki-67 LI of meningioma subgroups and clinical outcome. Although no definitive correlation between Ki-67 LI in meningioma and clinical parameters has been found in this study, other workers have found the determination of the proliferation rate in meningioma with Ki-67 is of value for the subgroups of meningioma, which sometime show no sign of increased proliferation with
conventional histology (Roggendorf et al., 1987). Ki-67 has been used extensively as a marker of proliferation in many other types of tumour. It has been shown that Ki-67 score is highly correlated with histological classification of non-Hodgkin's lymphoma and is of prognostic significance (Gerdes et al., 1984; Hall et al., 1988). Similarly, proliferative index in breast carcinoma was determined in situ by Ki-67 immunostaining and later correlated to clinical and pathological variables (Bernard et al., 1987). They found a weak negative correlation between age and the Ki-67 score and a positive association between the Ki-67 score and tumour grade but could not find any strong relationship between Ki-67 LI outcome in breast carcinoma.

4.2. Use of Ki-67 and PCNA in pituitary adenoma

The mean Ki-67 LI for 32 pituitary adenomas in this study was 1.8%, with a range of 0.1 - 9.7%. This is comparable with the spread of LIs (0.1 - 3.7%) reported in a group of 31 pituitary adenomas by Landolt et al. (1987). Similarly, Burger et al. (1988) also reported a mean Ki-67 LI 1.02% in pituitary adenoma with a range of 0.2-1.5%. The results in the present authors study with endocrinologically inactive adenomas, the mean LI of 0.7%, was similar to other studies for this subgroup of pituitary adenomas. Shibuya et al. (1992), in a histochemical study of 65 pituitary adenomas found low Ki-67 LIs of around 1% in primary tumours but higher Ki-67 LIs in recurrent tumours. The high LI in GH producing adenomas in the present study was similar to that of Landolt et al. (1987), which had also shown high LIs in GH producing tumours. The incidence of Ki-67 positive nuclei, however, varied markedly in pituitary adenomas with a wide range of Ki-67 LI between 0.1 and 9.7%. Burger et al. (1986), also showed a considerable variation in the percentage of stained cells in pituitary adenoma, although they had a smaller number of tumour samples included in the study. Similarly, Landolt et al. (1987) also reported a wide range of variation in the Ki-67 LI and this was particularly marked in prolactinomas.

In this study, PC 10 monoclonal antibody against PCNA was used in frozen sections of pituitary adenoma and this is the first time that such a study has been undertaken. In pituitary adenomas, the percentage of proliferating cells that are known for their slow growth is surprisingly high considering the rather slow growth of these tumours. The mean PCNA LI for 14 pituitary adenomas was 5.9% with a range of 1.04 - 20.7%.
4.2.1. Clinical parameters in pituitary adenoma

In this group of pituitary adenoma patients, the mean duration of symptoms was 36.4 months, with a range of 4 to 180 months, which was greater than the mean duration of symptoms of patients with meningiomas (20.5, range 1 - 264 months), and gliomas (12.04 months, range 1 - 120 months). There were only 2 patients (6.2%) with history of epilepsy at diagnosis as compared to 52.4% and 45.8%, for meningioma and glioma, respectively. It was inappropriate to apply the WHO 5-point performance scale in patients with pituitary adenoma. They were therefore categorised as symptomatic or asymptomatic preoperatively and postoperatively and at the final assessment as clinically the same, improved or worse. There were 31 patients (96.9%) with various symptoms, preoperatively and only 1 (3.1%) patient was clinically asymptomatic before operation. Twenty seven patients (84.4%), improved clinically after operation and 1 patient (3.1%) deteriorated while 4 patients (12.5%) remained clinically unchanged. At the end of the study it was found that 26 patients (81.2%) had improved clinically, 1 (3.1%) patient remained the same and 1 (3.1%) patient was worse after operation. Two patients with pituitary adenoma were lost to follow up and 2 (6.2%) patients died during the follow up period compared to 13.1% of meningioma patients and 54.1% of glioma patients. There was only 1 (3.12%) tumour recurrence as compared with 14.7% of meningiomas and 50% of gliomas.

4.2.2. Comparison between Ki-67 LI and PCNA LI

There was an interesting finding while comparing the LIs determined by these two methods in pituitary adenomas. The mean Ki-67 LI for 14 patients with pituitary adenoma was determined as 1.8%, whereas the PCNA LI for the same adenomas was 5.9%. There were 6 pituitary adenomas (42.9%) with Ki-67 LI less than 1%, while no pituitary adenomas had PCNA LIs below 1%. However, there were 7 (50%) pituitary adenomas with Ki-67 LIs between 1% and 5%, as compared with 10 (71.3%) pituitary adenomas labelled with PCNA. Similarly, there was only 1 (7.1%) pituitary adenoma with Ki-67 labelling which had LI greater than 5%, as but 4 (28.8%) pituitary adenomas with PCNA LIs greater than 5%. These results appear to indicate a tendency among pituitary adenomas to exhibit low Ki-67 LIs but high PCNA LIs. There is as yet no definite explanation for this, however, one possible hypothesis is that PCNA may be a more specific S-phase marker, although a less sensitive proliferation marker than Ki-67 (Louis et al., 1991) or alternatively PCNA expression may be influenced by the endocrine activity of these tumours. Dervan et al. (1992) carried out a comparative study between PCNA counts in formalin fixed paraffin-embedded tissue with Ki-67 in fresh tissue among 35 tumours of
various types and 11 normal tissues. However, they were unable to find any significant difference between Ki-67 and PCNA LI. In a similar study, using lymphomas, Kamel et al. (1991) showed a strong correlation between PCNA and Ki-67 counts. Dervan et al. (1992), found mean PCNA counts higher than Ki-67 counts. All pituitary adenoma stained with Ki-67, except one, had LIs less than 5%, whereas there were several pituitary adenoma stained with PCNA, had LIs greater than 5%, and the maximum LI was 20%. The pattern of the cumulative frequency distribution of the LIs of pituitary adenomas is shown in Figure 4.1. The individual Ki-67 LIs show a pattern with only small variation around the modal values, whereas the values obtained by PCNA show much wider variation with no single clear modal value. The mean differences between Ki-67 and PCNA LI in pituitary adenoma were compared, showed high PCNA LI and low Ki-67 LI. When the difference in both the LIs was plotted against the mean LIs, this produced a fan shaped distribution with marked trend towards low Ki-67 LIs with high PCNA LIs (Figure 4.3). Comparison based on histology among 14 pituitary adenomas, indicated higher PCNA LIs than Ki-67 LIs in chromophobe and basophil adenomas. However, in acidophil adenomas there was a small difference between the mean LIs. Similarly, when the comparisons were made between Ki-67 and PCNA LIs based on hormone secretion, although the small numbers of tumours in each group failed to reach statistical significance, it was evident that PCNA LIs tended to be considerably higher than Ki-67 LI in prolactinomas, ACTH producing adenomas and silent adenomas. However, there was no difference between the LIs in GH producing adenomas. The maximum mean value for Ki-67 LI was greatest for GH producing adenomas, followed by prolactinomas, silent adenomas and the lowest LIs were seen in ACTH producing adenomas. However highest PCNA LIs was seen in the silent adenomas subgroup, followed by ACTH adenomas, prolactinomas with the lowest LI in GH producing adenomas.

Opinion is divided about the potential clinical use of PCNA and Ki-67 in patients with CNS tumours. Louis et al. (1991), believed that the use of PCNA was more problematic than the use of Ki-67 because the staining pattern was variable and necessitated counting at high magnification to avoid missing lightly-stained nuclei and this introduced more difficulties in cell counting than the darker Ki-67 pattern. Therefore, Louis et al. (1991) were of the view that the 19F4 anti-PCNA antibody may be less useful than Ki-67 as a proliferation marker in frozen sections of human nervous system tumours. However, Dervan et al. (1992), were of the opinion that the extent and range of nonproliferative PCNA positivity needs further clarification before PC10 can be used as a general, all-purpose marker of tumour cell proliferation.
4.2.3. Ki-67 LI / PCNA LI and clinical parameters

The labelling indices for these two markers were analyzed and correlated with clinical parameters of patients with 32 pituitary adenomas. While correlating age and Ki-67 LI in pituitary adenoma, a negative relationship was found. Younger patients showed higher Ki-67 LIs than older patients. Only one patient with pituitary adenoma had tumour recurrence at 8 months, the LI with Ki-67 was 1.5% and PCNA 1.2%. The results of the present study show no relationship between rate of tumour recurrence and LI, although longer follow up may reveal some. This study was unable to demonstrate any correlation between the PCNA LI and clinical parameters in patients with pituitary adenomas.

4.3. Use of Ki-67 and PCNA in glioma

The Ki-67 monoclonal antibody has been used extensively in the past decade to evaluate the proliferative potential of various tumours, including gliomas. The mean Ki-67 LI for all gliomas in this study was 4.5%, 3.05% for 14 high grade malignant gliomas, 8.5% for grade III gliomas, 4.03% for grade II gliomas and a single case of ependymoma had Ki-67 LI of 0.81%. Burger et al. (1986), showed in 26 malignant gliomas that there was a mean Ki-67 LI of 3.5%, Patsouris et al. (1988), in a group of 30 gliomas, found a mean Ki-67 LI of 3.4% and Zuber et al. (1988), showed a mean Ki-67 LI of 1% in low grade glioma, 3.5% in anaplastic glioma and mean LI of 11.1% in high grade glioma. The results obtained in 53 GBM, by Shibata et al. (1988), were higher (10%) than results from this study for GBM and similarily, Deckert et al. (1989) reported a mean Ki-67 LI of 7% in 18 GBM and mean Ki-67 LI for low grade astrocytoma of 1.4%. Raghavan et al. (1990), showed mean Ki-67 LI of 0.5% in low grade glioma, 4.1% in anaplastic astrocytoma and 6.4% in GBM. All these findings, including the present study, show higher Ki-67 LI in gliomas as compared with meningioma and pituitary adenomas.

In the present study, a mean PCNA LI of 2.3% was found for a group of 15 gliomas of all types and grade. Breaking this down by histology, ten GBMs had a mean LI of 2.4%, two grade III astrocytomas had a mean LI of 6.5% and three cases of grade II astrocytoma had a mean PCNA LI of 1.14%. In this study, frozen sections, fixed in formalin were used, whereas Figge et al. (1992) and Revesz et al. (1993a), who both used the PC10 antibody, used formalin-fixed paraffin embedded sections. In the present study, results of mean PCNA LI appeared to be less than that of other two studies. Figge et al. (1992), retrospectively investigated a series of 40 GBM showed a wide range of LIs from 0.5% to 75% (mean 24.9%). Similarly, Revesz et al. (1993a), also in a retrospective study of 55 astrocytic tumours found a mean
PCNA LI of 4.1% in the grade II, 8.1% in the grade III and 26.1% in the grade IV astrocytomas. A wide variation of LIs observed in various studies may suggest that the extent of PCNA expression may be dependent on other technical factors such as tissue processing, fixation or the counting technique and above all in this study there was a relatively small number of glioma tumour samples studied with PCNA as compared to the other studies. However, it is difficult to explain the reason of low PCNA LI observed in this study.

4.3.1. Comparison between Ki-67 LI and PCNA LI

In this study, a comparative study was carried out between the two LIs in a small series of different types and grades of glioma and this showed a mean Ki-67 LI of 15 gliomas as 3.8% as compared with mean PCNA LI of 2.8%. When the gliomas were examined on the basis of histological grade, it was found that a mean Ki-67 LI was higher in grade IV (3.5%) and grade II (4.0%) astrocytomas as compared to mean PCNA LI of 2.4% in grade IV and 1.14% in grade II tumours. However, the mean Ki-67 LI (5.2%) in grade III was less than mean PCNA LI (6.6%) in tumours of the same grade. Louis et al. (1991), showed Ki-67 and PCNA LI of ≤ 1% in low grade glioma. The Ki-67 LI ranged from 2% to 10%, as compared to the PCNA LI which ranged from 1% to 1.5% in grade III gliomas and from 17% to 30% and from 6% to 15%, respectively, in glioma grade IV. These results confirm the data presented in this thesis that high-grade gliomas appear to have higher Ki-67 LIs than PCNA LIs. A possible explanation for this could be a greater affinity of Ki-67 antigen as compared to PCNA antigen for gliomas. In this study, there were fewer cases of glioma with Ki-67 LI of <1% than PCNA LI of <1% and there were more cases of glioma with Ki-67 LI between 1% and 5% than with PCNA. The number of cases of glioma with Ki-67 LI of > 5%, were less than PCNA. However, in grade II gliomas, the mean PCNA LI was greater than Ki-67 LI. None of the PCNA LIs was above 10%, while there was a glioma with Ki-67 LI greater than 10%. There seemed to be two definite groups of tumours in terms of distribution of values for both the LIs. One group of tumours had Ki-67 LIs greater than 5% and the other group had Ki-67 LIs less than 5%. Similarly, PCNA LI also showed two distinct groups of tumours, one with PCNA LI of ≤ 5% and a second group with PCNA LI greater than 5%. Similarly, 12.5% of gliomas had a Ki-67 LI less than 1%, as compared to 37.5% of gliomas stained with PCNA. Similarly, 68.7% of gliomas had a Ki-67 LI between 1% and 5%, as compared with 31.2% cases stained with PCNA. Lastly, there were only 18.7% of gliomas with Ki-67 LI greater than 5% as compared with 31.2% of glioma with PCNA LI greater than 5%. When the means of these LIs are compared, it appears that mean Ki-67 LI was greater than mean PCNA LI in glioma grade II and IV whereas, mean PCNA LI was greater than mean Ki-67 LI in
glioma grade III. The cumulative frequency distribution of Ki-67 LIs in gliomas is shown in Figure 4.1. This indicates that 20% of gliomas labelled with Ki-67 had one pattern of labelling, whereas 60% had another pattern and the remaining 20% a third staining pattern. In comparison, gliomas stained with anti-PCNA PC 10, 70% had the same pattern of labelling and the remaining 30% a different pattern. A positive correlation was found between Ki-67 LI and PCNA LI in high grade gliomas. For each tumour, where the LI had been determined using both Ki-67 and PCNA, the difference between LIs obtained using the two methods was plotted against the mean of LIs (Figure 4.4). This indicates that a rise in Ki-67 LI in associated with rise in PCNA LI. Both Hall et al. (1990) and Kamen et al., (1991), have demonstrated a linear relation between Ki-67 and PCNA staining in malignant lymphomas. Similarly, Dervan et al. (1992), found a strong correlation between PCNA LI and Ki-67 LI in a group of various malignant tumours and normal tonsil tissue. Sasaki et al. (1992), compared PCNA immunostaining with BUdR immunostaining in brain tumours and found a good correlation between the two methods.

4.3.2. Clinical parameters in glioma

The patients with gliomas were younger than patients with meningioma or pituitary adenoma (mean age, 46 years in males and 55.5 years in female patients). The total number of patients with glioma was 24 (13 male and 11 female). The mean duration of symptoms was shorter 12.04 months, as compared with 20.6 months in patients with pituitary adenoma and 36 months in patients with meningioma. Therefore, the patients with glioma had the shortest duration of symptoms which is consistent with the natural history of these malignant tumours. These patients also had the shortest mean follow up period of 17.05 months, as compared with mean follow up periods in patients with meningioma and pituitary adenoma because of their limited survival. The mean survival period for those patients who died during the follow up period was 9.5 months, less than the survival period in patients with meningioma and pituitary adenoma. The pre- and postoperative performance status of these patients was assessed using the WHO grading scale. Preoperatively, 12.5% of patients had a performance grade of 0; 33.3% patients were grade 1; 20.7% were grade 2; 33.3% were in grade 3 and none of the patients were in grade 4. When the comparison was made with the performance in the immediate postoperative period or by the time of hospital discharge, it was found that the percentage of patients in grade 0 had increased to 20.7% and there were 10 (41.7%) patients in grade 1. There was no change in the number of patients in grade 2, however, the percentage of patients in grade 3 was reduced to 8.3% and there were 2 patients (8.3%) in grade 4. At the time of last
follow up with a mean follow up period of 17.05 months, range 1 - 39 months, the percentage of patients in grade 0 remained the same, however 1 patient (4.1%) remained in grade 1 and there were no patients in grades 2 - 4. Thirteen patients with glioma (54.2%) died between 2 and 18 months postoperatively, as compared with 13.1% of patients with meningioma and only 6.2% of patients with pituitary adenoma. There was a high incidence of tumour recurrence (50%), as compared with 14.7% of patients with meningioma and only 3.12% of patients with pituitary adenoma. The relapse free interval was (12.3 months) was less than the RFI (18.2 months) of patients with meningioma who recurred.

4.3.3. Ki-67 LI / PCNA LI and clinical parameters

Malignant gliomas present a difficult therapeutic problem and a limited survival time. The concept of prognostic significance with reference to survival time and other clinical parameters of cell kinetics, specially the labelling index in patients with malignant glioma was advanced by Hoshino and Wilson in a series of papers beginning in 1972. The monoclonal antibody Ki-67 has been applied, often with the intention of obtaining prognostic information to define proliferative activity in different groups of human tumours including a wide range of intracranial tumours as described earlier. The value of Ki-67 as a prognostic marker in non-Hodgkin's lymphoma has been well established (Hall et al., 1990). Though there have been a number of studies performed on human brain tumours, particularly in malignant gliomas, no study has clearly demonstrated a relationship between the Ki-67 LI and prognosis (Siegers et al., 1989, Zuber et al., 1988, Roggendorf et al., 1987 and Burger et al., 1986). This study analyzed the relationship between labelling indices and clinical data from 24 glioma patients. There was no significant statistical correlation between Ki-67 LI and WHO pre and postoperative performance scores, duration of symptoms and the survival period. Similar results have been demonstrated by Pigott et al. (1991) and Zuber et al. (1988). There are other factors of important prognostic significance in glioma such as histological grade, performance status and age of the patient. Therefore, determination of Ki-67 labelling index in isolation may not correlate with the survival of malignant glioma patients. In order to evaluate a possible significance in the expression of proliferating cell nuclear antigen as a clinically useful prognostic parameter, this study also investigated small series of different types and grades of glioma by means of immunohistochemistry and compared the labelling indices to patient clinical parameters. This study could not find any correlation between the PCNA LI in gliomas and pre and postoperative WHO performance score, duration of symptoms and survival period. Figge et al. (1992), also could not find any significant correlation between PCNA LI and patient survival and their study
indicated that the expression of PCNA appears not to be a useful prognostic parameter for glioblastoma patients. The prognostic value of immunoperoxidase staining for PCNA was also studied in a series of 140 non-Hodgkin's lymphoma with median follow-up period of 9 years and PCNA staining did not prove to be an independent prognostic factor in a multivariate analysis (Klemi et al., 1992). These negative findings are disappointing but it remains possible that LI information taken together with other laboratory or clinical criteria may contribute useful diagnostic information.

4.4. Labelling of other brain tumours

There were three metastatic tumours in the brain derived from a renal adenocarcinoma, malignant melanoma and ovarian cancer. Secondaries were located in the occipital lobe, parieto-occipital lobes and the temporal lobe. The mean duration of symptoms was 24.3 months with one patient who had a history of epilepsy at diagnosis. The mean Ki-67 LI was 10.9%, which was higher than the rest of the tumours. One patient was lost to follow up. The WHO performance score between the preoperative and postoperative status remained the same in all the three cases. Two patients died during the follow-up period and two patients had tumour recurrence with a mean RFI of 11.5 months and a mean survival period of 18 months. These values were not compared with other values in rest of the tumours because of their small number. There was one male patient, aged 36 years with a PNET. The duration of symptoms was 4 months. The WHO preoperative performance score was 3 and it remained the same, postoperatively. The patient had a tumour recurrence after 2 months and died. The Ki-67 LI and PCNA LI were 2.2% and 1.4%, respectively. Deckert et al. (1989) also reported a high mean Ki-67 LI of 13.7% in PNET. There were one each patient with primary cerebral lymphoma and capillary haemangioblastoma, aged 55 years and 35 years, respectively. The duration for symptoms was only one month in the case of the lymphoma and 5 months in the one case of haemangioblastoma patient. The WHO pre- and postoperative performance scores remained the same at 2 in the lymphoma patient. However, the patient with the haemangioblastoma the WHO preoperative score improved from 3 to 2 in postoperative status and then to 1 at the last follow up. Although the lymphoma patient was given postoperative radiotherapy, the tumour recurred after 11 months and the patient died, while the other patient with haemangioblastoma was followed for 39 months and clinically improved at the last follow up.
4.5. Conclusions

The prognosis of an individual patient with a CNS tumour, particularly with a cerebral glioma depends on many factors including precise tumour type, grade and biological behaviour, as well as on the patient's age, pre- and postoperative performance score and treatment with radiotherapy or chemotherapy. A major stimulus to this study of cellular proliferation in human tumours of the central nervous system and correlation with the clinical parameters was an attempt to provide clinician and pathologist with an objective measurement with which more effective management of such patients could become possible. Tumour growth depends on a variety of factors including the growth fraction, cell cycle time and cell loss. Some of the factors are easier to quantify than others, however an assessment of the size of the proliferating population has been the aim of several groups working on CNS tumours. The experience in this study was with Ki-67 and PC10 in a series of astrocytic tumours, meningiomas and pituitary adenomas and was broadly in line with the results of other groups. However, several methodological and interpretative problems, mentioned earlier, limit the usefulness of these antibodies. A major obstacle to the routine use of Ki-67 lies in the fact that it could only be applied to fresh tissue, since the epitope which it detects is destroyed by tissue fixation in formalin and embedding. Therefore, a new monoclonal antibody, MIB1 has been produced (Cattovetti et al., 1992) by immunizing mice with a recombinant Ki-67 gene product. Initially in routine immunohistological staining of paraffin sections the result were disappointing, however it may be possible in future to evaluate cell kinetic parameters in routinely formalin-fixed and paraffin-embedded histological material using microwave irradiation techniques. Similarly, a number of theoretical and technical questions need further clarification in PC10 staining. Immunoreactivity of PC10 is reduced or lost by prolonged formalin fixation and heating of tissue sections, nevertheless in this study this was taken into account. It is likely that PCNA can be detected by PC10 in some nonproliferating cells. Therefore, the extent and range of nonproliferative PCNA positivity needs further clarification before PC10 can be used as a general, all-purpose marker of tumour cell proliferation. In this study the PCNA data for malignant glioma is lower than reported in studies which have used fixed paraffin sections and there may be several reasons for this. The heterogeneity in numbers of positive cells is greater in high grade tumours than lower grade tumours, and it is possible that tumour have been selected for study with unrepresentative low labelling indices (sampling error). Therefore, with high grade tumours it may be difficult to rely on one sample and future studies may require multiple samples. It is more likely that necrosis is present in high grade tumours. Although the necrotic tissue was not counted in this study, it may be
possible that PCNA expression in cells shuts down in anoxic tissue before there is frank necrosis. There may be a balance between unmasking PCNA antigen with short period of fixation and destroying it with longer periods. Although there is data to suggest that long periods of fixation followed by embedding reduce PCNA labelling in cells, it is possible that some "unmasking" of this antigen occurs during short periods of fixation or processing which produce the higher LIs seen in studies using conventionally processed tissue rather than frozen sections. The value of Ki-67 as a prognostic marker in non-Hodgkin's lymphoma has been well established. However, there have been a number of studies performed on human brain tumour tissue where a definitive relationship between Ki-67 LI and the prognosis has not been demonstrated. This study also could not show a strong relationship between Ki-67 or PCNA LI and prognosis. This may be due to several reasons. Cell proliferation is a complex process and is not well understood. The nature of the antigen recognised by Ki-67 and the epitope recognised by PC10 are not fully characterised. Moreover, the labelling indices produced by these markers give no information about the length of the cell cycle time or the rate of cell loss in brain tumours in situ. Furthermore, the relatively small number of cases included in this study may contribute towards the lack of success in establishing a relationship between labelling index and survival. However, with the introduction of MIB1 or other new markers of cell proliferation, a better relationship may be found with prognosis.

4.6. Future perspectives

Although, currently there seems to be some methodological and interpretative problems which limit the usefulness of Ki-67 and PC10 as markers of prognostic importance, the recent introduction of MIB1 monoclonal antibody should make it possible to evaluate cell kinetic parameters in routinely formalin-fixed and paraffin-embedded histological material with greater reliability. The assessment of cell proliferation with the application of this immunohistological method may allow, in future, routine evaluation of kinetic data to aid patient management, for example assessing the response to therapy. However, further work is still to be done to produce convincing evidence that these antibodies have anything to offer with reference to patient's prognosis. The following are suggestions for future work in this area:

1. There is no doubt that large number of patients with tumours of similar histology are required. This means that patients entered into large scale, well defined trials will have to be studied. It is unlikely that Ki-67 would be useful in this situation as frozen sections would be required, which would not be possible to
get in prospective clinical trials. Therefore it may be better to use MIB 1 or PCNA on archival material.

2. The question of antigen unmasking should be addressed using either frozen sections or paraffin wax material and techniques such as microwaving.

3. Use of other molecular genetic markers such as oncogene expression or loss of function in some tumour suppressor genes eg, those on chromosome 10 may well provide additional important prognostic information and also to provide a rationale for selecting treatment. Such studies may include the use of interphase cytogenetics to look for losses of individual chromosomes.

4. There are differences in the intensity of staining between nuclei in the same section. The data can be reanalysed using different criteria to determine which nuclei were important for predicting prognosis eg, whether only strongly stained nuclei are important or all nuclei. Perhaps the use of image analysis equipment may be able to separate lightly stained nuclei from strongly stained ones.

5. PCNA is a marker of DNA repair as well as DNA replication (ie. proliferation). Can it can be used to predict chemo- or radiosensitivity in tissue culture experiments or to provide a correlation between in situ studies and in vivo ones?

6. It is important to assess the biological bases for the differences between Ki-67 and PCNA LIIs in meningiomas and pituitary tumours. Is there really a difference between the histological/immunocytochemical subgroups and is it related to expression of peptide growth factors like platelet derived growth factor or fibroblast growth factor and what is the relationship between peptide growth factor expression and endocrine function in pituitary adenomas?

7. The effects of bromocriptine on Ki-67/PCNA expression in vitro and in vivo require further study to see if it influences expression of these proliferation associated markers.
Figure 4.1. Cumulative frequency distribution.
Figure 4.2. Mean difference between Ki-67 LI & PCNA LI in meningioma

Difference in LIs between Ki-67 LI & PCNA LI in meningioma
Figure 4.3. Mean differences between Ki-67 LI & PCNA LI in pituitary adenoma.

Difference in LIs between Ki-67 LI & PCNA LI in pituitary adenoma.
Figure 4.4. Mean differences between Ki-67 LI & PCNA LI in malignant glioma IV

Difference in LIs between Ki-67 & PCNA LI in malignant glioma IV
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Appendix II

Ki-67 Staining Protocol

1. Cryostat sections, fix in cold acetone (4° C) for 10 minutes at room temperature.
2. Air dry at room temperature for 5 minutes.
3. Cold PBS wash for 5 minutes.
4. Add 1/10 solution of primary antibody for 30-45 minutes in room temperature.
5. Cold PBS wash for 5 minutes.
6. Add 1/20 solution of secondary antibody (IgG) for 30 minutes at room temperature.
7. Cold PBS wash for 5 minutes.
8. Apply activated DAB solution (freshly prepared) for 10 minutes, add 3 µl. of hydrogen peroxide and filter the solution.
9. Cold PBS wash for 5 minutes.
10. Counter stain with haematoxylin for between 5 and 10 minutes.
11. Cold PBS wash for 5 minutes.
12. Dehydrate in 70%, 80%, 90% and 100% alcohol and in Xylene for 1 minute.
13. Mount with DPX.
Appfndh in
PC 10 Staining Protocol

1. Cryostat sections, fix in 4% formal saline at room temperature for 2 minutes, follow by ethanol for 10 minutes.
2. PBS wash for 5 minutes.
3. Add 1/10 solution of primary antibody for 30-45 minutes at room temperature.
4. PBS wash for 5 minutes.
5. Add 1/20 dilution of secondary antibody (IgG) for 30 minutes at room temperature.
6. PBS wash for 5 minutes.
7. Apply activated DAB solution for 10 minutes at room temperature.
8. PBS wash for 5 minutes.
9. Counter stain with haematoxylin for 10 minutes at room temperature.
10. PBS wash for 5 minutes.
11. Dehydrate in 70%, 80%, 90% and absolute alcohol and then in Xylene for 1 minute.
12. Mount with DPX.
**Appendix IV**

**FILM PROTOCOL**

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Appendix V

SURNAME: ........................................
SEX:  MALE ☐  FEMALE ☐
HOSPITAL NO: ..............................
ADDRESS: ........................................
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........................................
CONSULTANT: ..............................
DIAGNOSIS: .................................

AGE:  ............................. Years
G.P:  .................................
WARD:  .................................

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very brisk = +++; brisk = ++; present = +; absent = o; clonus = cl

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**DATE OF 2ND OPERATION:** 

**Operative Findings:** 

**Post Op. CT/MRI Finding:** 

**Follow Up:** 

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### X-RAY SKULL:

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### ANGIOGRAM:

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### CAT PRE-CONTRAST:

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### POST-CONTRAST:

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### MRI:

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### DATE OF OPERATION:

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### OPERATIVE FINDINGS:

Tumour Site / Size / Consistency

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### HISTOPATHOLOGICAL REPORT:

---
a. Meningotheliomatous meningioma, showing a few cells positively stained with Ki-67

b. Recurrent meningotheliomatous meningioma showing many cells positively stained with Ki-67
c. Meningotheliomatous meningioma showing few cells positively stained with PC10

d. Malignant glioma grade IV showing many cells positively stained with PC10
e. Meningotheliomatous meningioma showing a few cells positively stained with Ki-67

f. Meningotheliomatous meningioma showing a few cells positively stained with PC10
i. Pituitary adenoma, chromophobe, GH producing showing a few cells positively
stained with Ki-67

j. Pituitary adenoma, chromophobe, GH producing showing many cells positively
stained with PC10
k. Pituitary adenoma, chromophobe, ACTH producing showing a few cells positively stained with Ki-67

l. Pituitary adenoma, chromophobe, prolactin producing showing many cells positively stained with PC10
m. Pituitary adenoma, chromophobe, silent showing few cells positively stained with Ki-67

n. Pituitary adenoma, chromophobe, silent showing many cells positively stained with PC10
q. Malignant glioma grade IV showing many cells positively stained with Ki-67

r. Astrocytoma grade II showing a few cells positively stained with Ki-67
Oligodendroglioma grade III showing many cells positively stained with K-67.

Oligodendroglioma grade III showing few cells positively stained with PC10.
u. RT 112 showing many cells positively stained with Ki-67

v. Recurrent anaplastic meningioma showing many cells positively stained with Ki-67
Appendix VI

General & Prognostic Information

Serial No: 
First Name: 
Surname: 
Sex: 
Age: 
Diagnosis: 
Tumour subtype: 
Grade: 

Duration of symptoms: 
Operation type: 
Epilepsy: 
Site: 
Side: 
Operation date: 
Extent of removal: 
Date of recurrence: 

Radiotherapy: 
Chemotherapy

Performance status: 
Preoperatively: 
Postoperatively: 
Last Follow-up: 

Patient Alive? 
Follow up period: 

Relapse Free Interval:

Ki-67 LI%: 
PCNA LI%: