"A STUDY ON EPIDERMAL GROWTH FACTOR RECEPTOR AND KI 67 EXPRESSION IN CORRELATION WITH GRADING OF GLIOMA"

Dissertation submitted to

The Tamil Nadu Dr. MGR Medical University

In partial fulfillment of the regulations for the award of the degree of

M.D. PATHOLOGY

Branch III

INSTITUTE OF PATHOLOGY

Madras Medical College and Rajiv Gandhi Government General Hospital

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MAY 2018

CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON EPIDERMAL GROWTH FACTOR RECEPTOR AND KI 67 EXPRESSION IN CORRELATION WITH GRADING OF GLIOMA"** is the original work of **Dr. SNEHA SURESH,** in partial fulfilment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R. Medical University to be held in May 2018.

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DECLARATION

I, **Dr.SNEHA SURESH,** solemnly declare that the dissertation titled **"A STUDY ON EPIDERMAL GROWTH FACTOR RECEPTOR AND KI 67 EXPRESSION IN CORRELATION WITH GRADING OF GLIOMA"** is the bonafide work done by me at the Institute of pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr. Bharathi Vidhya Jayanthi** M.D., Director & Professor of Pathology, Institute of pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfilment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr. SNEHA SURESH**

ACKNOWLEDGEMENT

I express my sincere thanks to Dean, **Prof. Dr. R. Narayana Babu, MD, DCH,** Madras Medical College and Rajiv Gandhi Government General Hospital, for permitting me to utilize the facilities of the institution.

I take this opportunity to express my thanks and heartfelt gratitude to **Prof. Dr. Bharathi Vidhya Jayanthi, M.D.,** Director and Professor of Pathology, Institute of pathology, Madras Medical College for her keen interest, constant encouragement and valuable suggestions throughout the study.

I am extremely thankful to my guide **Prof.Dr.Ramamoorthy M.D.,and my professors Prof.Dr.R.Padmavathi M.D., , Prof.Dr.Geetha Devadas M.D., Prof.Dr.Sudha Venkatesh M.D., Prof.Dr.M.P.Kanchana M.D., Prof.Dr.K.Rama M.D., Prof.Dr.Rajavelu Indira M.D., Prof.Dr.S.Pappathi M.D., Prof.Dr.Selvambigai M.D.,** for their valuable suggestions and constant encouragement throughout the study.

I express my heartfelt thanks to all my assistant professors for their help and suggestions during the study.

I also would like to thank the Institutional Ethics Committee for approving my study.

I would like to express my heartfelt thanks to my husband for his constant support, guidance and patience throughout the study. My parents and sister for their unconditional love and support.

I thank my friends, colleagues, senior & junior postgraduates, technicians and staff for their continuous support and helpful advice.

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CERTIFICATE OF APPROVAL

To
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Dear Dr.Sneha Suresh,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY ON EPIDERMAL GOWTH FACTOR RECEPTOR AND KI 67 EXPRESSION IN CORRELATION WITH GRADING OF GLIOMA " NO. 28102016.**

The following members of Ethics Committee were present in the meeting hold on **04.10.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.M.K.Muralidharan,MS.,M.Ch.,Dean, MMC,Ch-3	:Deputy Chairperson
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11.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

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This is to certify that this dissertation work titled **"A STUDY ON EPIDERMAL GROWTH FACTOR RECEPTOR AND KI 67 EXPRESSION IN CORRELATION WITH GRADING OF GLIOMA"** of the candidate **DR.SNEHA SURESH** with registration Number **201513007** for the award of **M.D PATHOLOGY** (Branch-III). I personally verified the urkund.com website for the purpose of Plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion and result shows **1 percentage** of plagiarism in the dissertation.

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ABBREVIATIONS

CNS	-	Central Nervous System
EGFR	-	Epidermal Growth Factor Receptor
LI	-	Labelling Index
WHO	-	World Health Organisation
NSC	-	Neuronal Stem Cells
SVZ	-	Sub ventricular zone
ICMR	-	Indian Council of Medical Research Programme
NCRP	-	National Cancer Registry
CBTRUS	-	The Central Brain Tumor Registry of the United States
IARC	-	International Agency for Research on Cancer
HL	-	Hodgkin Lymphoma
NHL	-	Non Hodgkin Lymphoma
PA	-	Pilocytic Astrocytoma
EPEN	-	Ependymoma
CCEP	-	Clear cell ependymoma
MPEP	-	Myxopapillary Ependymoma
DA	-	Diffuse Astrocytoma
PXA	-	Pleomorphic Astrocytoma
ODG	-	Oligodendroglioma

AA	-	Anaplastic Astrocytoma
GB	-	Glioblastoma
GS	-	Gliosarcoma
NF	-	Neurofibromatosis
TSC	-	Tuberous Sclerosis Complex
CT	-	Computed Tomography
MRI	-	Magnetic Resonance Imaging
PET	-	Positron Emission Tomography
SPECT	-	Single Photon Emission Computerised Tomography
IDH	-	Isocitrate Dehydrogenase
IHC	-	Immunohistochemistry
PCNA	-	Proliferating Cell Nuclear Antigen
ATRX	-	Alpha Thalassemia/Mental Retardation X linked
MIB-1	-	Molecular Immunology Borstel-1
NIG	-	Non Infiltrative Glioma

CONTENTS

Introduction

INTRODUCTION

Tumors of Central Nervous System pose a major diagnostic dilemma due to varied presentations and innumerable morphological entities. Among them, Gliomas form a major portion of all brain tumors. Glial cells form the support system of the Central Nervous System, and are composed of four main cell types: Astrocytes, Oligodendrocytes, Microglia and Ependymal cells. The neoplasms in the brain are hence named according to the cell of origin.

Gliomas are the most common brain tumor in adults, accounting for about 70% of primary neoplasms of the central nervous system (CNS). Glioblastoma, is the most common glioma, accounting for approximately 70% of astrocytomas and 15% of all intracranial neoplasms[1].

WHO grades Gliomas from Grade I to Grade IV, with Glioblastoma being graded as Grade IV. Along with the highest-grade Glioblastoma is also associated with a fatal outcome as Grade IV tumors are associated with a shorter survival period. Histopathologically, high grade gliomas are seen to frequently exhibit necrosis, vascular proliferation and increased mitotic activity[2].

The most frequent genetic alteration associated with Glioblastoma is amplification of the epidermal growth factor receptor (EGFR) gene, which results in over expression of EGFR, a transmembrane tyrosine kinase receptor. High protein levels of EGFR occur in about 90% of astrocytic tumors, suggesting that

alterations in transcription and translation of this gene may also participate in tumorigenesis[1,2].

Amplifications and rearrangements of EGFR are highly indicative of high-grade gliomas.

High grade gliomas are also associated with increased mitotic activity due to high cell turnover, which can be assessed by Ki 67 Labelling Index. Expression of EGFR and Ki-67 has been seen to significantly correlate with the histological grade of the glioma[2].

Few studies show that EGFR immunopositivity was significantly higher in grade III and IV gliomas compared with grades I and II. The mean Ki-67 labelling index (LI) was also seen to significantly increase in the higher glioma grades[2].

It has also been observed that dysregulation of EGFR enhances tumor growth, migration, angiogenesis, and metastatic spread. Additionally, EGFR overexpression is a poor prognostic factor and correlates with decreased overall survival in Glioblastoma patients[3].

In medicine, the study of EGFR expression has become important due to targeted therapy. Role of EGFR inhibitors aiming to promote apoptosis of cancer cells and possible adjuvant therapies is being extensively researched.

Considering the evidence from above stated studies, higher grade gliomas have poor prognosis and also show EGFR and Ki67 positivity. Their evaluation and grading have further implications in management of such cases. Hence to elaborate this, we aimed to study the correlation of EGFR and Ki 67 immunolabelling in conjunction with grading of glioma.

Aims and Objectives

AIMS & OBJECTIVES

- The aim of the study was to evaluate the expression of EGFR and Ki-67 immunolabelling in gliomas.

The objectives of the study were,

1. To correlate the expression of EGFR and Ki 67 with WHO grading of gliomas.
2. To study the age, sex, site and few histological parameters like necrosis and vascular proliferation in different types of gliomas
3. To evaluate EGFR expression in gliomas as a useful tool for diagnostic and therapeutic purposes.

Review of Literature

REVIEW OF LITERATURE

The Central Nervous System comprises of two principal types of cells, the neurons and the supporting cells or neuroglia. Glial cells are of two types Macroglia and Microglia.

Neuroglial cells are primarily of four types[1,2]-

1. Oligodendrocytes - Responsible for formation of myelin in the central nervous system

2. Astrocytes - These are highly branched cells that pack the interstices between the neurons, neuronal processes and oligodendrocytes. Their function is to provide mechanical support, help in exchange of metabolites between the neurons and the vascular system, take part in the formation of blood brain barrier, and also play an important role in repair of tissue following damage. They are so called because of their star shaped appearance, caused by multipolar branching cytoplasmic processes which have cytoplasmic intermediate filaments and Glial Fibrillary Acidic Protein.

3. Microglia - Represent the Monocyte-Macrophage system. Their function is to phagocytose neurons that undergo programmed cell death during development. These cells respond to injury by proliferation and by producing nodules of microglia.

4. Ependymal cells - Cuboidal cells that lines the ventricles and spinal canal.

Macroglia are composed of astrocytes, oligodendrocytes and ependymal cells, and are derived from the neuroectoderm, while Microglial cells are derived from the mesoderm[1,2].

CNS neoplasms were first discovered by a Russian Scientist, Gupta Longati in the year 1873. Primary CNS neoplasms account for 2% of all cancers, and 20% of these cancers occur in children under 15 years of age[3].

Neurogenesis in adults is thought to be responsible for the replacement of neurons and glia for the purposes of cellular replenishment, remodeling and response to injury [4].

There are two identified neurogenic niches in the adult mammalian brain: the sub ventricular zone (SVZ) and the sub granular zone[4,5].

The sub ventricular zone (SVZ) is an extensive germinal layer adjacent to the ependyma that concentrates stem cells on the walls of the lateral ventricles[6].

Gliomas are derived from neuronal progenitors or Neuronal Stem Cells (NSCs)[4]. Neural stem cells and their progeny have become candidates for the cell of origin of glioma since the discovery of neurogenesis in the adult brain. These cancer stem cells provide a reservoir of cells with self-renewal capabilities and maintain tumors by generating differentiated tumor cells. They are also regarded

to play an essential role in recurrence after chemo-radiotherapy. Therefore, the identification of cancer stem cells and understanding the mechanisms by which cancer stem cells act appear to be crucial for the development of treatments for these intractable tumors, as lately cancer stem cells have been shown to exhibit resistance to radiation and chemotherapeutic drugs[4,5,6]

EPIDEMIOLOGY OF BRAIN TUMORS

- As of 2015, worldwide, brain tumors are the most common cancer in age group 0-19 years and is also estimated to be the leading cause of cancer related deaths in children of the same age group. Meningiomas represent 36.4% of all primary brain tumors, making them the most common primary brain tumor[7-9].
- Gliomas, represent 27% of all brain tumors and 80% of all malignant tumors.
- Glioblastomas represent 15.1% of all primary brain tumors, and 55.1% of all gliomas[7-10].
- Astrocytomas, represent 75% of all gliomas.
- Oligodendrogliomas represent nearly 2% of all primary brain tumors[7-10].
- Medulloblastomas/embryonal/primitive tumors represent 1% of all primary brain tumors.
- Pituitary tumors represent 15.5% of all primary brain tumors[7-10].

Highlighting the Indian Scenario, in 2015 the incidence (newly diagnosed cases of cancer in a year) of Brain Tumors was about 2 patients per 1,00,000

population, while the mortality rate (deaths due to brain cancer) was less than 2 patients per 1,00,000 population. Glioblastoma (GBM) was the most common brain and Central Nervous System (CNS) malignancy, accounting for about 45.2% of malignant primary CNS tumors, 54% of all gliomas and 16% of all primary CNS tumors[10,11].

National Cancer Registry Programme (NCRP) of Indian Council of Medical Research (ICMR)[11], studied the trends in cancers of central nervous system in both the sexes, in five population based cancer registries (Mumbai, Chennai, Bangalore, Delhi and Bhopal) over a period of two decades from 1982-2003. Age adjusted incidences were used and it was derived separately for each of the five cancer registries. Separate data was arrived at for males and females. The starting period was taken as (1982-83) and the end of period (2002-03) in both the sexes for the registry[11].

According to the Chennai registry, CNS tumors ranked 9th in males and 8th in females. The average age adjusted incidence rates for CNS cancers, ranged in males from 2.53 to 4.14, while in females it ranged from 1.46 to 2.66[11].

Pediatric brain tumors vary considerably in their histological, topographical and gender distribution throughout childhood and adolescence, reflecting different dynamics of individual tumor entities [12,13,14].

Overall the prevalence for boys (60.9%) was higher and supra-tentorial locations predominated (53.3%); there was an even distribution of low-grade

WHO I/II (51.5%) and high-grade WHO III/IV (48.5%) tumors. Boys were more commonly affected in all age groups throughout childhood and adolescence. Infratentorial location was more common between the ages of 3 and 11 years (57. 5%).The main histological entities were pilocytic astrocytomas (23.5%), followed by medulloblastomas (16.3%), ependymomas (10.1%), anaplastic astrocytomas and glioblastomas (7.2% each), and craniopharyngiomas (5.6%); astrocytomas overall accounted for 47.3% of pediatric brain tumors[13,14].

In India, a study conducted by Satyanarayana et al revealed that the age adjusted incidence rates of pediatric CNS tumors in boys was 6.6-19.8 and in girls was 3.0-16.0[128].

In a study conducted by Arora et al, CNS tumors are the second most common childhood cancer (22-25%) and lymphomas are the third common (10%). In India lymphomas often exceed CNS tumors, particularly in males. Not only is the proportion of lymphomas higher in India, Hodgkin's Lymphoma (HL) exceeds Non-Hodgkin's lymphoma (NHL). This specifically seems to be a result of the high incidence of HL in male children in India in the larger urban areas of Bangalore, Chennai, Delhi, and Mumbai. The incidence rate of CNS tumors is 10-20 per million children, per year, which is half of that when compared to the statistics from all around the world[14].

In a study conducted by Jain et al, the most common primary pediatric brain tumors were astrocytic tumors (34.7%), followed by medulloblastoma and

supratentorial primitive neuro-ectodermal tumors (22.4%), craniopharyngiomas (10.2%) and ependymal tumors (9.8%)[15].

CBTRUS-The Central Brain Tumor Registry of the United States-analyzed data from 2009 to 2013, which consisted of new cases and incidence rates of malignant and benign primary tumors of CNS, pituitary and olfactory tumors of nasal cavity (lymphoma, leukemia)[16]. Data was obtained from Center for Disease Control and prevention & National Program of Cancer Registries. Incidence of all primary malignant CNS tumors is 22.36 per 1,00,000 of a total count of 3,68,000 incident tumors. Rate was found to be higher in females (24.46/1,00,000) than males (20.10/1,00,000). In the age group of 0-19 years according to CBTRUS the incidence was 5.67/1,00,000[16].

The 5-year survival rate according to the age group was as follows,

- 0-19 years-73.8%
- 20-44 years-61.5%
- 45-54 years-33.5%
- 55-64 years-18.55%
- 65-74 years-11.2%
- >75 years-6.3 %

EPIDEMIOLOGY OF GLIOMAS

- Diffuse astrocytomas- account for 10-15% of all brain tumors. According to a study conducted by Dasgupta et al, in India Astrocytomas represent the largest and most prevalent tumor of adult brain[17-20].
- Pilocytic Astrocytoma- Pilocytic astrocytoma is the most common primary brain tumor of childhood. They constitute 70-85% of all cerebellar astrocytomas, 5.2% of all primary central nervous system gliomas. Pilocytic astrocytoma constitutes approximately 19% of all central nervous system tumors among patients aged 0-14 years and approximately 12% among those aged 15-19 years[17-20].
- Oligodendroglioma- Oligodendroglioma is the least common of all glial tumors accounts for 2%–5% of primary brain tumors and 5%–18% of all glial neoplasms. Age adjusted incidence rates are 0.32/100000-person years. The peak age of incidence is 4^{th} to 5^{th} decade [21].
- Anaplastic Oligodendroglioma-it is quite rare accounting to <2% of glial tumors. It constitutes 1.2% of all primary brain tumors with an age adjusted annual incidence of 0.17/100000-person years [22].
- Ependymoma-In children aged 0-14 years, ependymomas accounted for 5.7% of all tumors diagnosed. Approximately 185 children are diagnosed with ependymoma per year. In children aged 15-19 years, ependymoma accounted for 4% of all tumors diagnosed. For tumors involving the spinal cord, spinal meninges and cauda equina, ependymomas accounted for

20.5% of all tumors diagnosed. In adults 20+ years, ependymomas accounted for 1.9% of all tumors diagnosed [23].

- Anaplastic Ependymoma-the incidence was equal in males and females
- For those with ependymoma, the overall 5-year relative survival rate is 83.4%. 5-year relative survival rates are highest for those aged 20-44 years (91%), and decrease with increasing age at diagnosis with a 5-year relative survival rate of 57.8% for those aged 75+ years. For children aged 0-19, the 5-year relative survival rate is 75.2% [23].
- Subependymomas-10% of ependymomas, most often present as incidental autopsy findings in the brains of the elderly [22,23].
- Myxopapillary ependymomas-arise most frequently in adults; approximately 20% occur in children, in whom there is a 2:1 male-to-female tendency for dissemination through Cerebrospinal Fluid (CSF) pathways. Anaplastic ependymomas are far more common in the pediatric age group, frequently arising in a supratentorial location [22,23].
- Sub ependymoma- represents 8% of all ependymal tumors[23].
- Pleomorphic Xantho-astrocytoma- is an uncommon astrocytic neoplasm which constitutes <1% of gliomas. So far only 200 cases have been reported in literature. This tumor occurs more commonly in young adults, between second to third decade [24,25].
- Glioblastoma (GB) accounts for 15-17% of all primary brain tumors. GB accounts for about 50-65% of all astrocytic tumors. GB has an age adjusted incidence of 2-3/100,000-person years. For adults with more aggressive

glioblastoma, treated with concurrent temozolamide and radiation therapy, median survival is about 14.6 months and two-year survival is 30%. However, a 2009 study reported that almost 10% of patients with glioblastoma may live five years or longer. Supra-tentorial and cerebellar tumors are more amenable to surgical treatment and thus carry better prognosis than tumors in the brainstem or diencephalon [26,27,28].

- Gliosarcoma- It is the neoplasm with mixed glial and sarcomatous elements. It has an increased rate of metastases and has not been found to carry the hallmark of EGFR overexpression generally found in glioblastomas [29,30].

SYMPTOMS OF CNS TUMORS

The location of CNS tumors will determine the symptoms of patients. The usual presentations are

1. Epilepsy (focal or generalized)
2. Focal neurologic deficits
3. Symptoms and signs of raised intracranial include-headache (particularly postural and nocturnal or early morning), vomiting (particularly children), clouding of consciousness and coma, papilledema [31,32,33].

Neoplasms and cysts that often present with hydrocephalus include:

1. Any posterior fossa neoplasm (particularly medulloblastoma and ependymoma).

2. Pineal gland neoplasms.
3. Sub ependymal giant cell astrocytoma.
4. hypothalamic pilocytic astrocytoma.
5. central neurocytoma.
6. chordoid glioma of the third ventricle.
7. colloid cyst of the third ventricle.

Lesions such as oligodendroglioma, anaplastic oligodendroglioma and gliosarcoma have a predilection for the frontal lobe, hence present with behavioral changes [31,32,33].

Neoplasms that present with raised intracranial pressure are-

1. Ependymoma.
2. Anaplastic ependymoma.
3. Oligodendroglioma.
4. Glioblastoma [31,32,33.]

RISK FACTORSAND ETIOLOGICAL FACTORS-

1. AGE & SEX-

   Studies show that the incidence of glioma increase exponentially after the 4^{th} decade. Gliomas are more common in males than females in the ratio of 60:40 [34].

2. RADIATION-

Ionizing radiation in the form of x-rays and gamma rays is the only exposure which the International Agency for Research on Cancer (IARC) classifies as a cause of brain and CNS tumors [34,35]. Evidence on the effects of ionizing radiation came initially from studies of atomic bomb survivors, ionizing radiation overall generally appears to be more strongly associated with meningioma risk than with glioma risk. The risk of meningioma is increased by 64-100% with each Gray (Gy) of ionizing radiation exposure received. Age at exposure, sex, and time since exposure does not appear to modify the effect of radiation on meningioma risk. The risk of glioma appears to be increased by 8%-56% per Gy. Younger age at exposure confers a stronger effect on glioma risk[35].

Brain tumor risk is almost tripled in people who received 1-2 CT scans (total average X-ray dose around 60 milli-gray (mGy)) during childhood or adolescence, large cohort studies have shown [36,37].

Radiotherapy for a primary brain tumor (compared with no radiotherapy) was associated with around 55% higher risk of secondary brain tumor, in a study of US patients treated between 1973 and 2002 [38].

People who received radiotherapy for cancer during childhood have a 14-fold higher risk of developing glioma later in life, compared with those who did not receive radiotherapy for their childhood cancer [39,40].

The risk of second primary brain tumor increased linearly with increasing radiotherapy dose, with this effect much stronger for meningioma than glioma[39,40].

NON IONIZNG RADIATION

Interphone International Study in 13 countries in 2765 gliomas and 2409 meningiomas showed no association between mobile phone usage and brain tumors [41,42]. IARC classifies mobile phone use as a possible cause of glioma and acoustic neuroma, based on limited evidence [41,42,43]. Most studies have reported no significant association between mobile phone use and brain tumor risk at the population or individual level [44,45].

GENETIC SYNDROMES AND FAMILY HISTORY-

- Neurofibromatosis (NF) are a group of genetic conditions. The risk of brain and CNS tumors is at least 23-43 times higher in NF1 patients than in the general population [46,47]. Relative risk of brain tumors in NF1 patients increases with patient age [48]. Most NF1-associated brain tumors are gliomas [48,49]. Both children and adults with NF1 have an increased risk of astrocytoma, with the tumor grade often higher in adults than children [49,50]. 5-25% of children with NF1 develop optic pathway gliomas [50].
- Tuberous Sclerosis Complex (TSC) is associated with benign CNS tumors. The prevalence of TSC is estimated at around 1 in 25,000 [51,52]. Up to 20% of TSC patients develop sub ependymal giant-cell astrocytomas[53].

- Li-Fraumeni syndrome is a very rare genetic condition associated with increased risk of early-onset brain tumors and other cancer types. Astrocytoma's, glioblastomas, medulloblastomas, and choroid plexus carcinomas are the most common brain tumors seen in this population [54-57].
- Turcot's syndrome is associated with both brain and bowel tumors. Turcot's syndrome type 1 is associated with early-onset gliomas [58,59,60].

Having a family history of CNS tumors is associated with increased risk of developing brain tumors, those with any first-degree relative diagnosed with glioma have a 77% increased risk of developing, and a 2.2- to 2.6-fold risk increase if the affected relative is a sibling [61-64].

HORMONAL FACTORS

A meta-analysis showed that the Glioma risk is 32% lower in postmenopausal hormone replacement therapy (HRT) [65].

OCCUPATIONAL EXPOSURE -

Early studies focused on nitroso compounds and polycyclic aromatic hydrocarbons because of their abilities to induce brain tumors in animal models, but studies have yet to conclusively link brain tumors to exposures to these or other neuro-carcinogenic compounds in humans [66-69].

Cadmium is a type I carcinogen associated with human lung, renal, bladder, breast, liver, and gastric cancers and ranks first among suspect metals for brain tumors. The major sources of personal exposure are occupation, smoking, and diet. Studies support the carcinogenic effects of cadmium and show its effects on increasing the permeability of the Blood Brain Barrier [70,71,72].

NEUROIMAGING IN GLIOMAS-

MRI, CT Scan are the most important investigations in work up of gliomas. MRI helps in adjudging the extent of soft tissue involvement.

Neuroimaging gives valuable information regarding the exact location, cerebral edema, fluid collection, extent of involvement, vascularity.

Other modalities of imaging are PET CT, Magnetic Resonance spectrophotometry, Single Photon Emission Computerized Tomography (SPECT) 70-72

WHO CLASSIFICATION OF CNS TUMORS-

For the past century, the classification of brain tumors has been primarily dependent on light microscopic features in hematoxylin and eosin-stained sections, immune-histochemical expression of lineage associated proteins and ultrastructural characterization. The current update (2016 CNS WHO) incorporates molecular parameters into the classification of CNS tumor entities [73].

The current WHO Classification-2016 has the following advantages:

- Integrated phenotypic and genotypic parameters for CNS tumor classification brings in a level of objectivity.
- Greater diagnostic accuracy.
- Improved patient management.
- Accurate determinations of prognosis and treatment response.

Histological grading remains the single most important factor in the grading of gliomas followed by genotyping of the tumor.

Whilst reporting of CNS tumors, diagnoses should consist of a histopathological name followed by the genetic features - for example, Oligodendroglioma, IDH-mutant and 1p/19q-codeleted.

For a tumor lacking a genetic mutation, the term wildtype can be used if an official “wildtype” entity exists: Glioblastoma, IDH-wildtype [73].

The revised Grading of Gliomas are-

GRADE I-Pilocytic Astrocytoma.

- Sub ependymal Giant Cell Astrocytoma.
- Myxopapillary Ependymoma.
- Sub ependymoma.
- Desmoplastic Infantile Astrocytoma and Ganglioglioma.
- Angiocentric Glioma.

GRADE II-Diffuse Astrocytoma-IDH mutant.

- Oligodendroglioma-1p/19q co deletion.
- Pleomorphic Xanthoastrocytoma.
- Ependymoma.

GRADE III-Anaplastic Astrocytoma-IDH mutant.

- Anaplastic Oligodendroglioma-IDH mutant and 1p/19q co deletion.
- Anaplastic Ependymoma.
- Anaplastic Pleomorphic Xantho-astrocytoma.
- Anaplastic Oligodendroglioma.

GRADE IV-

- Glioblastoma
- Gliosarcoma

PILOCYTIC ASTROCYTOMA

Pilocytic astrocytoma is classified separately from diffuse astrocytic neoplasms because -

- It does not share their genetic abnormalities
- Does not infiltrate surrounding tissue in the same manner
- Very rarely progresses to an anaplastic form
- Has a predilection for sites not commonly involved by other astrocytic neoplasms
- Has a significantly better prognosis.

It is seen to involve the cerebellum, hypothalamus, optic pathway and third ventricle. Children and young adults are commonly affected. The MAPK/ERK pathway aberrations, most commonly due to generation of a KIAA1549 - BRAF fusion protein with tandem duplication of the BRAF gene. In contrast to diffuse astrocytoma's, PAs lack either IDH1 / 2 or TP53 mutations. Of patients with neurofibromatosis type 1, approximately 15% develop a PA. Grossly, it is a differential for cyst with a mural nodule. Microscopically, it has a biphasic pattern, with microcystic and fascicular pattern. Fascicular pattern is visualized as bipolar cytoplasmic processes, these cells have a spindle to oval nucleus with fibrillary cytoplasm. Rosenthal fibers and eosinophilic granular bodies are seen associated with PA [74-76].

SUBEPENDYMAL GIANT CELL ASTROCYTOMA

The most common CNS neoplasm in TSC patients, develops in about 5% of TSC patients. Usually occurs in adolescence. Typically situated in the wall of the lateral ventricle. It shows autosomal dominant inheritance with high penetrance gene on chromosome 9q34 in about half of affected families. A well-defined soft mass of gray tissue protrudes into the ventricle. Grossly, it is predominantly exophytic, and does not invade surrounding parenchyma. Calcification may be present. At the microscopic level, many large oval cells have abundant glassy eosinophilic cytoplasm and a large eccentric nucleus, but elongated cells with smaller nuclei and fibrillary processes may surround groups

of the plump cells, forming a nodular pattern. Mitoses are unusual, but their presence does not signify aggressive behavior [74,75,76].

OLIGODENDROGLIOMA

Has a predilection for younger age group. The following mutations are responsible,

- Co deletion of Chromosomes 1p/19q, the presence of which is favorable,
- MGMT methylation, presence of which is favorable,
- IDH1 mutation, presence of which is favorable in anaplastic oligodendroglioma,
- CDKN2A deletion, absence of which is favorable,
- EGFR over expression in anaplastic oligodendroglioma.

Oligodendrogliomas are composed of uniform cells, these infiltrate gray matter. A delicate vasculature consisting of branching capillaries runs through the neoplasm. Cytoplasm is sparse. Artefactual clearing of the cytoplasm is common in paraffin-embedded material, particularly when fixation is delayed, and gives a 'fried-egg'appearance to the cells. Nuclei are round and usually contain faintly speckled chromatin. A single small nucleolus may be present [77,78].

ANAPLASTIC OLIGODENDROGLIOMA -

It is classified under WHO Grade III. These neoplasms are associated with necrosis, increased mitosis, endothelial proliferation and nuclear atypia. There is also overexpression of EGFR and increased Ki 67 expression [79,80].

EPENDYMOMA -

Classic ependymoma presents as an intracranial neoplasm in childhood. The myxopapillary ependymoma, which has a more indolent behavior than the classic ependymoma, nearly always presents as a spinal neoplasm in adulthood. Ependymomas are generally composed of sheets of uniform cells with indistinct cytoplasmic borders and round or oval nuclei. Mitoses should be infrequent. The pseudo rosette is a perivascular anuclear zone of fibrillary processes that taper towards the vessel. Pseudo rosettes are a frequent, not specific, finding in ependymomas. Ependymal (true)rosettes with a central lumen and a halo of neoplastic cells are more specific, but found less consistently [81,82].

Cellular ependymomas contain cells with a high nuclear: cytoplasmic ratio. Differentiating this variant from an anaplastic ependymoma is difficult, and depends on the identification of an increased mitotic count and cytologic pleomorphism in the latter. Microvascular proliferation and abundant necrosis are also features of most anaplastic ependymomas [82,83,84].

Clear cell ependymoma-may be confused with an oligodendroglioma, but the presence of pseudo rosettes helps in differentiating.

Criteria for the diagnosis of Anaplastic ependymoma-WHO GRADE III are Necrosis and capillary endothelial proliferation. The WHO classification emphasizes atypical cytologic features (i.e. nuclear pleomorphism), a high cell density, and plentiful mitoses in its diagnosis.

It is important to note that no histologic feature of ependymomas correlates reliably with prognosis [82-84].

MYXOPAPILLARY EPENDYMOMA -

Myxopapillary ependymoma is a slow-growing tumor, arising predominantly in the region of the filum terminale It has also been described in extra-spinal locations including extradural/subcutaneous tissue, cervical thoracic spine, lateral ventricle, and the brain. There is a slight male predominance. Grossly, they are well defined, sausage shaped tumors with a smooth surface. Histologic sections reveal numerous small papillary structures surrounded by single layer of cuboidal or columnar cells. The cells have round nuclei and delicate chromatin and lack obvious cytoplasmic processes. The cores of the papillae are either completely filled up with the mucinous/myxoid matrix or have a central blood vessel surrounded by the matrix. Other areas can show a loose structure with microcystic spaces formation or more compact gliofibrillar areas resembling typical ependymomas with perivascular pseudo rosettes or even true ependymal rosettes. Mitotic figures are not usually seen. Clusters of bland nuclei embedded in a dense, fine, glial fibrillary background. Mild nuclear pleomorphism, microcystic formations (only in lateral ventricular tumors. occasional ependymal pseudo rosettes, hemorrhage, calcification may be seen. Mitoses is very rare [85,86].

Sub ependymomas- are composed of cells with uniform, round or oval nuclei and generally scanty perinuclear cytoplasm. The cells are clustered in small, scattered groups and separated by broad bands of closely packed fibrillary processes. Occasional cells have more abundant, homogeneous, perinuclear cytoplasm. Mitoses are absent [86].

DIFFUSE ASTROCYTOMA -

Astrocytoma, anaplastic astrocytoma, and glioblastoma constitute a range of diffusely infiltrating astrocytic neoplasms, which occur throughout the CNS. They are referred to as 'diffuse astrocytic tumors' to distinguish them from the pilocytic astrocytoma and other rarer forms of localized or circumscribed astrocytic neoplasms (e.g. pleomorphic xantho-astrocytoma and sub ependymal giant cell astrocytoma). The diffuse astrocytic neoplasms are commonest in the cerebrum in adults and brain stem in children [87-89].

GENETICS OF DIFFUSE ASTROCYTOMA

The origin of astrocytic neoplasms may include neural stem cells, progenitor cells, or differentiated glial cells. Isocitrate dehydrogenase (IDH1, IDH2) and TP53 gene mutations are considered to be early events in neoplastic progression. PDGFRA overexpression is seen in about 60% of cases. Promoter hypermethylation of p14ARF gene occurs in 30% of cases. There is loss of chromosome 19q accounts to progression to anaplastic astrocytoma. IDH1 mutation is associated with better prognosis. IDH1/2 mutation is common in

astrocytoma, oligodendroglioma, oligoastrocytoma, and their anaplastic counterparts [87-89,90,91].

Macroscopically, Cerebral astrocytoma's diffusely expand the white matter, sometimes distorting the overlying gray matter. The neoplastic process is poorly demarcated. Macroscopic cyst formation can occur. Cysts in astrocytoma's contain clear yellow fluid, in contrast to the slightly turbid fluid in glioblastoma cysts. Diffuse astrocytoma's insidiously invade brain tissue, but necrosis and microvascular proliferation are not features of the diffuse astrocytoma.

Cytologically, neoplastic cells in diffuse astrocytoma's show mild atypia, particularly nuclear pleomorphism and hyperchromasia. The cells of previously called fibrillary astrocytoma's may appear as bare nuclei, their tenuous fibrillary processes blending with the brain's parenchyma. Mitotic activity is not found in diffuse astrocytoma's; it denotes anaplastic progression [90,91].

ANAPLASTIC ASTROCYTOMA

Anaplastic transformation of an astrocytoma is often preceded by the development of rapidly worsening neurologic symptoms and signs. Focal increase in cell proliferation, nuclear pleomorphism is present and the nuclear: cytoplasmic ratio increased. Mitotic activity distinguishes the anaplastic astrocytoma. Necrosis is not found [92-94].

GLIOBLASTOMA-

Glioblastomas arise de novo or ultimately be the endpoint of neoplastic progression from an astrocytoma. Genetics of GB has been studied extensively, and based on the gene expression it has been classified into four types:

- Classical,
- Pro-neural,
- Mesenchymal,
- Neural.

Classical type is associated with chromosome 7 and 10 amplification and it occurs in younger individuals and is commonly associated with secondary GBM. The Proneural Type is associated with EGFR overexpression and alterations of PDGFRA it is responsive to therapy and is associated with primary GB. The Mesenchymal type is associated with NF mutation and deletions and offers a survival advantage and is associated with primary GB. About 90% of glioblastomas are primary or de novo. Grossly, GB is an infiltrating neoplasm. Glioblastomas appear as a spherical mass with a necrotic center, which is seen on MRI imaging as a ring of contrast-enhancing tissue around a region of low attenuation. The differential diagnosis will be an abscess, but the center of the glioblastoma is usually filled with straw-colored fluid and scanty necrotic debris. Sometimes, the tumor crosses the corpus callosum, sometimes forming a bi-hemispheric mass called a ‘butterfly glioma’. Foci of cyst formation, necrosis, and hemorrhage. Microscopically, it resembles anaplastic astrocytoma, necrosis and a

florid microvascular proliferation are the key features distinguishing glioblastomas [95-108].

GLIOMATOSIS CEREBRI-

Gliomatosis cerebri is characterized by disseminated infiltration of the CNS by small neoplastic glial cells. Typically, the cerebrum is involved, and this is sometimes accompanied by spread of neoplastic cells into the brain stem and even the spinal cord.

The molecular genetics of this entity are poorly characterized. Gliomatosis cerebri can present like other neuroepithelial neoplasms, with headaches, focal neurological deficits, or epilepsy. Neuroimaging studies help us reach the diagnosis [109,110].

MACROSCOPIC AND MICROSCOPIC APPEARANCES

Classic gliomatosis cerebri produces diffuse expansion of at least two cerebral lobes without an obvious tumor mass (type 1 gliomatosis cerebri). However, some produce the characteristic diffuse expansion of CNS tissue plus a mass of neoplastic cells (type 2 gliomatosis cerebri), which most often has the features of a glioblastoma. Generally, a moderate increase in cell density is seen in regions infiltrated by neoplastic cells. These cells have mildly pleomorphic nuclei and indistinct cytoplasm. Neoplastic cells tend to cluster around neurons and in subpial and perivascular spaces [109,110].

IMMUNOHISTOCHEMISTRY IN GLIOMA-

Immunohistochemistry(IHC) is of immense value in CNS neoplasms. IHC is a combination of immunology and histology and is based on the principle of localizing specific antigens in tissues or cells based on antigen-antibody recognition. It works on the principle of binding of an antibody with the corresponding antigen and its recognition therefore with the help of a chromogen. The different methods are Direct, Indirect and Polymer based methods. Out of these, Polymer based methods, by utilizing Horse Radish Peroxidase is commonly utilized as it has an increased sensitivity with minimal background staining and it also cuts short many of the steps involved with direct and indirect methods [111,112].

In CNS neoplasms, IHC plays a significant role in diagnosis and prognostication. Of great importance is the markers used for prognostication, which are –

1. Cell cycle/proliferation markers-like KI 67, Proliferating Cell Nuclear Antigen(PCNA), Bromodeoxyuridine (BrDU)
2. Tumor suppressor genes/oncogene protiens-p53 tumor suppressor gene, Retinoblastoma tumor suppressor gene, C-myc oncogene.
3. Growth factors - Epidermal Growth Factor receptor
4. Others - Isocitrate Dehydrogenase (IDH 1 and 2), ATRX (Alpha Thalassemia/mental retardation X linked) [111,112].

Ki 67 and MIB-1

Molecular immunology borstel-1 (MIB-1) antibody is an improvised version of Ki-67, which correlates with mitotic activity of the tumor. It recognizes an antigen expressed in all the active phases of cell cycle- G1, S, G2, and M in paraffin-embedded tissue. It helps in indicating the aggressiveness of the tumor. It is used as a prognostic marker, and correlates with the prognosis, including time to recurrence and survival and also correlates well with the histological grade of the tumor [113].

P53 **-**

p53 is a tumor suppresser gene. It is coded by a tumor suppressor gene (p53 or TP53 gene) on chromosome 17p13.1. It is mainly responsible for the genomic stability of the cell.

It is considered as a marker of astrocytic tumor with a frequency that is in the range of 58–83%. Low-grade gliomas carrying this positivity are associated with a shorter survival and a shorter time interval to progress to high-grade gliomas. Secondary GB commonly show p53 mutations while primary GB. s rarely shows p53 mutations[114,115].

Iso-citrate dehydrogenase-1 and -2

Iso-citrate dehydrogenases (IDHs) are the enzymes that are involved in tricarboxylic acid cycle. They de-carboxylate isocitrate to α-ketoglutarate with the production of NADH and/or NADPH. In most of the IDH mutated genes, only

one copy of IDH gene is mutated. When IDH gene is mutated, there is a reduction of α-ketoglutarate to 2-hydroxyglutarate, which is increased in mutant gliomas and this has been implicated for the malignant progression of the tumor. IDH-1 mutation is present in 70-80% of WHO Grades II and III diffuse gliomas, ODGs (80%), anaplastic ODGs (85%), and mixed oligoastrocytomas (71%), as well secondary GBMs (82%). IDH-1 mutation is rare in primary GBMs (5%), pilocytic astrocytoma's (10%), and is absent in ependymomas. IDH-2 mutation is seen in a smaller proportion of gliomas, and that too mainly in oligodendroglial tumors. IDH mutations have now been incorporated into the recent WHO classification. Several studies have shown that IDH-1 mutation is associated with a longer survival [113-116].

ATRX

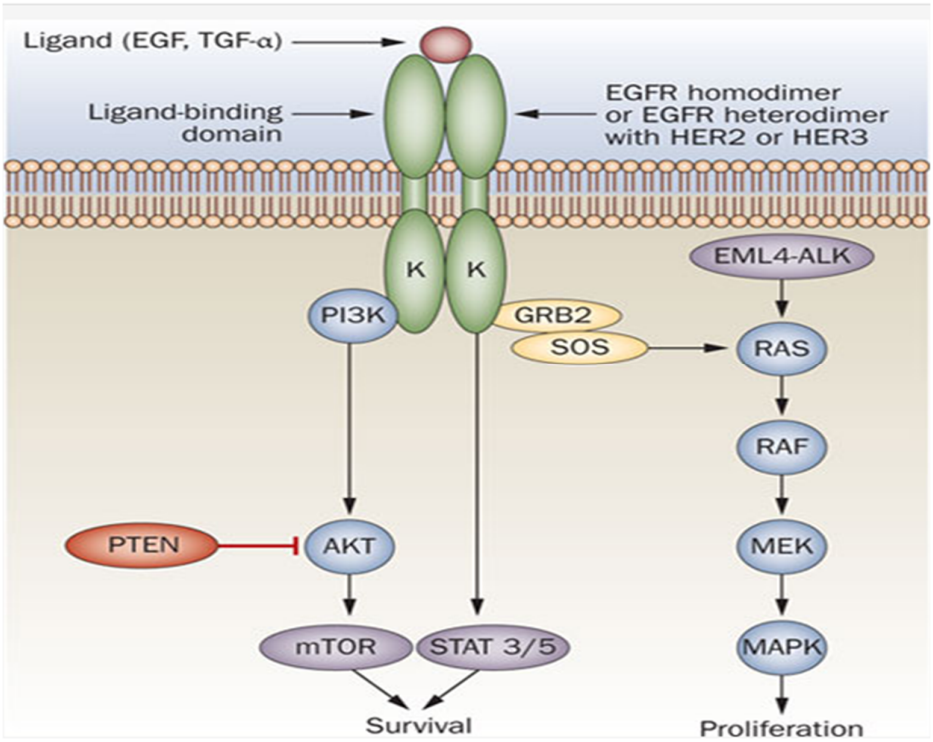
α-thalassemia/mental retardation syndrome X-linked (ATRX) protein is also seen in the nuclei of all normal cells. Mutation of ATRX gene leads to loss of its expression in tumor cells. ATRX mutation is considered as a specific marker for astrocytic lineage [117].

EGFR AND MALIGNANCIES –

EGFR is a member of the ErbB family of tyrosine kinase receptors and they transmit a growth-inducing signal to cells that have been stimulated by an EGFR ligand (e.g., TGFα and EGF). In normal tissues, the EGFR ligands are tightly orchestrated to keep in check the kinetics of cell proliferation which maintains homeostasis.

In malignancies, EGFR is often constitutively activated because of the sustained production of EGFR ligands in the tumor microenvironment Aberrant expression of EGFR by tumors typically confers a more aggressive phenotype and is thus predictive of poor prognosis. EGFR is a principal in targeted therapeutic intervention [118,119].

Figure 1. EGFR protien and gene transcription.[127]



Filip Janku, David J. Stewart & Razelle Kurzrock *Nature Reviews Clinical Oncology* 7, 401-414 (July 2010)-127

EGFR AND GLIOMA -

The most frequent genetic alteration associated with Glioblastoma is amplification of the (EGFR) gene, which results in over expression of the EGFR. High protein levels of EGFR occur in about 90% of astrocytic tumors, suggesting

that alterations in transcription and translation of this gene may also participate in tumorigenesis [120-127].

Amplifications and rearrangements of EGFR are highly indicative of high-grade gliomas. Expression of EGFR has been seen to significantly correlate with the histological grade of the glioma. Few studies show that EGFR immune-positivity was significantly higher in grade III and IV gliomas compared with grades I and II [120-127].

EGFR as a marker serves for prognostication, Dysregulation of EGFR has also been shown to enhance tumor growth, migration, angiogenesis, and metastatic spread. Additionally, EGFR overexpression is a poor prognostic factor and correlates with decreased overall survival in GB patients [120-127].

Role of EGFR inhibitors aiming to promote apoptosis of cancer cells and increasing tumor sensitivity to possible adjuvant therapies is being extensively researched.

Recently, a study conducted by Fanny Burel et al, showed that EGFR expression in cell with high Nuclear Cytoplasmic ratio can enable differentiation between infiltrative gliomas and other non-infiltrative glial lesions (NIG), including gliosis, benign tumors, and demyelinating disease. This helps in distinguishing even low grade gliomas from other benign lesions, thereby

showing that EGFR is expressed in tumor cells of low grade glioma also, rather than only high grade gliomas of Grade 3 and 4 [119-127].

KI 67 AND MALIGNANCIES-

Ki-67 is a nuclear non-histone protein that is present at low levels in quiescent cells but is increased in proliferating cells, the expression of the Ki-67 protein is strongly associated with cell proliferation. The fact that the Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2), but is absent from resting cells (G0) makes it an ideal marker for determining the fraction of cells undergoing active cell proliferation [123-125].

KI 67 AND GLIOMA-

Various studies have shown that tumour grade in CNS neoplasms is the single most important factor in determining prognosis, it positively correlates with patient survival. Ki 67 is one of the most important prognostic markers that would help in classifying glioma.

Ki 67 expression is seen to increase positively with increasing grade of glioma. Higher the Ki 67 labelling index, higher is the grade which in turn related to bad prognosis and reduced survival of patients [123-125].

Materials and Methods

MATERIALS AND METHODS

This study is both a retrospective and prospective descriptive clinicopathological analysis of gliomas in Department of Neuropathology, Rajiv Gandhi Government General Hospital, Chennai for a period of October 2015 to June 2017.

SOURCES OF DATA:

Cases of glioma that were reported in the Department of Neuropathology, Rajiv Gandhi Government General Hospital and Madras Medical College, Chennai during a period of October 2015 to June 2017.

INCLUSION CRITERIA-

1. Patients diagnosed with gliomas-Ependymoma, Oligodendroglioma, Diffuse Astrocytoma, Pilocytic Astrocytoma, Anaplastic Astrocytoma, Glioblastoma.
2. According to WHO grading, tumors are either grouped as Grade I and II- which are considered as low grade and Grade III and IV-considered as high grade

EXCLUSION CRITERIA-

Non- neoplastic lesions of CNS, benign lesions of the CNS, Non- Glial tumors such as meningiomas, schwannomas etc.

METHOD OF DATA COLLECTION-

This is both a retrospective and prospective study where cases were between 2015 to 2017 were included. The relevant clinical details such as age, gender, symptoms, radiological findings, per operative squash findings, macroscopic and microscopic findings were retrieved from the Neuropathology registers between October 2015 to June 2017 and investigations were collected from medical records of Institute of Pathology, Madras Medical College, Chennai. Out of these, a total of 50 cases of Gliomas were randomly selected,25 cases of Low Grade Glioma and 25 cases of High Grade Glioma. There were Grade I (9 cases), Grade II (16 cases), Grade III (4 cases), Grade IV (21 cases).

Corresponding paraffin blocks were collected, histopathological sections were prepared on a glass slide from formalin fixed paraffin embedded tissue of resected specimens. They were subjected to H and E staining and Immunohistochemical analysis by two markers EGFR and Ki 67. This was done for a total of 50 cases. The results were tabulated in a master chart and recorded with photographs. As much as possible, follow up data regarding adjuvant therapy, recurrence, disease free survival was obtained from Medical Records Section of Neurosurgery and oncology.

IMMUNOHISTOCHEMICAL EVALUATION-

IHC analysis was done using EGFR and Ki 67 in paraffin embedded tissue samples using poly excel detection system protocol.IHC was done for 50 cases.4-micron thickness of paraffin embedded sections were taken and transferred to positively charged slides. Heat induced antigen retrieval was done using Tris EDTA buffer. The antigen is bound with rabbit monoclonal antibody (Pathinsitu) EGFR and Ki 67 followed by the addition of secondary antibody conjugated with horse radish peroxidase, this step is followed by addition of a chromogen Diaminobenzidine which imparts color. Hematoxylin is used as at the end as a counter stain.

INTERPRETATION AND SCORING SYSTEM-

The immunohistochemically stained slides were analysed under the microscope for the presence or absence of reactivity as detected by the brown colour imparted by the brown product of Diaminobenzidine and various parameters were analysed namely-

1. Cellular localization-for EGFR-membrane positivity and nuclear positivity for Ki 67
2. Scoring for EGFR-For the marker, percentage of immunopositive cells was estimated by counting the number of positive cells in ten high power fields(40x), which were randomized throughout the section. For each field the ratio of positive cells/total number of cells was calculated in

percentage, then the mean value of ten fields was taken as estimated percentage of immunopositivity for that case and was allocated a value according to the following,

a. 0-25%-Score 1
b. 26%-50%-Score 2
c. 51%-75%-Score 3
d. 76%-100%-Score 4

3. Ki 67-Nuclear positivity is looked for this marker and is scored as percentage of immunopositive cells per thousand cells

Observation and Results

OBSERVATION AND RESULTS

We have received a total of 181 cases of various types of glioma in our institute of neuropathology from a period of October 2015 to June 2017.

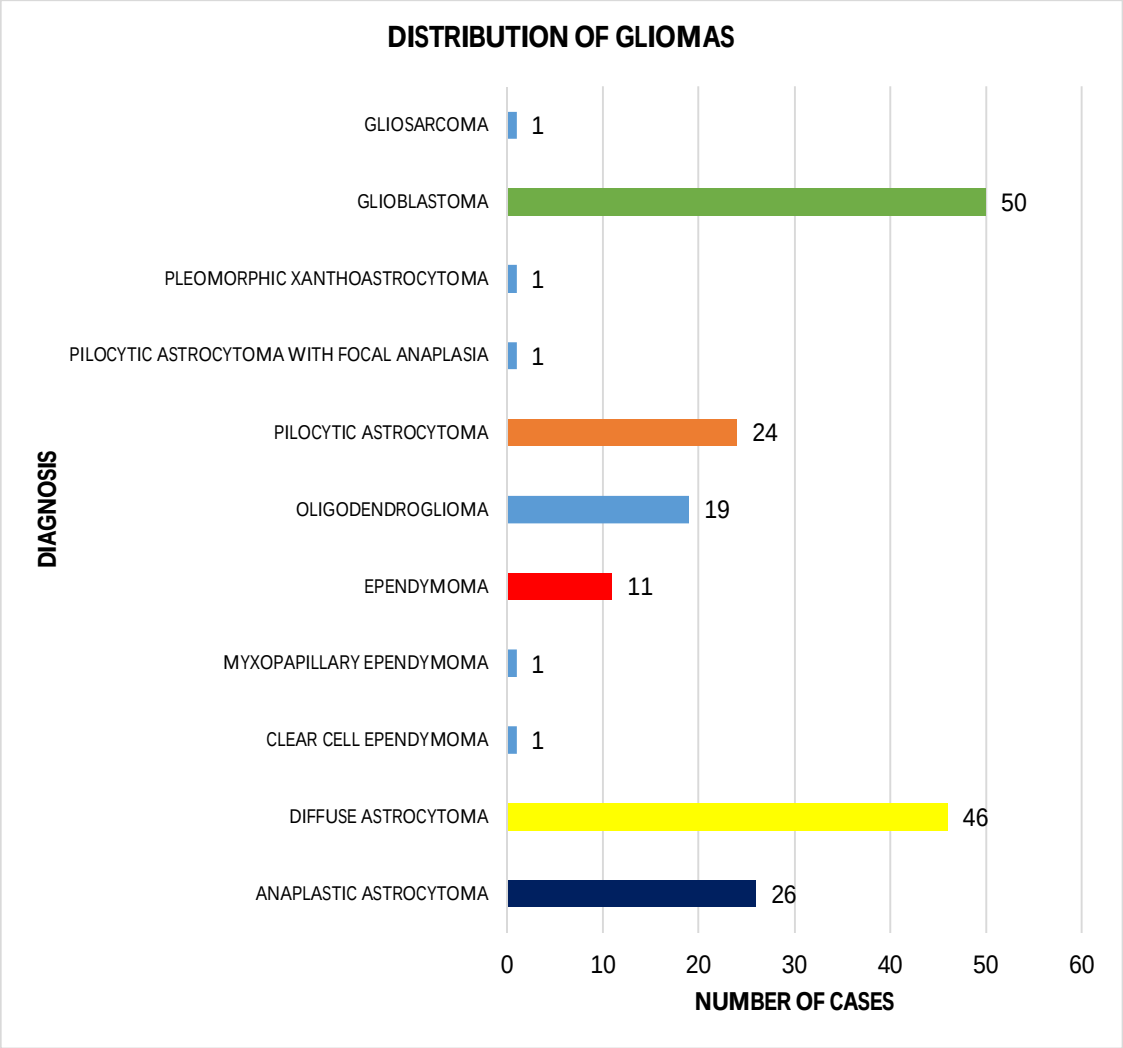
Table.1: Distribution of cases in the institute of neuropathology

Diagnosis	Number of cases	Percentage
Anaplastic astrocytoma	26	14.4
Diffuse astrocytoma	46	25.5
Clear cell ependymoma	1	0.5
Myxopapillary ependymoma	1	0.5
Ependymoma	11	6.1
Oligodendroglioma	19	10.5
Pilocytic astrocytoma	24	13.3
Pilocytic astrocytoma with focal anaplasia	1	0.5
Pleomorphic xanthoastrocytoma	1	0.5
Glioblastoma	50	27.7
Gliosarcoma	1	0.5
Total	181	100

Among the 181 cases of glioma, the most commonly observed diagnosis was Glioblastoma, with a prevalence of 27.7%, followed by Diffuse Astrocytoma with a prevalence of 25.5%, Anaplastic Astrocytoma with a prevalence of 14.4%, Pilocytic Astrocytoma with 13.3% and Oligodendroglioma with 10.5%. Entities like Gliosarcoma, Myxopapillary Ependymoma, Clear cell ependymoma,

Pleomorphic Xanthoastrocytoma are rare, and were associated with lower prevalence rates.

Figure 2. Distribution of Gliomas.



AGE DISTRIBUTION-

Age distribution was tabulated individually for each glioma to highlight the correlation of each glioma pertaining to age of the patient.

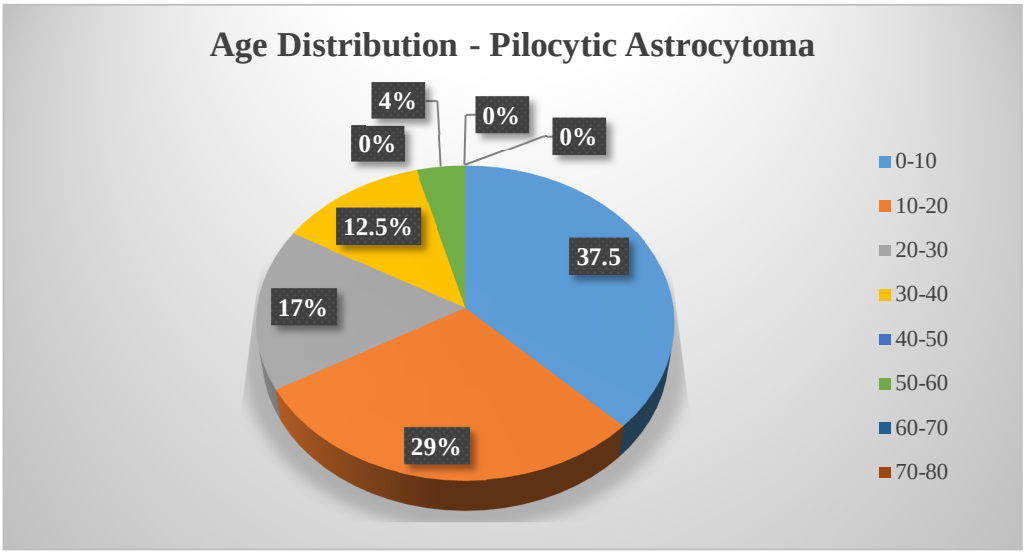
PILOCYTIC ASTROCYTOMA:

These group of gliomas showed the maximum prevalence in the age group of 0-20 years amounting up to 66% of the cases. This age trend was found to be significant with a p value of 0.0002.

Table 2. Distribution of Pilocytic Astrocytoma across various age groups.

Age Group	Number of cases	Percentage
0-10	9	37.5
10-20	7	29
20-30	4	17
30-40	3	12.5
40-50	0	0
50-60	1	4
60-70	0	0
70-80	0	0
Total	24	100

Figure 3. Distribution of Pilocytic Astrocytoma across age groups.



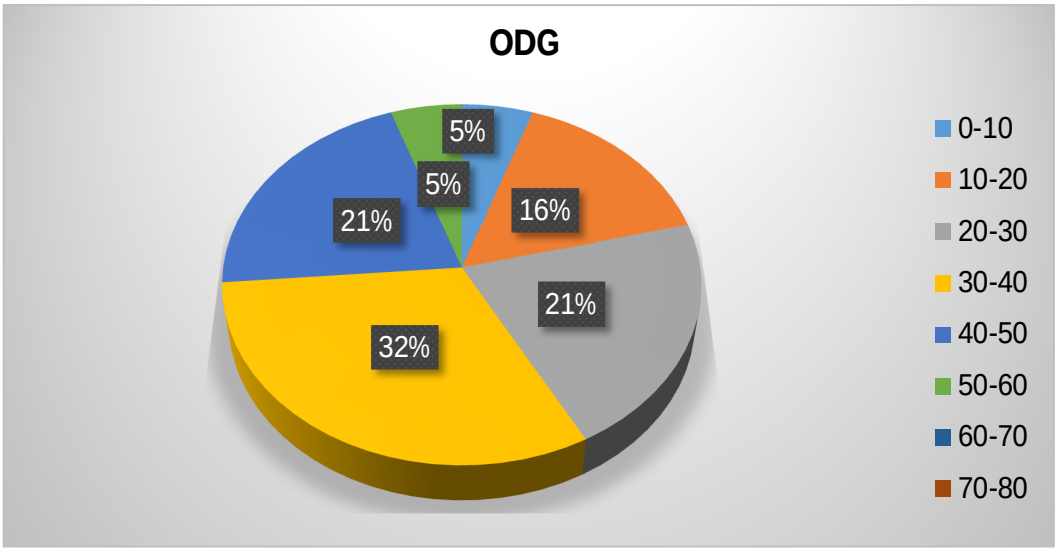
OLIGODENDROGLIOMA-

About 32% of oligodendroglioma were seen to occur in the age group of 30-40 years.

Table 3. Age distribution of Oligodendrogliomas.

Age Group	Number of cases	Percentage
0-10	1	5.7
10-20	3	15.8
20-30	4	21.3
30-40	6	31.6
40-50	4	21.3
50-60	1	5.3
60-70	0	0
70-80	0	0
Total	19	100
		p=0.0466

Figure 4. Percentage Age Distribution of Oligodendrogliomas.



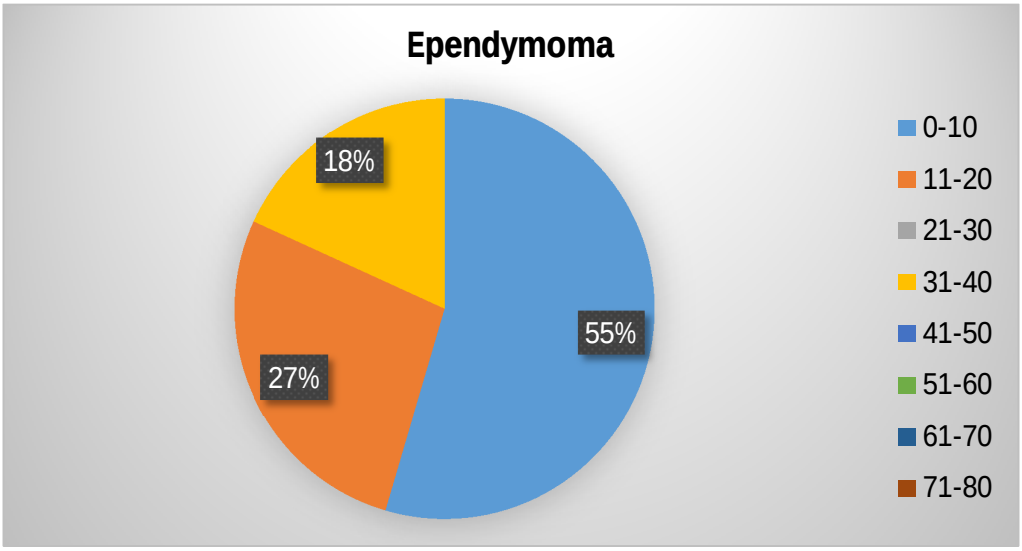
EPENDYMOMA-

Ependymoma was seen to occur most commonly in the age group between 0 to 20 years, with 88% of cases occurring in this age group. This was found to be significant with a p value of 0.000088.

Table 4. Age Distribution of Ependymoma.

Age Group	Number of Cases	Percentage
0-10	6	55
10-20	3	27
20-30	0	0
30-40	2	18
40-50	0	0
50-60	0	0
60-70	0	0
70-80	0	0
Total	11	100

Figure 5. Age distribution of Ependymoma.



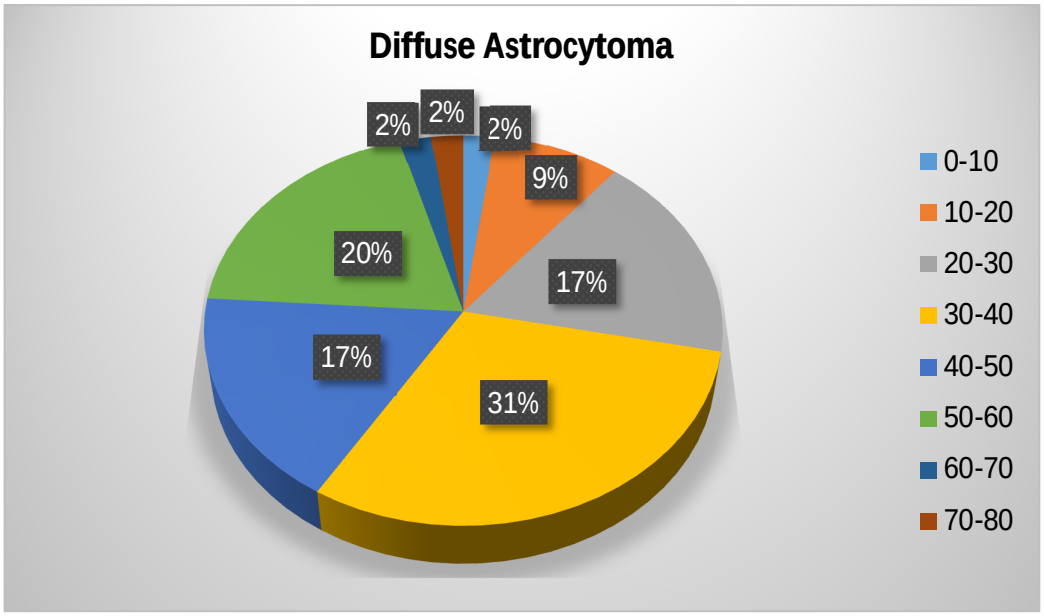
DIFFUSE ASTROCYTOMA-

Diffuse astrocytoma was distributed with majority of cases lying between the age group of 20 to 60 years, But the maximum percentage of patients (about 30.4%) were seen in the age group of 30 to 40 years with a p value of 0.0002.

Table 5. Age Distribution of Diffuse Astrocytoma.

Age Group	Number of Cases	Percentage
0-10	1	2.1
10-20	4	8.7
20-30	8	17.4
30-40	14	30.4
40-50	8	17.4
50-60	9	19.6
60-70	1	2.2
70-80	1	2.2
Total	46	100
		p=0.0002

Figure 6. Age Distribution of Diffuse Astrocytoma.



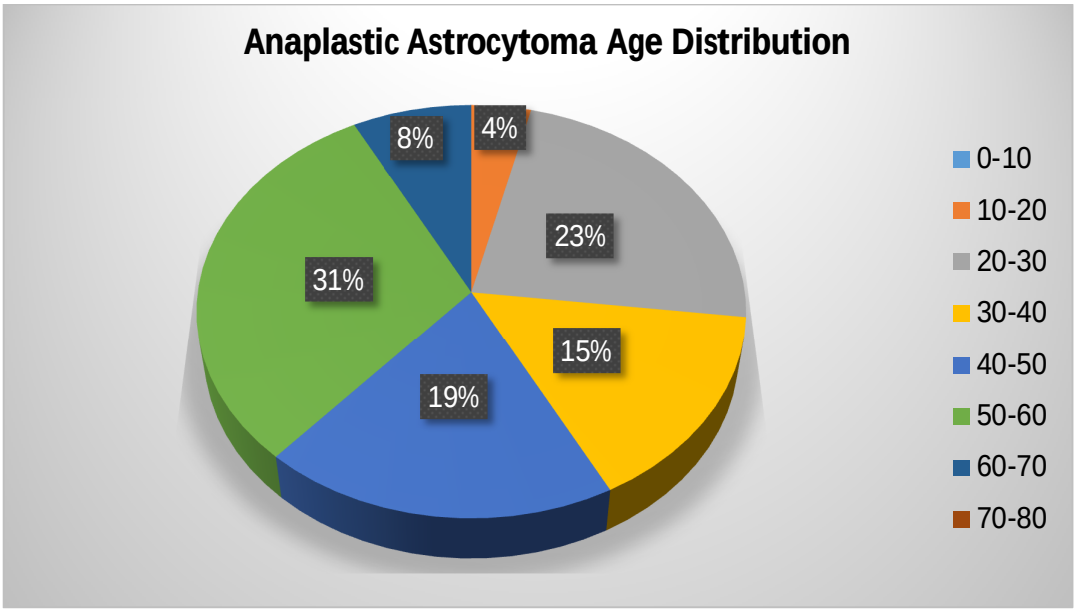
ANAPLASTIC ASTROCYTOMA

Anaplastic Astrocytoma cases showed an increasing distribution across the age groups and had maximum proportion (30.8%) of cases falling in the 51 to 60 years age groups with a significant p value of 0.00081.

Table 6. Age Distribution of Anaplastic astrocytoma.

Age Group	Number of Cases	Percentage
0-10	0	0
11-20	1	3.8
21-30	6	23.1
31-40	4	15.4
41-50	5	19.2
51-60	8	30.8
61-70	2	7.7
71-80	0	0
Total	26	100
		p=0.00081

Figure 7. Age Distribution of Anaplastic astrocytoma.



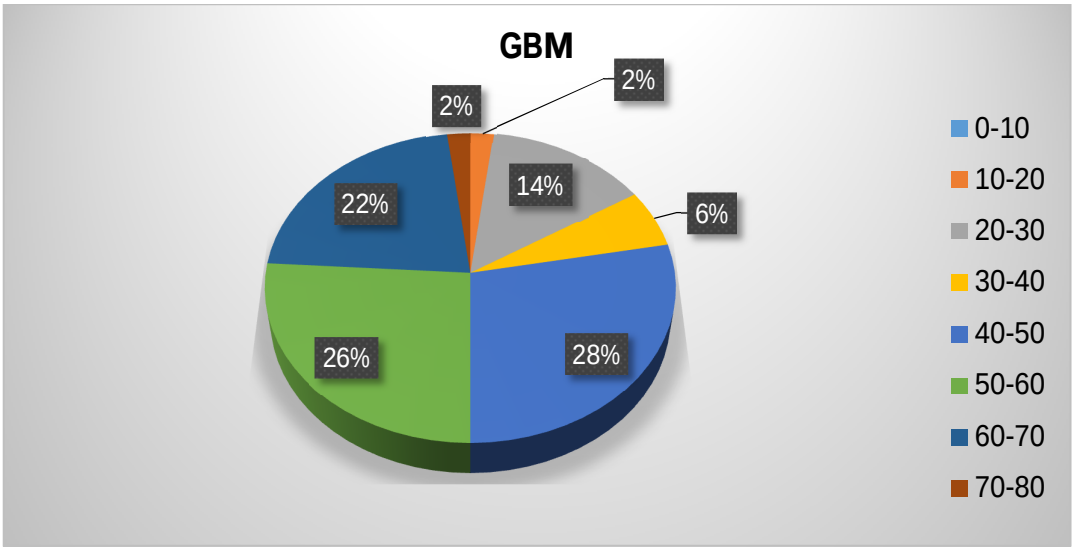
GLIOBLASTOMA-

Maximum proportion of cases of Glioblastoma were distributed between the ages of 40 to 70. The maximum percentage of cases were in the 40-50-year age group with a representation of 28% with a significant p value less than 0.0001.

Table 7. Age Distribution of Glioblastoma Multiforme.

Age Group	Number of Cases	Percentage
0-10	0	0
10-20	1	2
20-30	7	14
30-40	3	6
40-50	14	28
50-60	13	26
60-70	11	22
70-80	1	2
Total	50	100
		p<0.0001

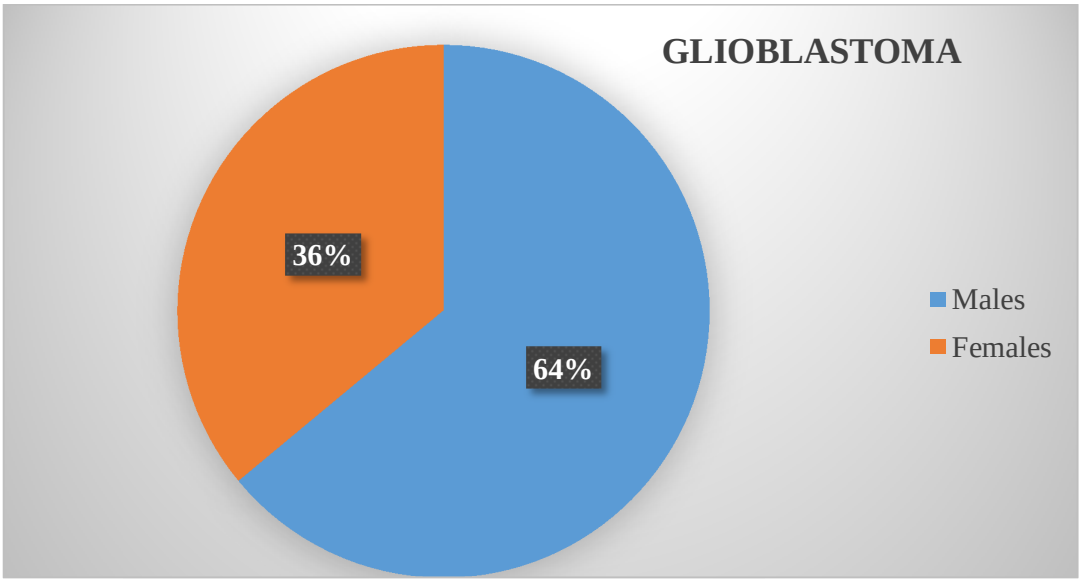
Figure 8. Age Distribution of Glioblastoma Multiforme.



GENDER DISTRIBUTION

The gender distribution was studied for 181 cases and it was found that among the gliomas, Glioblastoma was significantly seen to occur in males with 32 out of 50 cases occurring in males, with a significant p value of <0.047.

Figure 9. Gender Distribution of Glioblastoma.



Significant p value <0.047 was observed.

Table 9. Gender Distribution of Cases.

Diagnosis	Males	Percentage	Females	Percentage
Anaplastic Astrocytoma	15	57.7	11	42.3
Clear Cell Ependymoma	1	100	0	0
Diffuse Astrocytoma	27	58.7	19	41.3
Ependymoma	7	63.6	4	36.4
Glioblastoma(N=50)	32	64	18	36
Gliosarcoma	1	100	0	0
Myxopapillary Ependymoma	0	0	1	100
Oligodendroglioma	12	63.2	7	36.8
Pilocytic Astrocytoma	8	33.3	16	66.9
Pilocytic Astrocytoma with Focal Anaplasia	1	100	0	0
Pleomorphic Xanthoastrocytoma	1	100	0	0
Total	105	58	76	42

SITES AND SIDE OF GLIOMAS

The commonest site was Frontal with 24.3% of patients presenting here, while 11% and 9.9% of patients presented with glioma in the cerebellar and temporo-Parietal regions.

While observing the common side of occurrence of gliomas, it was seen that 45.3% of cases had gliomas on the left side and 29.3% of cases had it on the right side. Rest of the cases had a midline distribution.

Table 10. Distribution of Gliomas according to various sites in the Brain.

Site	Number of cases	Percentage
Fronto Temporal	8	4.4
Thalamic	1	0.6
Temporo Parietal	18	9.9
4th ventricle	2	1.1
Bifrontal	3	1.7
Brainstem	2	1.1
Corpus Callosal	10	5.5
Cerebellar	20	11
Cerebrum	1	0.6
Cerebello Pontine Angle	1	0.6
D12-L1 Intradural Intramedullary	1	0.6
Dorsolumbar	1	0.6
Fronto Parietal	14	7.7
Frontal	**44**	**24.3**
Intradural Extramedullary	1	0.6
Insular	2	1.1
Lateral Ventricle	1	0.6
Middle Cranial Fossa	1	0.6
Occipital	1	0.6
Optic Chiasma	1	0.6
Optic Nerve	1	0.6
Para-sagittal	1	0.6
Parasellar	1	0.6
Parietal	13	7.3
Parieto Occipital	9	4.9
Posterior Fossa	6	3.3
Supra-Sellar	1	0.6
Temporal	10	5.6
Temporo Occipital	1	0.6
Thalamus	5	2.7
TOTAL	181	100

Table 11. Distribution of Gliomas in different sides of the Brain.

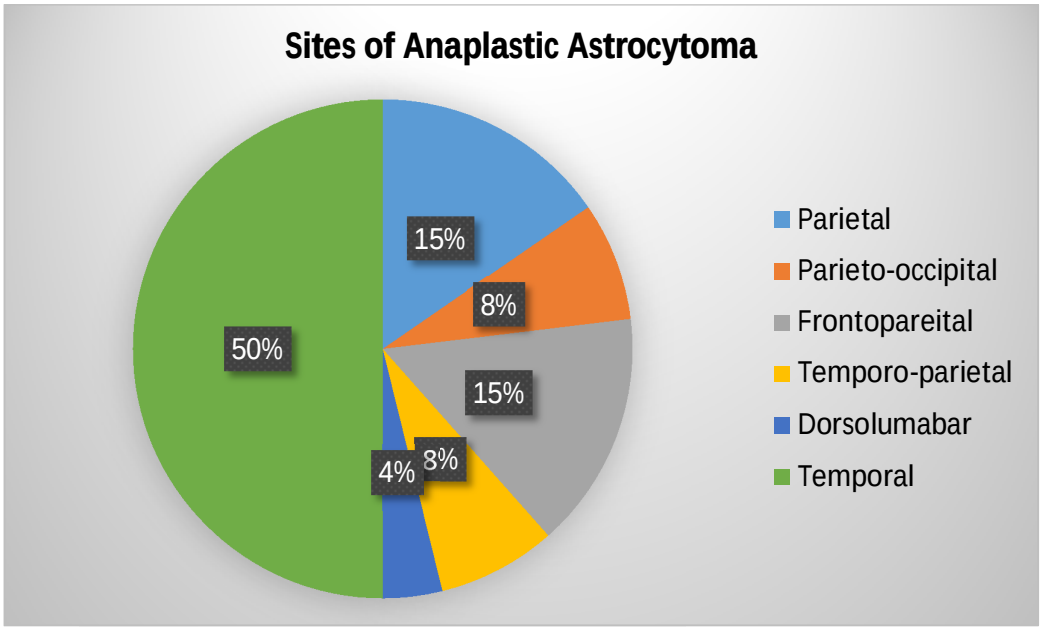
Side	Number of cases	Percentage
Left	82	45.3
Right	53	29.3
Midline	46	25.4
Total	181	100

When Observing Individual types of gliomas the presentation of site and sides changed accordingly.

Anaplastic astrocytoma-

Showed a temporal lobe predilection in maximum number of cases (50%), while Parietal and Frontoparietal lobe presentation was seen in 15% cases each.

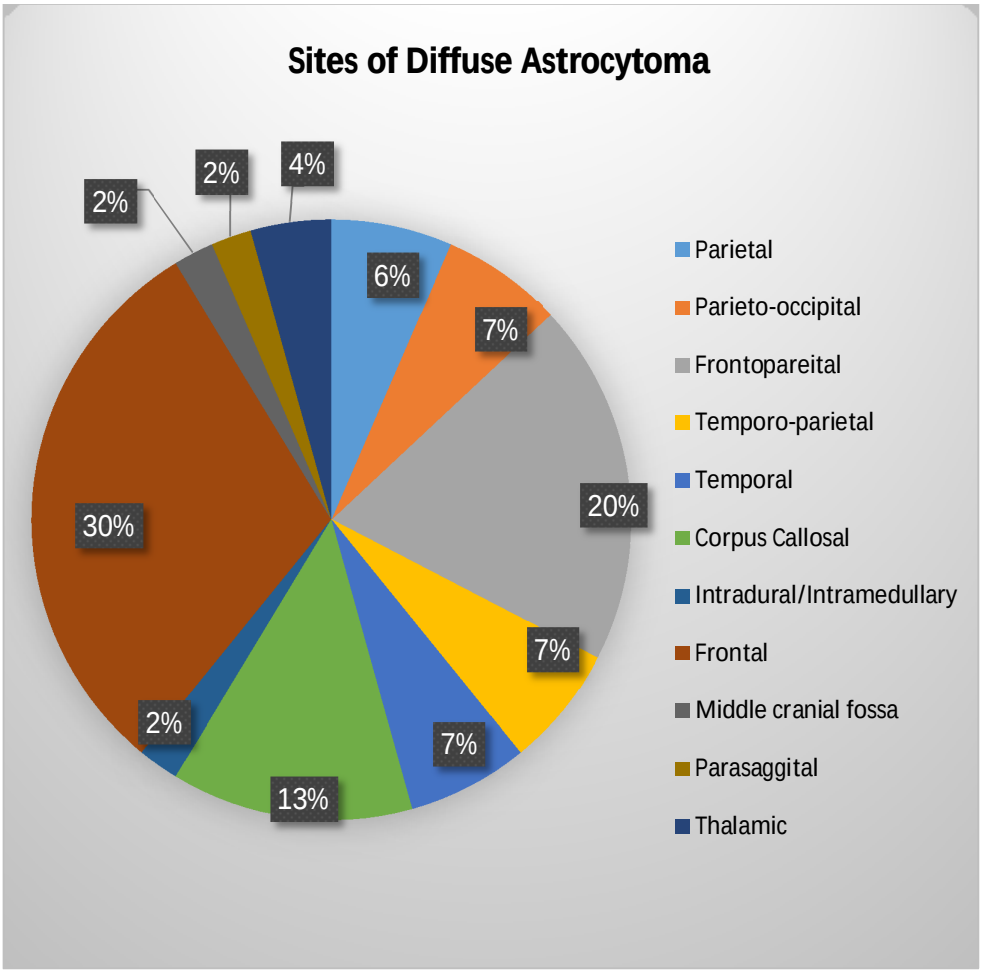
Figure 10. Distribution of Anaplastic Astrocytoma according to site.



DIFFUSE ASTROCYTOMA

Showed maximum predilection for frontal region with 30%, followed by frontoparietal region with 19.6%

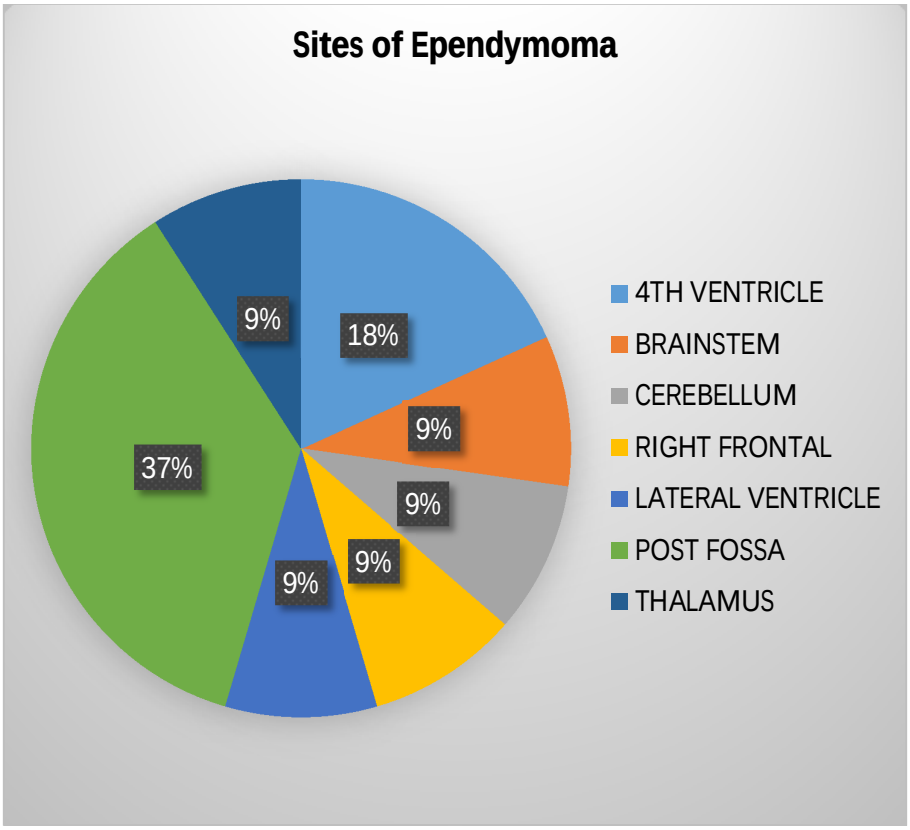
Figure 11. Distribution of Diffuse Astrocytoma according to site



EPENDYMOMA

37% of cases were seen to afflict the posterior fossa followed by 18% of cases occurring in the fourth ventricle.

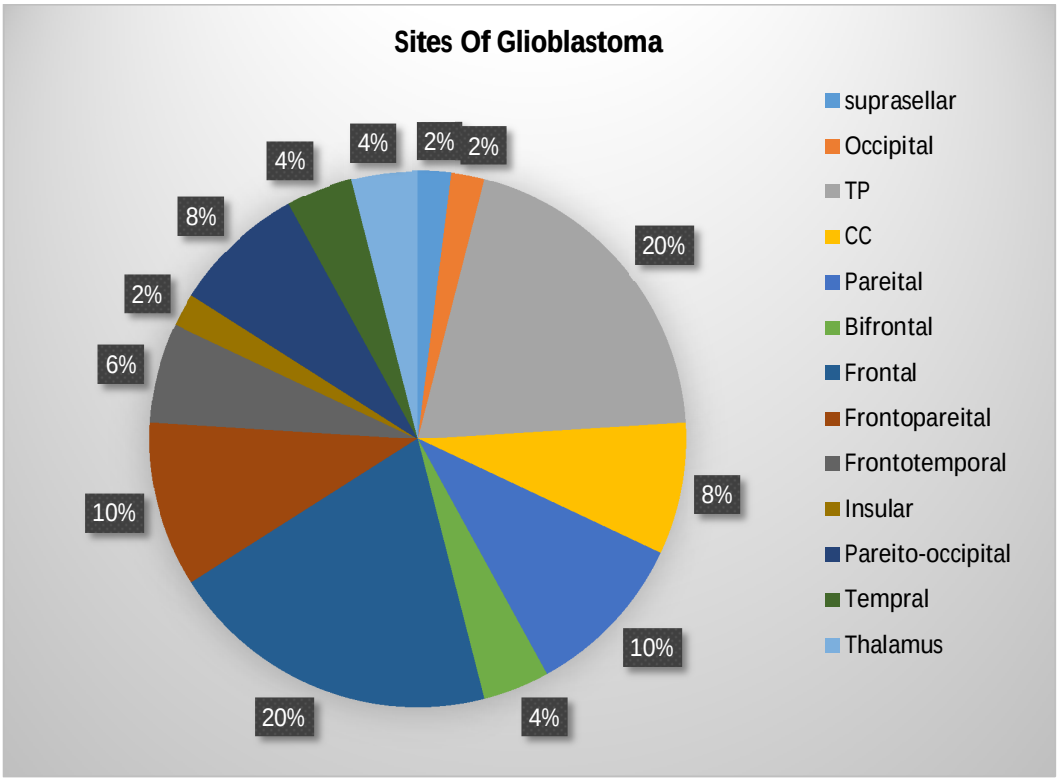
Figure 12.Distribution of Ependymoma according to site



GLIOBLASTOMA-

20 Percent of cases having Glioblastoma showed presentation of the disease, in Frontal and Temporo- Parietal areas. 10% of cases presented in Fronto-Parietal and Parietal areas respectively.

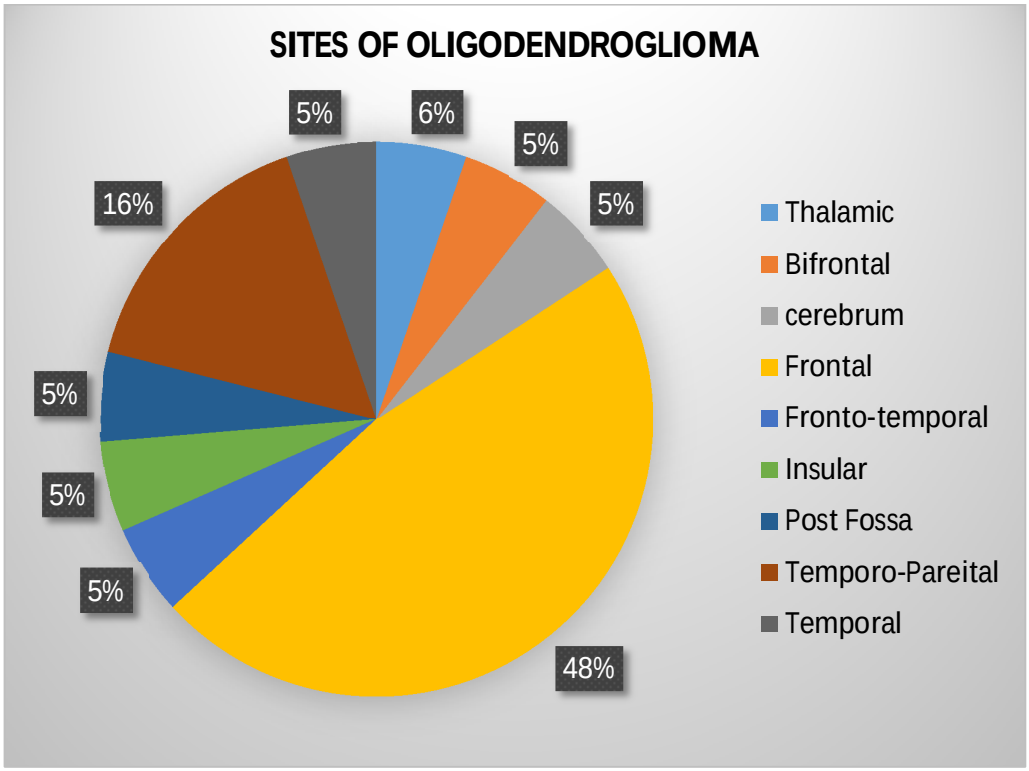
Figure 13. Distribution of Glioblastoma according to site



OLIGODENDROGLIOMA-

Maximum predilection was seen for the frontal lobe (48%), followed by temporo parietal region (16%).

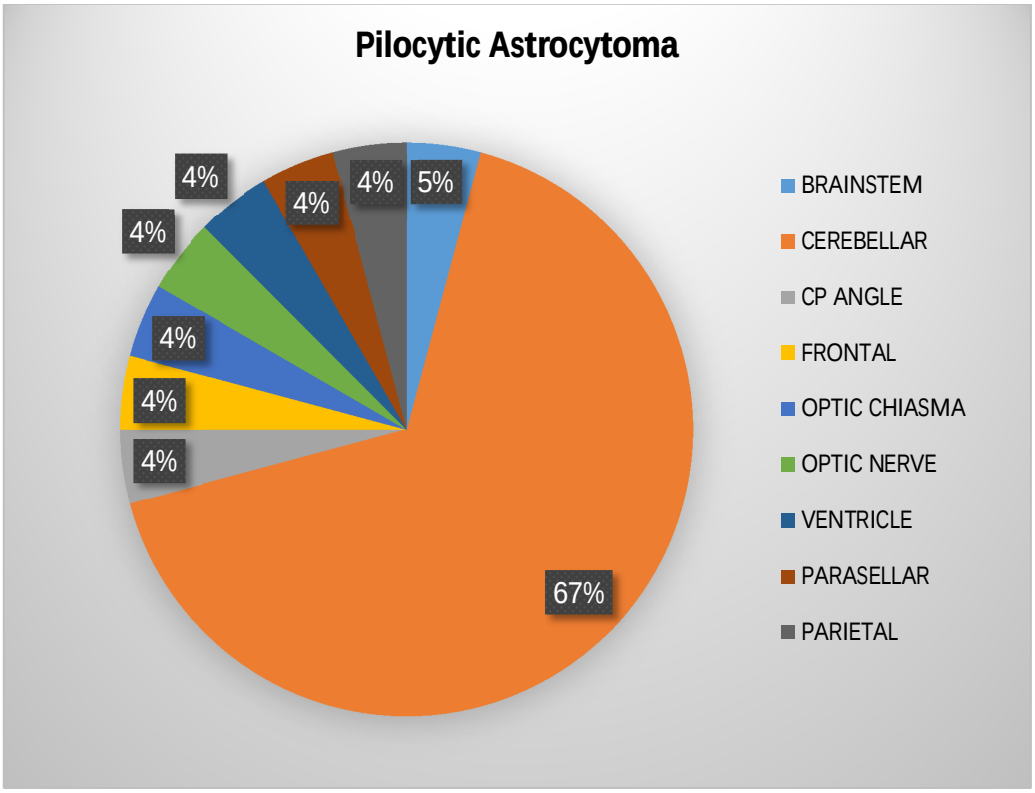
Figure 14. Distribution of oligodendroglioma according to site



PILOCYTIC ASTROCYTOMA-

Majority of the cases (67%), were seen to occur in the cerebellar region, the percentage of its occurrence in the other areas was 4% each.

Figure 15. Distribution of Pilocytic Astrocytoma according to site



CORRELATION WITH HISTOLOGICAL PARAMETERS-

Histological parameters such as nuclear pleomorphism, vascular proliferation and mitosis were studied.

NUCLEAR PLEOMORPHISM-

Nuclear pleomorphism was scored as Mild (1+), Moderate (2+). It was seen that 100% of Glioblastoma cases showed marked nuclear pleomorphism with a significant p value of <0.00078. It was also seen that 67.4% of Diffuse Astrocytoma exhibited mild nuclear pleomorphism. 100% of Ependymomas exhibited mild nuclear pleomorphism, whereas Oligodendroglioma and Pilocytic Astrocytoma showed mild nuclear pleomorphism in 94.7% and 95.8% of cases respectively. WHO Grade IV tumors were associated with marked nuclear pleomorphism.

Table 12. Distribution of nuclear pleomorphism in glioma

Diagnosis	Nuclear Pleomorphism				
	1+	Percentage	2+	Percentage	P value
Anaplastic Astrocytoma (n=26)	1	4%	25	96	0.00001
Diffuse Astrocytoma (n=46)	31	67.4%	15	32.6%	0.0183
Ependymoma (n=11)	11	100%	0	0%	0.0009
Glioblastoma(n=50)	0	0%	50	100%	0.000078
Oligodendroglioma(n=19)	18	94.7%	1	5.3%	0.000096
Pilocytic astrocytoma (n=24)	23	95.8%	1	4.2%	0.000709

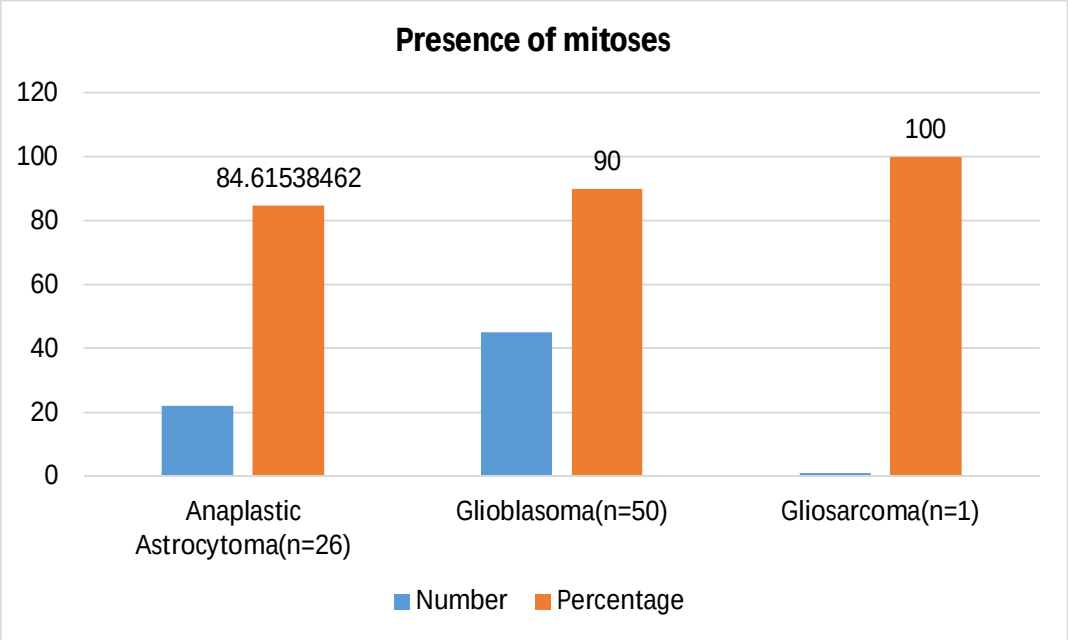
MITOSES-

Mitotic count was scored on the basis of presence (P) or absence (A) of mitosis. In Anaplastic Astrocytoma, 84.6% of cases show presence of mitosis, 90% of Glioblastoma showed increased mitotic activity. The p value was found to be highly significant for Anaplastic Astrocytoma and Glioblastoma with $p<0.01$. Highly significant p values <0.01 was obtained for ependymoma, oligodendroglioma and diffuse astrocytoma, as 100% of the cases showed no mitoses.

Table 13. Distribution of mitoses in glioma

DIAGNOSIS	Mitosis			
	Present	**Percentage**	**Absent**	**Percentage**
Anaplastic astrocytoma	22	84.6	4	15.4
Diffuse astrocytoma	0	0	46	100
Ependymoma	0	0	11	100
Glioblastoma	45	90	5	10
Oligodendroglioma	0	0	19	100
Pilocytic astrocytoma	0	0	24	100

Figure 16. Distribution of mitoses in glioma



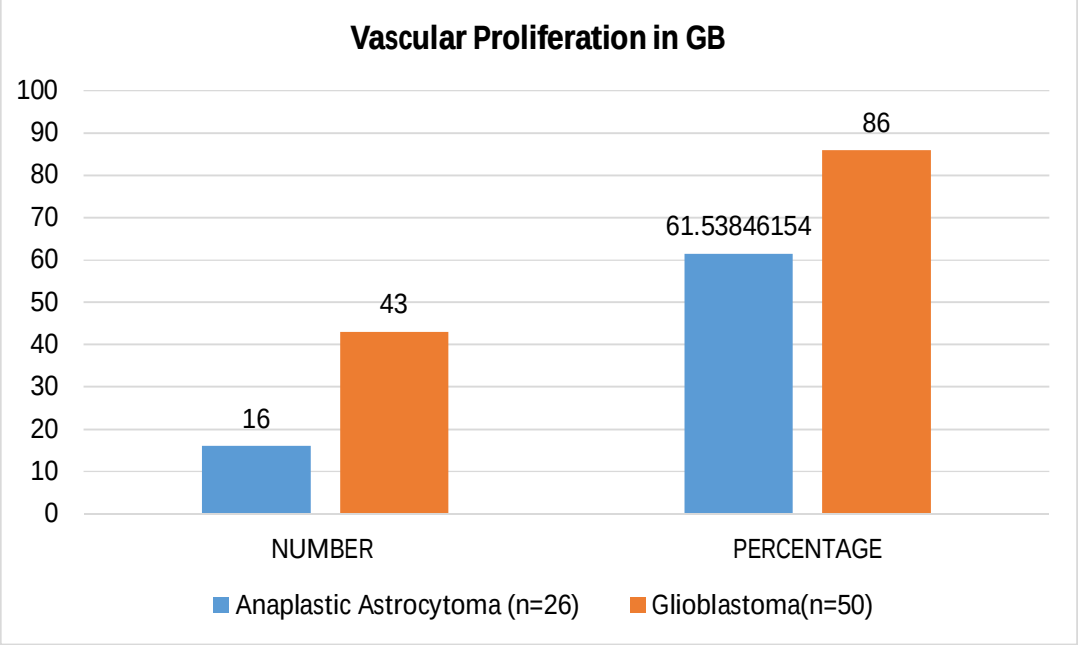
VASCULAR PROLIFERATION-

Vascular proliferation observed in 181 cases was scored based upon its presence (P) or Absence (A). 86% of Glioblastoma showed vascular proliferation and this was found to be highly significant. None of the Diffuse Astrocytoma, Ependymoma, Oligodendroglioma and Pilocytic Astrocytoma showed vascular proliferation and this was found to be statistically significant (with p value <0.01).

Table 14. Distribution of vascular proliferation in glioma

DIAGNOSIS	Vascular Proliferation			
	Present	%	Absent	%
Anaplastic Astrocytoma	16	61.5	10	38.5
Diffuse Astrocytoma	0	0	46	100
Ependymoma	0	0	11	100
Glioblastoma	43	86	7	14
Oligodendroglioma	0	0	19	100
Pilocytic Astrocytoma	0	0	24	100

Figure 17. Distribution of vascular proliferation in glioblastoma



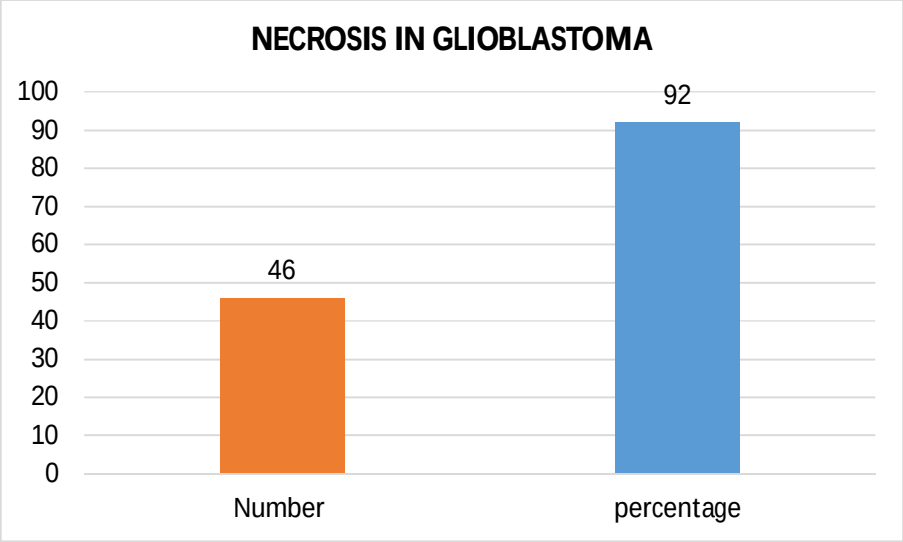
NECROSIS-

Necrosis was assessed based on its Presence (P), or Absence (A). Of all the 181 cases studied, 92% of Glioblastoma showed necrosis. It was the only malignant glioma associated with necrosis, and this was statistically very significant with a p value <0.00025.

Table 15. Distribution of necrosis in glioma

Diagnosis	Necrosis				p value
	Present	%	Absent	%	
Anaplastic Astrocytoma (n=26)	0	0	26	100	0.000314
Diffuse Astrocytoma (n=46)	0	0	46	100	<0.0001
Ependymoma (n=11)	0	0	11	100	<0.0001
Glioblastoma (n=50)	46	**92**	4	8	0.000025
Oligodendroglioma (n=19)	0	0	19	100	<0.0001
Pilocytic Astrocytoma (n=24)	0	0	24	100	<0.0001

Figure 18. Distribution of necrosis in glioblastoma



GRADING OF GLIOMAS-

Gliomas are graded according to WHO grading system, with Grade I and II correlating with Low grade gliomas and III and IV corresponding to high grade gliomas. In our Institute, 43.1% of the cases were Grade II tumors closely followed by Grade IV tumors with 28.2%.

Table 16. Distribution of gliomas according to WHO Grade

Grade	Number of cases	Percentage
Grade I	25	13.8
Grade II	78	43.1
Grade III	27	14.9
Grade IV	51	28.2
Total	181	100

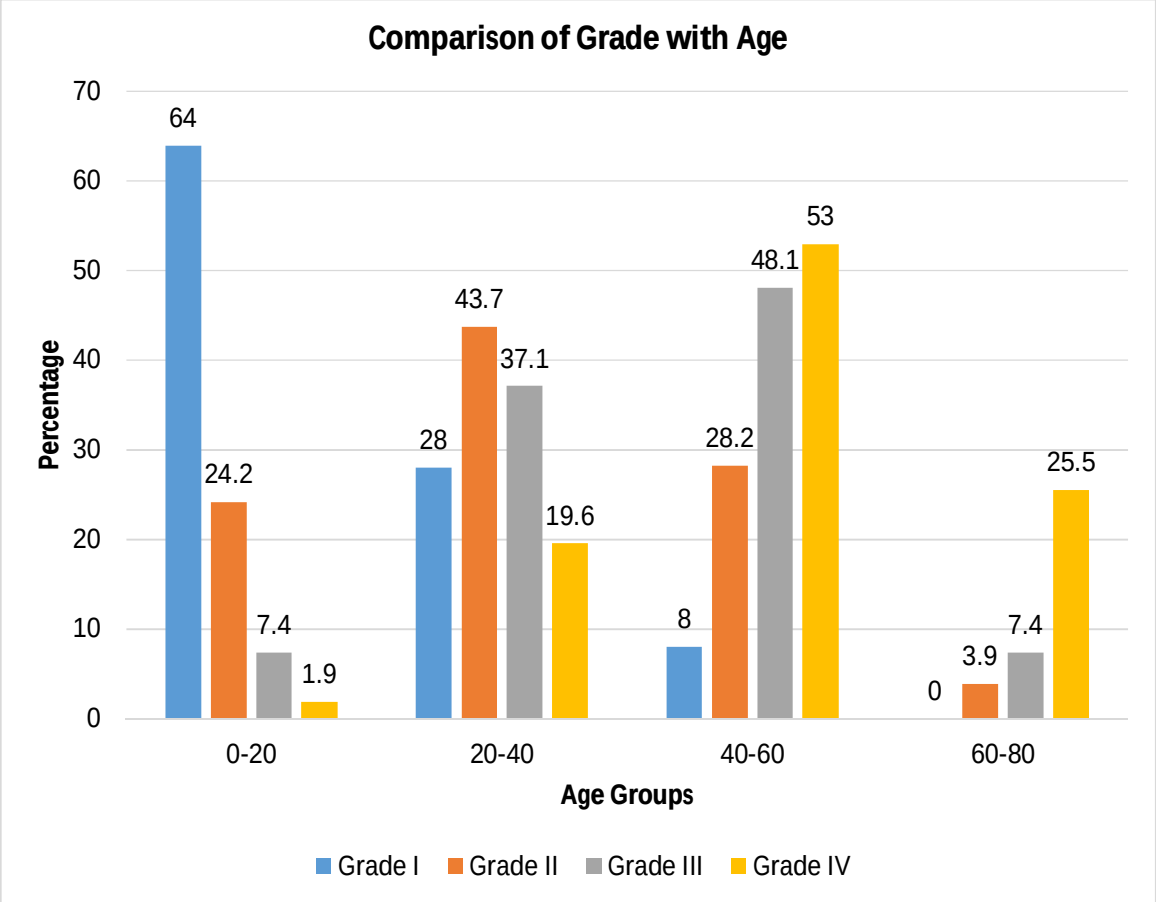
CORRELATION OF AGE WITH GRADING-

The age of 181 patients was correlated with grading of glioma and it was found that **64% of patients with Grade I glioma were in the age group of 0-20 years and this was found to be statistically significant with a p value of .0077.** 43.7% of patients with Grade II glioma were in the age group of 20-40 years. This was not statistically significant.

53% of patients with Grade III glioma were in the age group of 40-60 years. This was statistically significant with p value <0.00778.

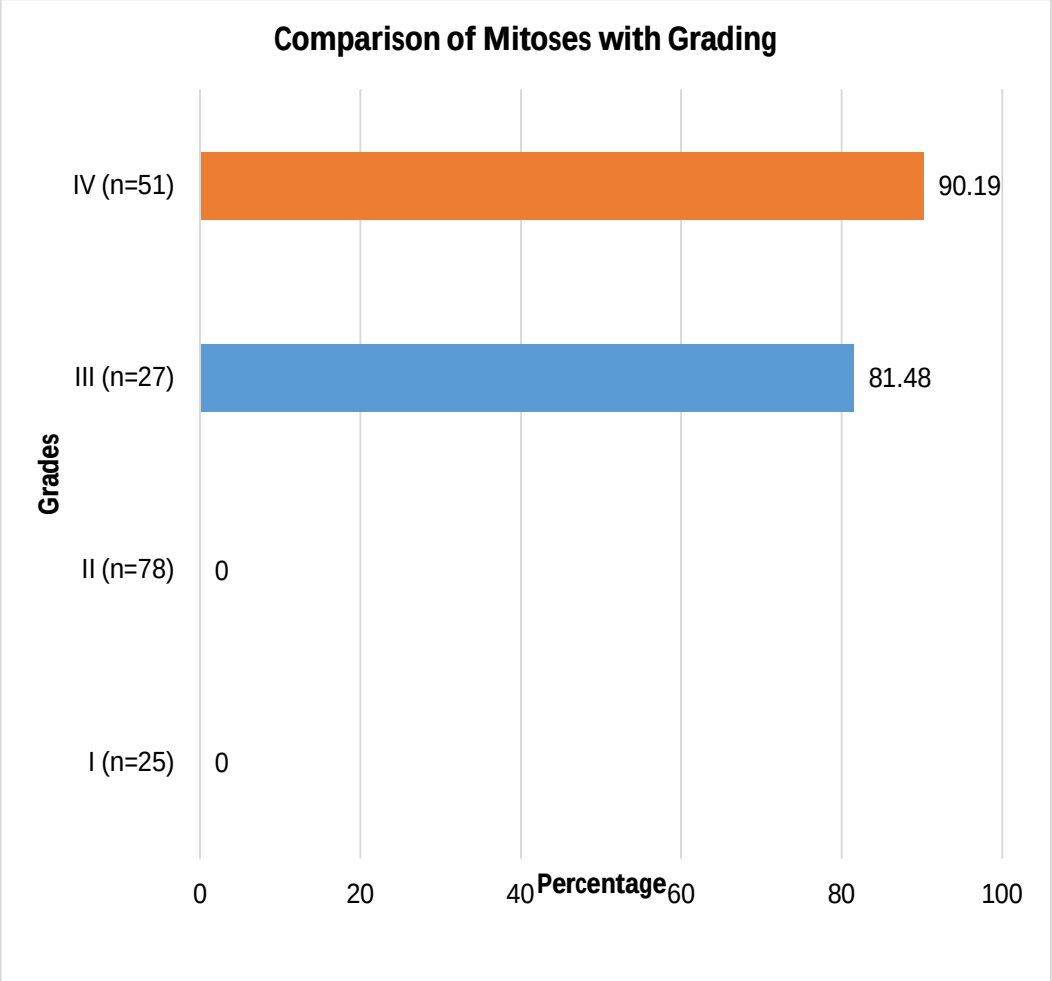
25.5% of patients with Grade IV glioma were in the age group of 60-80 years. This was statistically significant with a p value of 0.0088

Figure 19.Correlation of age with grading of gliomas



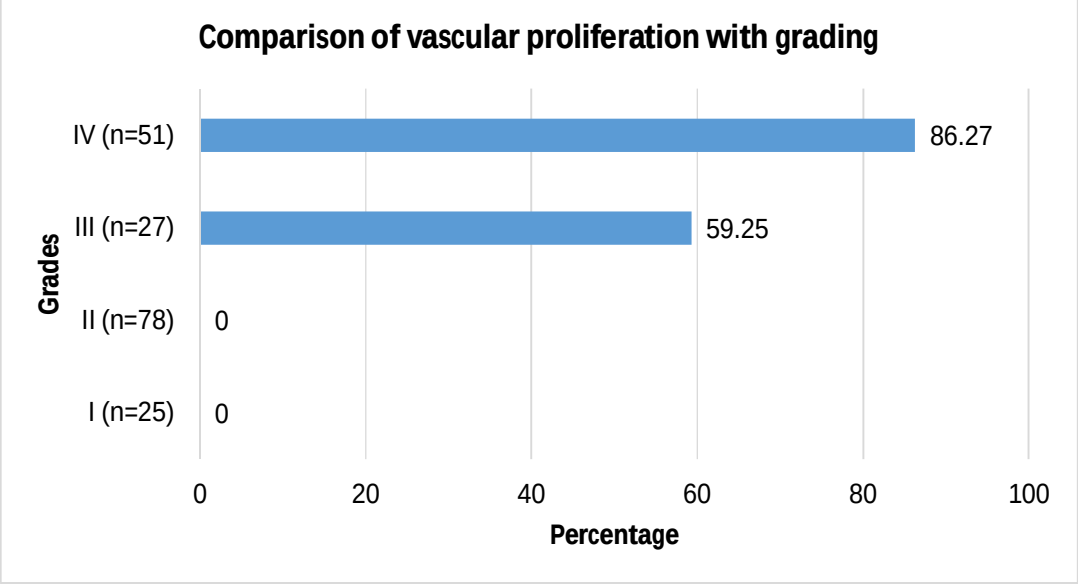
CORRELATION WITH HISTOLOGICAL PARAMETERS-

Figure 20. Correlation of mitotic activity with grading of gliomas.



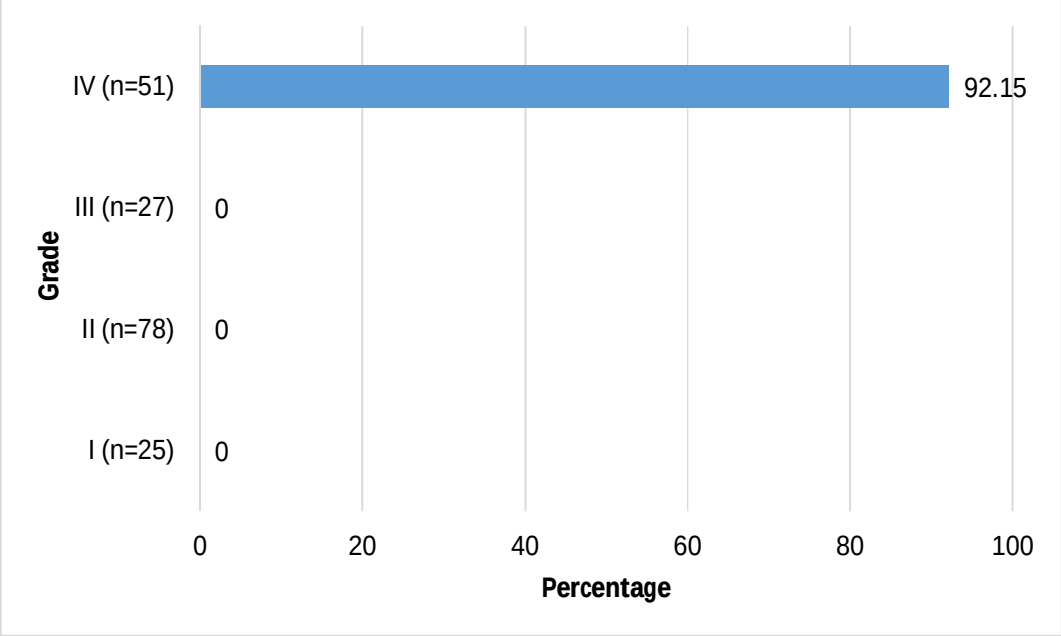
90.19% of patients having Grade IV glioma were found to be associated with mitoses and 81.48% of patients with Grade IV were found to be associated with mitoses. Both these were found to be highly significant with a p value of <0.01.

Figure 21. Correlation of vascular proliferation with grading of glioma



86.27% and 59.25% of patients with Grade IV and Grade III respectively, were seen to be associated with vascular proliferation and this was found to be highly significant with a p value of <0.01.

Figure 22. Correlation between necrosis and Grading of Glioma



In our study 92.15% of patients with Grade IV were found to have necrosis. Necrosis was not seen in any other grade and this was statistically very significant with a p value <0.001.

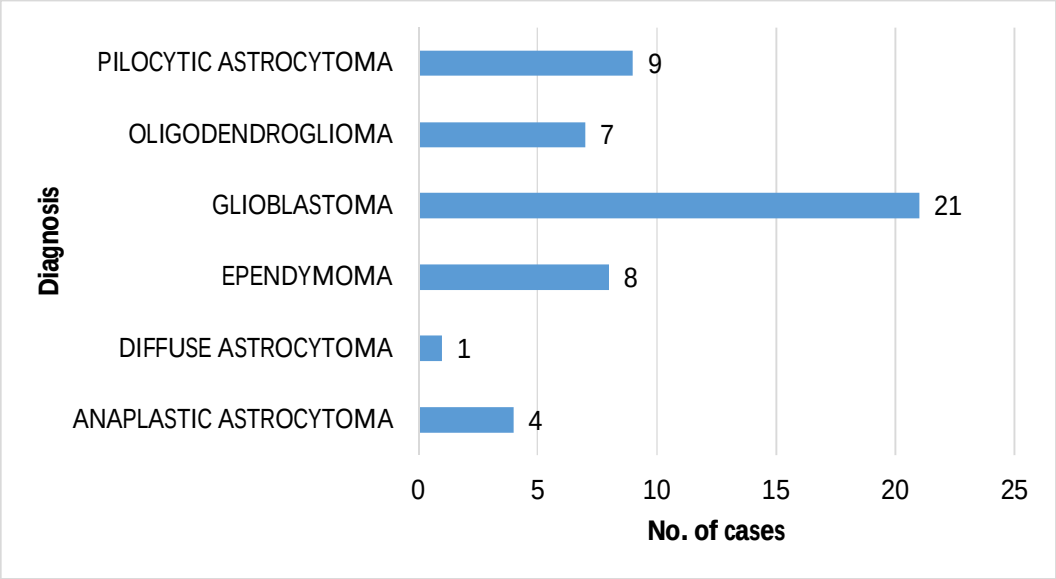
IMMUNOHISTOCHEMICAL EXAMINATION OF GLIOMA- CORRELATION WITH EGFR AND KI 67 EXPRESSION

The expression of EGFR and Ki 67 was studied in different types of glioma. A subset of 50 cases constituting 25 cases of low grade (WHO Grade I and II) and 25 cases of high grade (WHO grade III and IV) representative of the whole sample of 181 cases was chosen. The formalin fixed paraffin embedded sections of selected 50 cases was subjected to immunohistochemical analysis with EGFR and Ki 67. The list of cases are as follows.

Table 17. List of cases for which immunohistochemical analysis was done

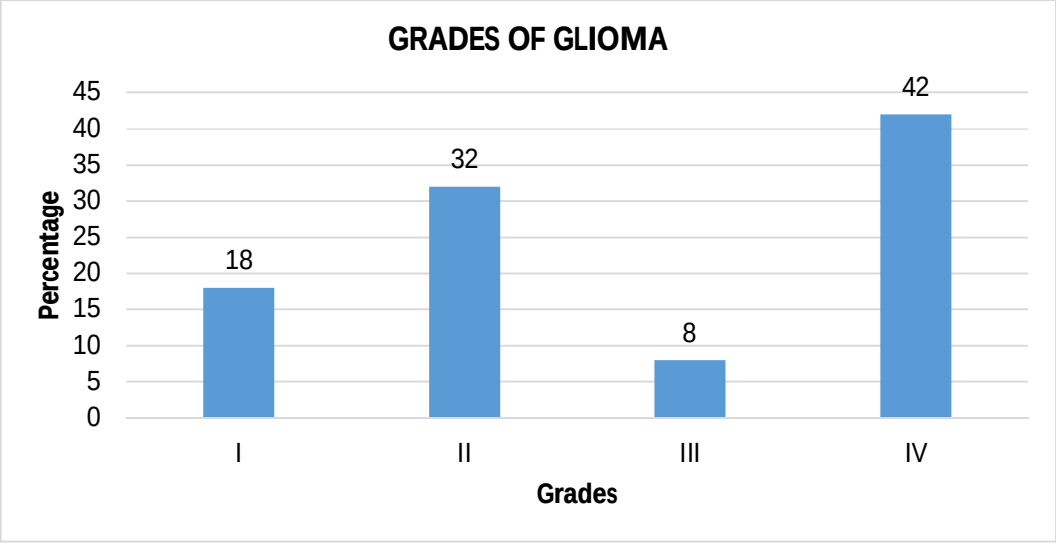
Diagnosis	Number of cases	Percentage
Anaplastic astrocytoma	4	8
Diffuse astrocytoma	1	2
Ependymoma	8	16
Glioblastoma	21	42
Oligodendroglioma	7	14
Pilocytic astrocytoma	9	18
Total	50	100

Figure 23.Distribution of cases for which immunohistchemical analysis was done



WHO grading of gliomas was done for all the 50 cases. Amongst these, 42% of the cases were Grade IV,32% were Grade II,18% of cases were Grade I and 8% of cases were Grade III.

Figure 24. Distribution of cases according to WHO grading system



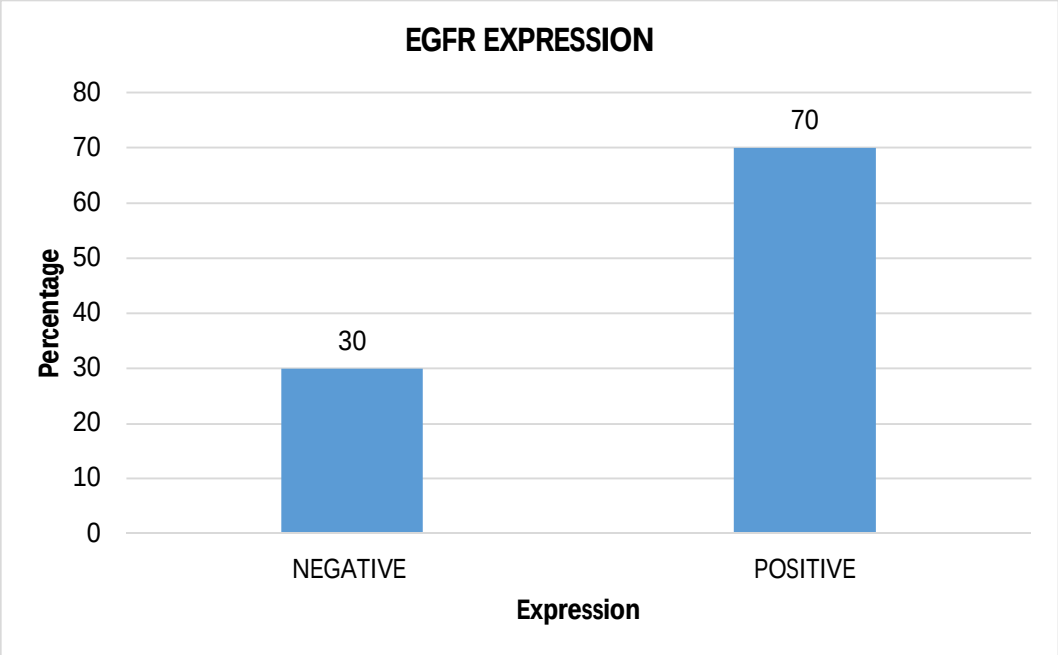
DISTRIBUTION OF EGFR EXPRESSION IN GLIOMAS-

Out of 50 cases in total, 35 cases (70%) stained positive with EGFR.

Table 18. Cases with expression of EGFR

EGFR EXPRESSION	NUMBER OF CASES	PERCENT
NEGATIVE	15	30
POSITIVE	35	70

Figure 25. Cases with expression of EGFR



DISTRIBUTION OF Ki 67 EXPRESSION IN GLIOMAS-

In the scoring done for Ki 67, the scoring was assigned based on percentage of immunopositive cells per thousand cells.26 out of 50 cases(52%) showed more than >5% positivity.

Table 19. Cases showing expression of Ki 67

KI 67 EXPRESSION	NUMBER OF CASES	PERCENT
<5%	24	48.0
>5%	26	52.0

Figure 26. Cases showing expression of Ki67

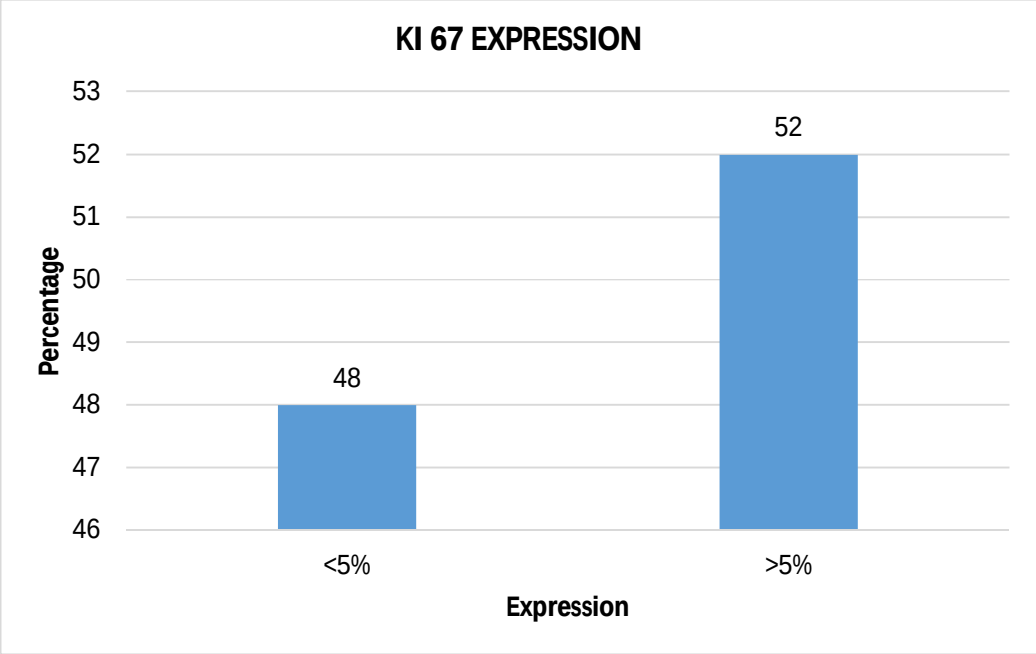


Table 20. Correlation between expression of Ki67 and EGFR staining

KI67_SCORE		EGFR		Total
		NEGATIVE	POSITIVE	
<5% NEGATIVE	Count	15	9	24
	% within EGFR	100.0%	25.7%	48.0%
>5% POSITIVE	Count	0	26	26
	% within EGFR	0.0%	74.3%	52.0%
Total	Count	15	35	50
	% within EGFR	100.0%	100.0%	100.0%

Pearson Chi-Square=23.214** P<0.001

A total of 26 cases showed positivity (>5%) for Ki 67. All these 26 cases also showed positivity for EGFR (100%).

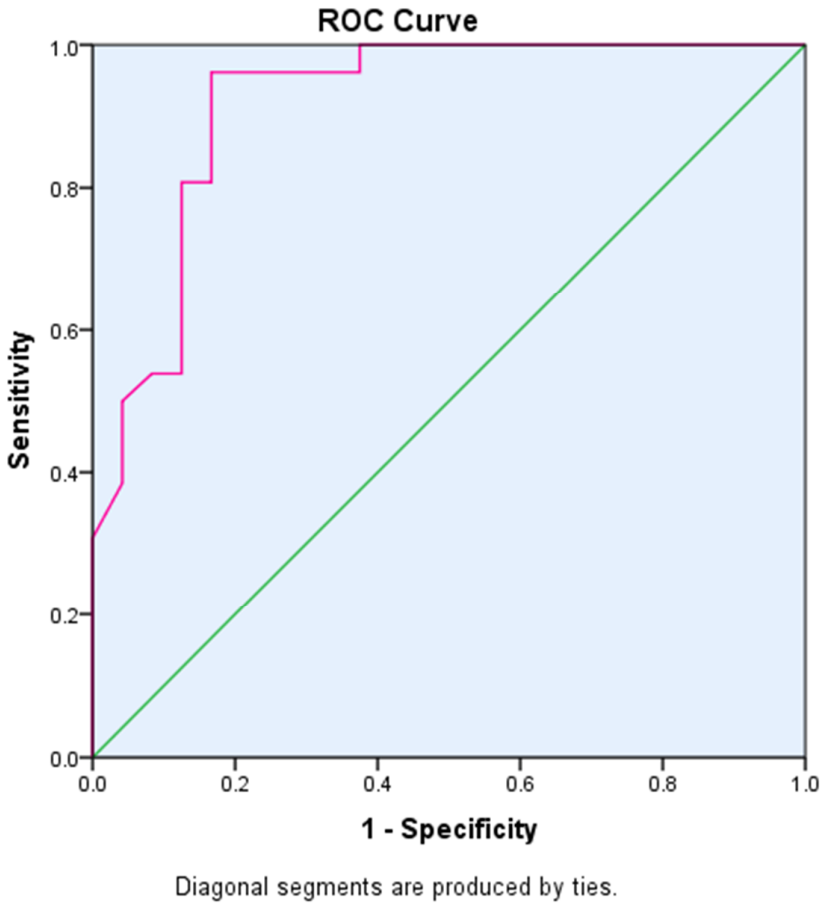
Of 35 cases that showed positivity for EGFR,74.3% of the cases also showed positivity for Ki 67 and 25.7% of cases were negative for Ki 67.

This was found to be significant with a p value <0.001.

Utilizing the above comparison between EGFR and Ki 67, a Sensitivity and Specificity analysis of EGFR was done. It showed a sensitivity of 74.29% (56.74-87.51%: -95% CI) and a Specificity of 100% (78.20%-100%). This showed a positive predictive value of 100%.

Based on the above sensitivity and specificity results an ROC curve was plotted to visualize the results. The plot showed a straight line for Specificity, hence proving 100% Positive Predictive value

Figure 27. ROC curve for sensitivity and Specificity.



The area under the straight line was calculated as 0.917 with a standard error of 0.041 (0.838-0.997: -95% CI).

Table 21. Correlation between EGFR and WHO grading of gliomas

EGFR		WHO_GRADING				Total
		I	II	III	IV	
NEGATIVE	Count	6	7	0	2	15
	% within WHO Grading	66.7%	43.8%	0.0%	9.5%	30.0%
POSITIVE	Count	3	9	4	19	35
	% within WHO Grading	33.3%	56.2%	100.0%	90.5%	70.0%
Total	Count	9	16	4	21	50
	% within WHO Grading	100.0%	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=13.109** P=0.004

While comparing cases with EGFR positivity with WHO grading, it was observed that only 33.3% of Grade I patients, 56.2% of Grade II patients, 100% of Grade III patients, 90.5% of Grade IV showed positivity for EGFR.

These results show that Grade III and IV cases showed higher positivity for EGFR with a significant value of p=0.004.

Table 22. Correlation between Ki 67 and WHO grading of gliomas

KI67_SCORE		WHO_GRADING				Total
		I	II	III	IV	
<5%	Count	7	11	1	5	24
	% within WHO Grading	77.8%	68.8%	25.0%	23.8%	48.0%
>5%	Count	2	5	3	16	26
	% within WHO Grading	22.2%	31.2%	75.0%	76.2%	52.0%
Total	Count	9	16	4	21	50
	% within WHO Grading	100.0%	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=11.728** P=0.008

On further examining correlation between Ki 67 and WHO grading, it was observed that 22.2% of Grade I patients, 31.2% of Grade II patients, 75% of Grade III and 76.2% of Grade IV patients showed increased mitotic activity of >5%. These results show that as and when compared to Grade I and II, Grade III and IV showed increased mitotic activity and staining for Ki 67.

COLOUR PLATES

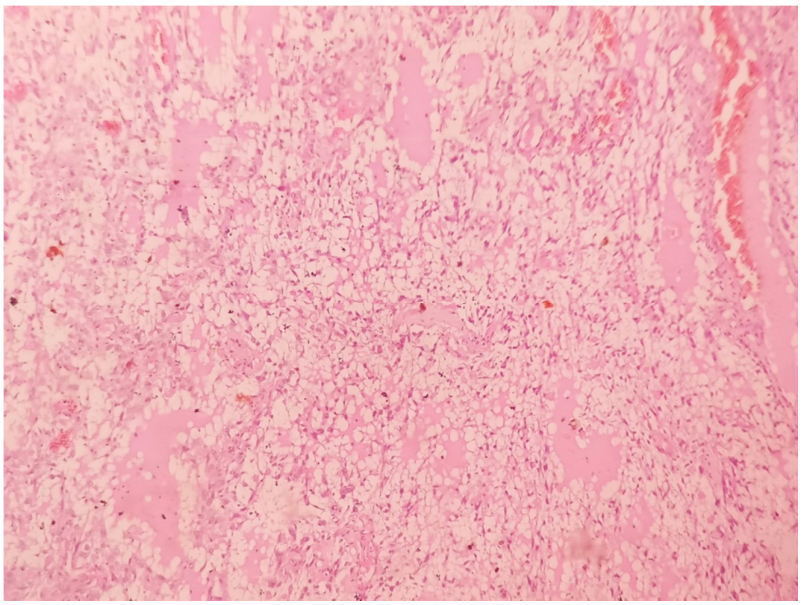


Figure 1-100x view of Pilocytic Astrocytoma showing microcystic areas.

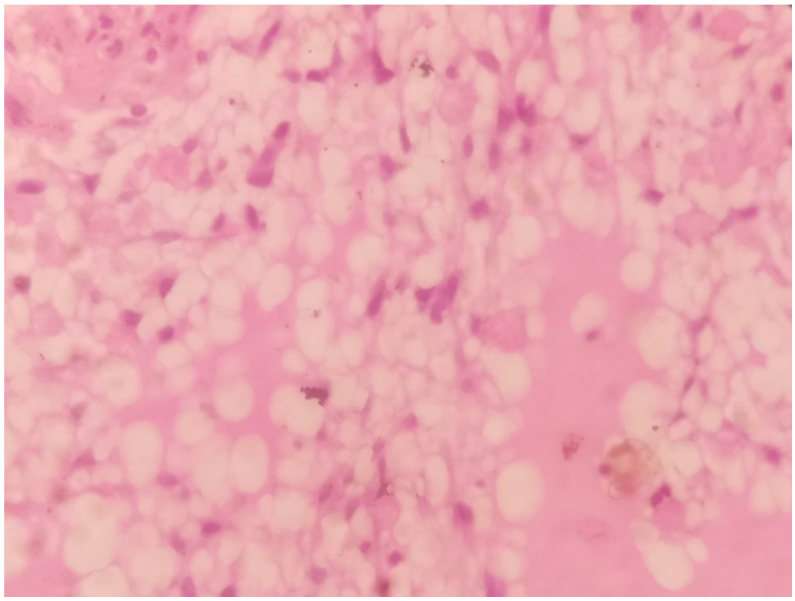


Figure 2-400x view of Pilocytic Astrocytoma.

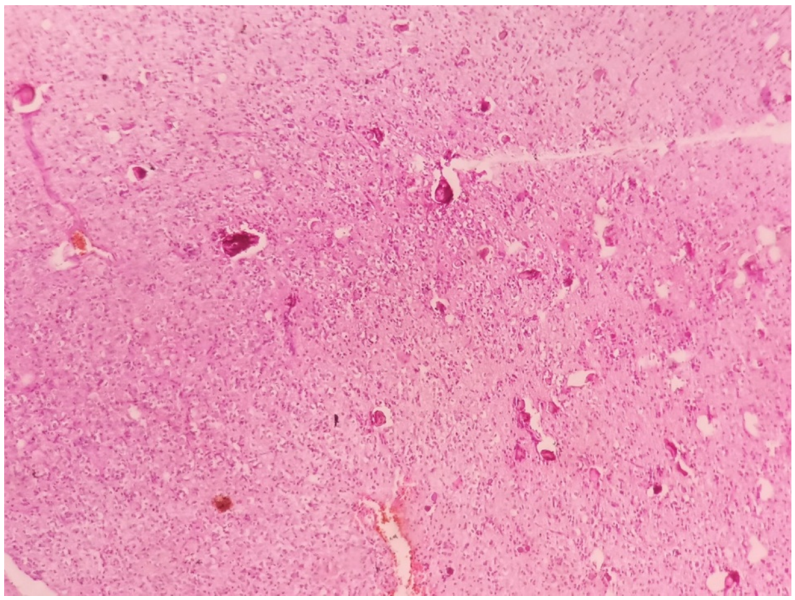


Figure 3-100x view of Oligodendroglioma-with areas showing calcification.

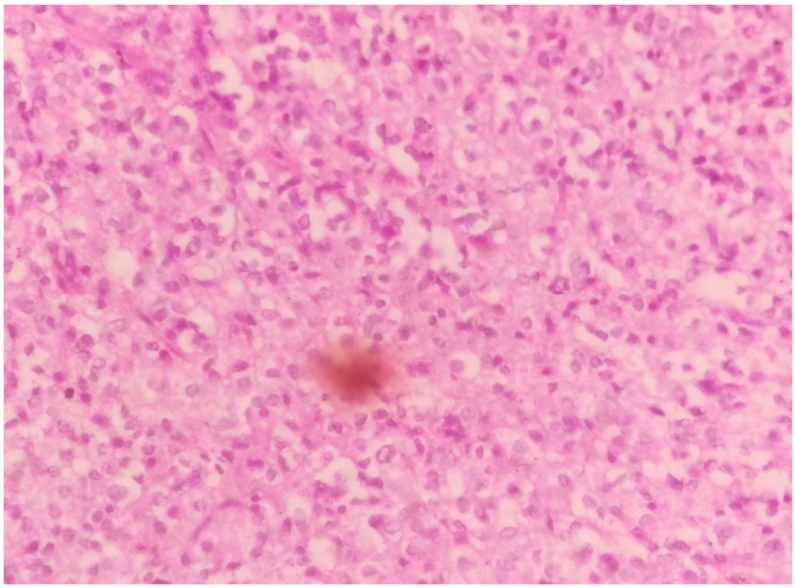


Figure 4-400 x view of Oligodendroglioma with halos seen around individual tumour cells-fried egg appearance.

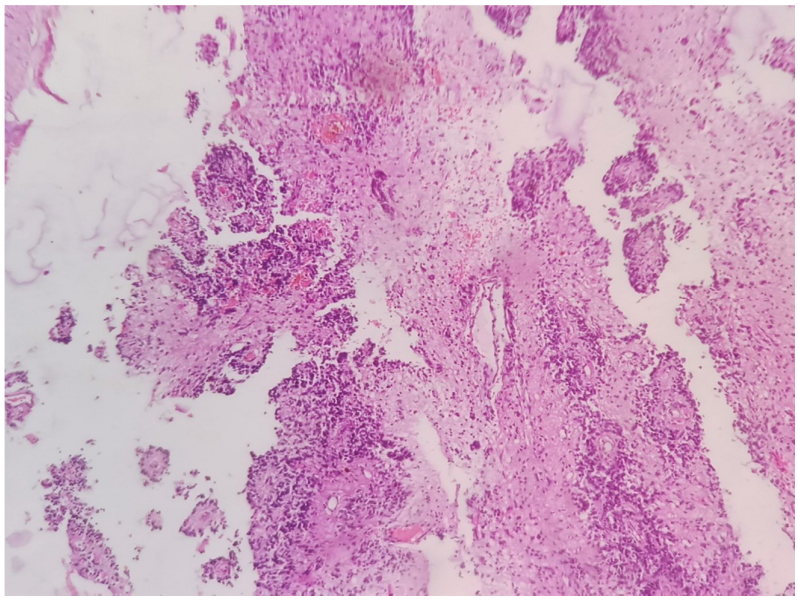


Figure 5-100x view of Ependymoma-perivascular pseudo rosettes seen

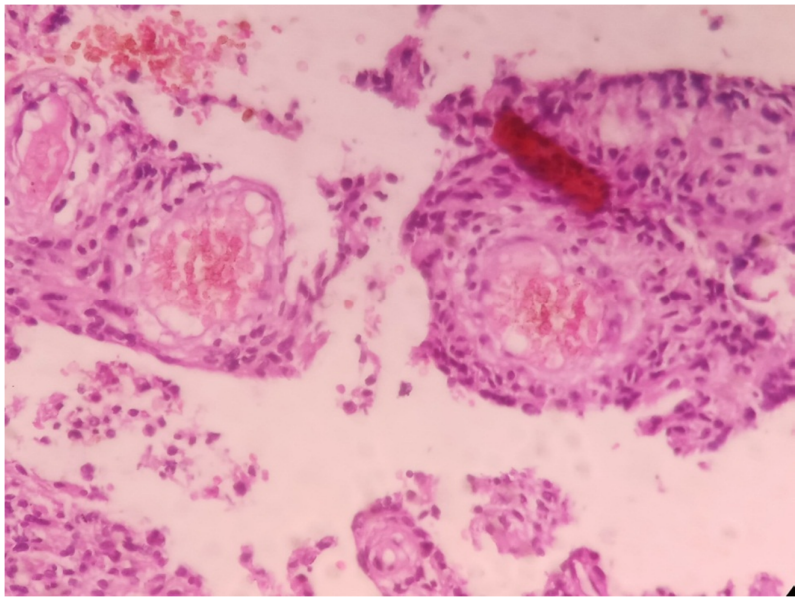


Figure 6-400x view of Ependymoma

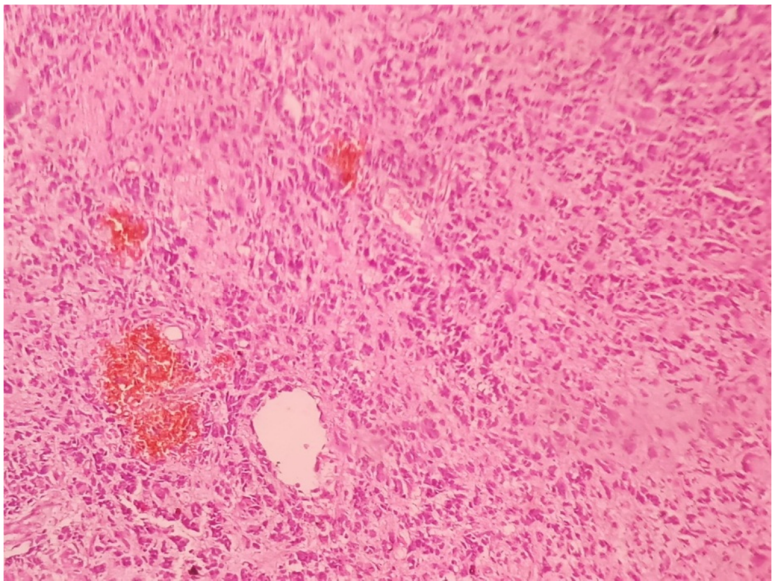


Figure 7-100x view of Anaplastic Astrocytoma

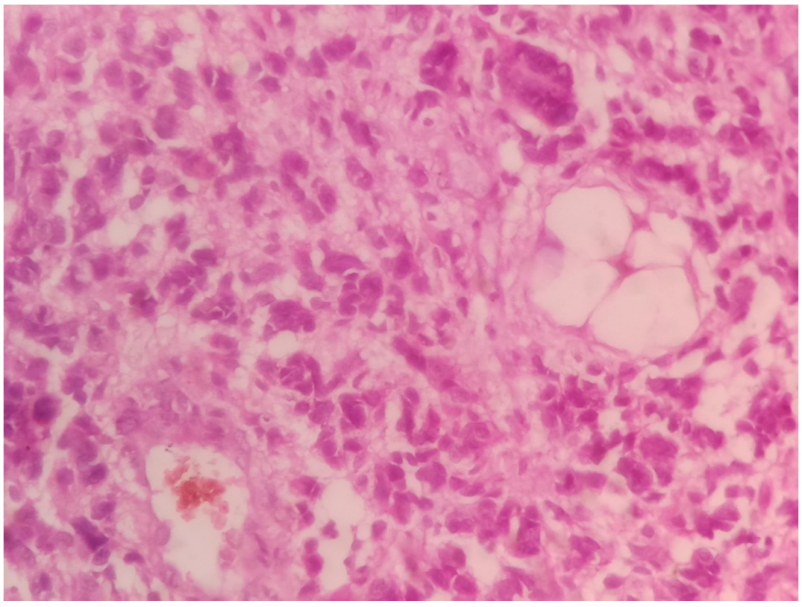


Figure 8-400x view of Anaplastic Astrocytoma

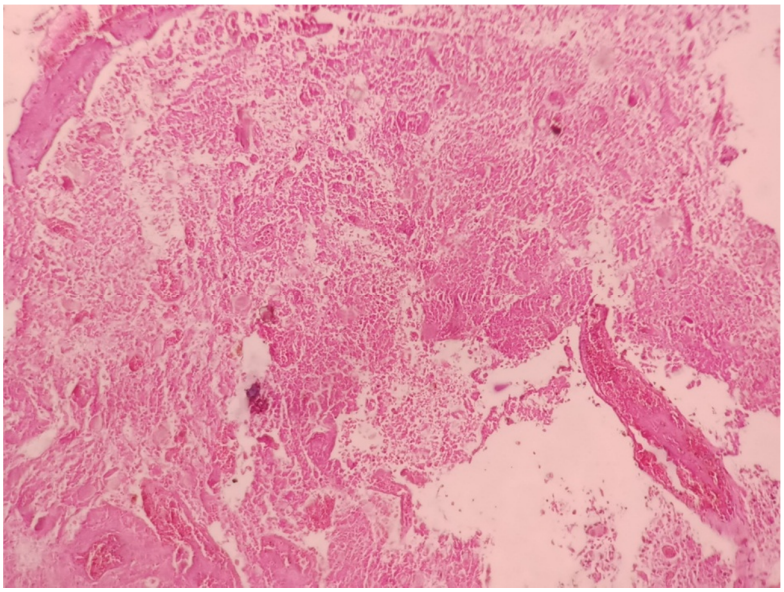


Figure 9-100x view of Glioblastoma-with areas of extensive necrosis

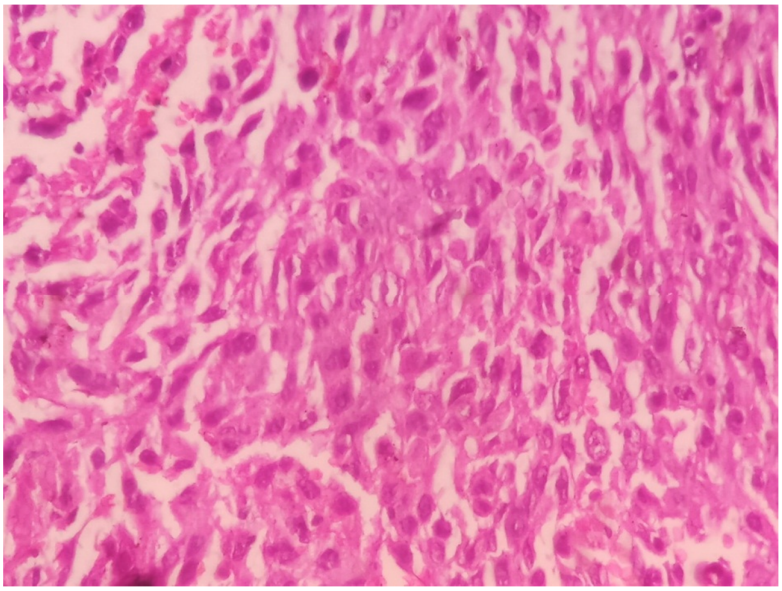


Figure 10-400x view of Glioblastoma

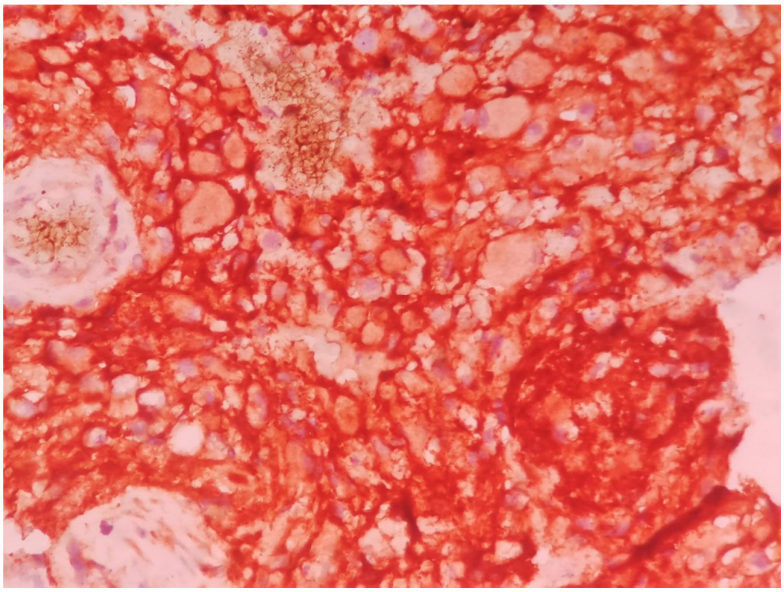


Figure 11-IHC-EGFR showing strong membranous positivity in Glioblastoma

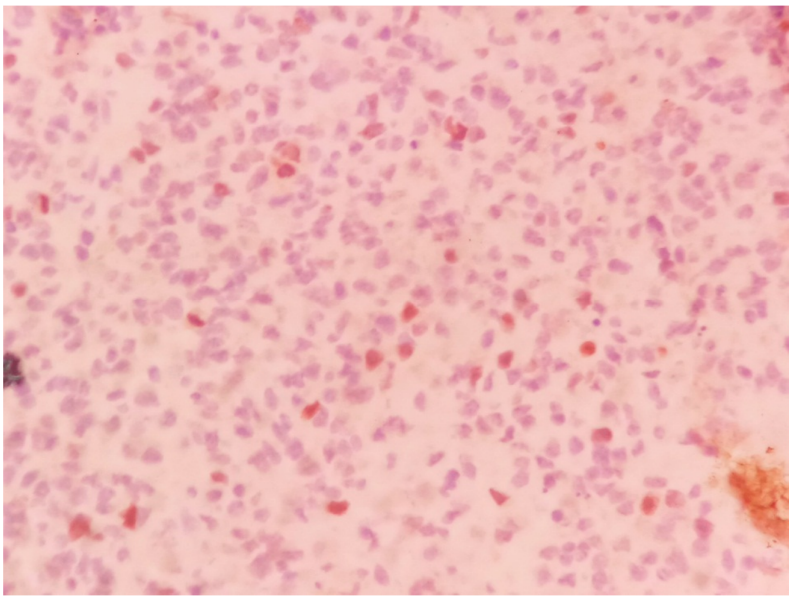


Figure 12-IHC-Ki 67 labelling index->5% positivity in Glioblastoma

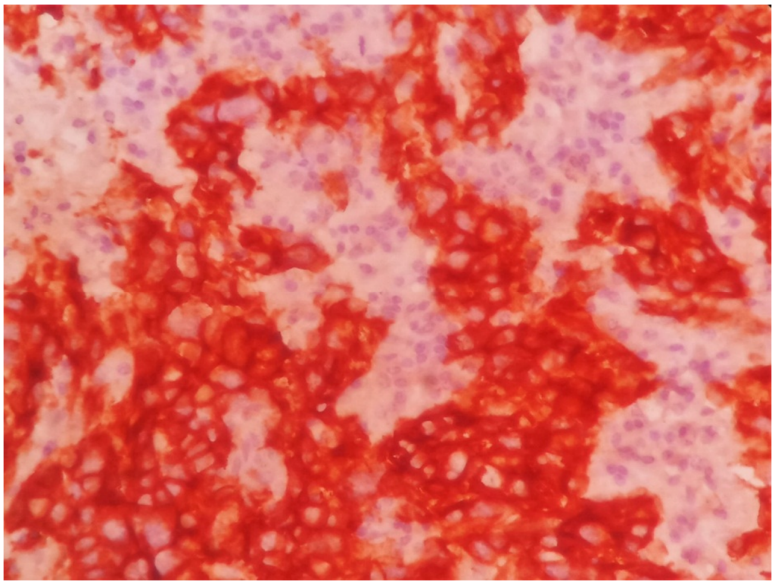


Figure 13-IHC-EGFR showing strong membranous positivity in Anaplastic Astrocytoma

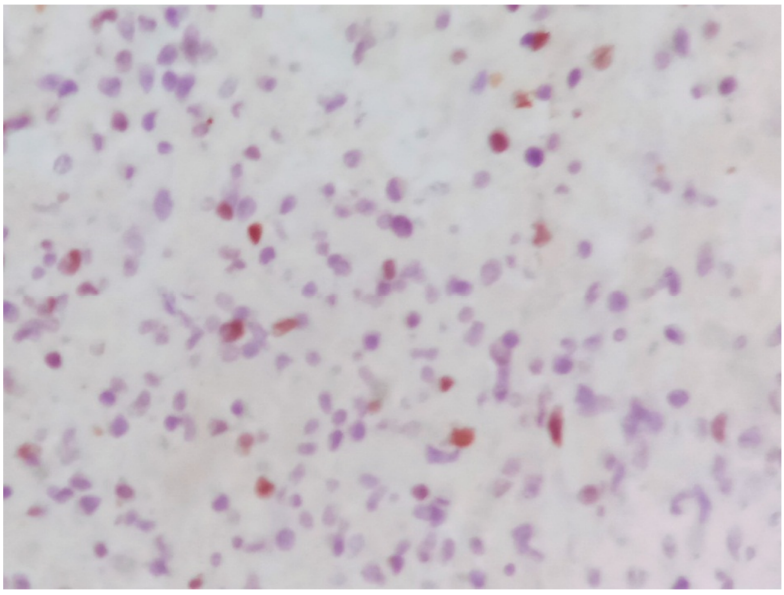


Figure 14-IHC-Ki 67 labelling index in anaplastic astrocytoma

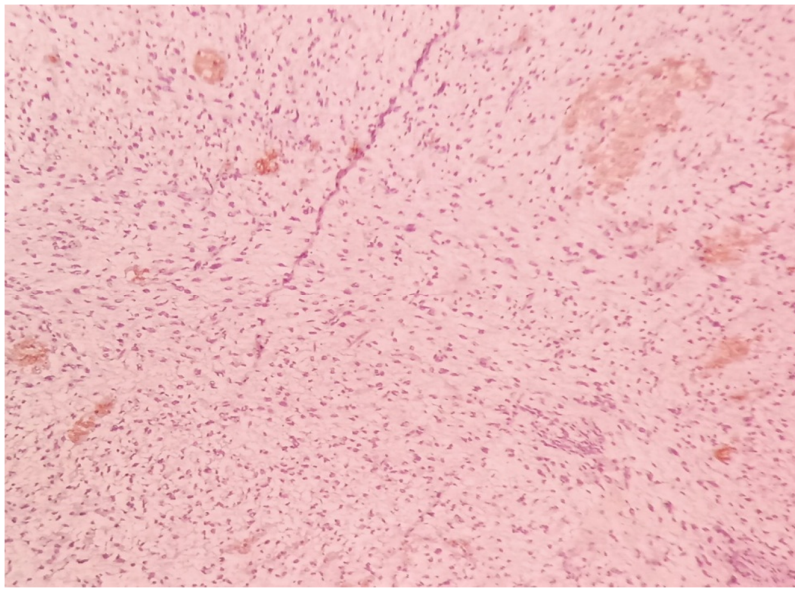


Figure 15-IHC- EGFR showing weak staining in Pilocytic Astrocytoma

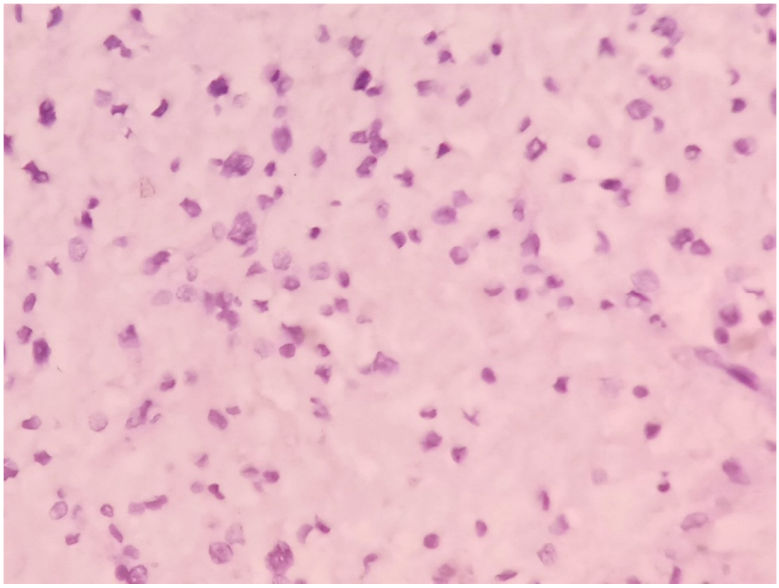


Figure 16-IHC of Ki 67-showing <5% positivity in Pilocytic Astrocytoma

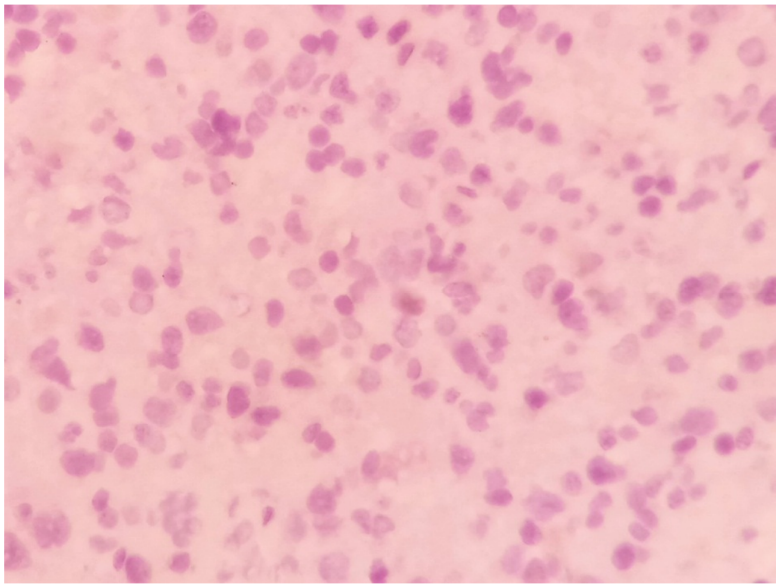


Figure 17-IHC of EGFR showing weak staining in Oligodendroglioma

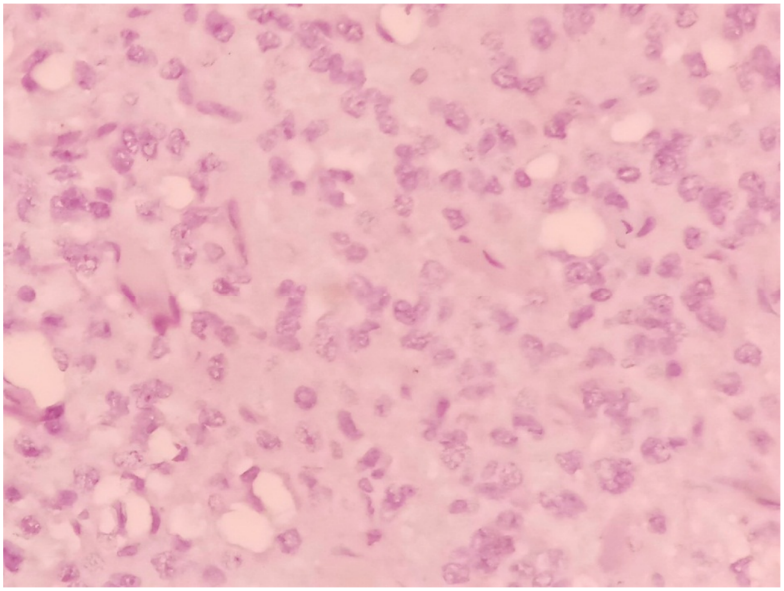


Figure 18-IHC of Ki 67-showing <5% positivity in Oligodendroglioma

Discussion

DISCUSSION

Gliomas are the most common primary brain tumors in adults[118]. They are associated with a poorer survival outcome.

In the present study, histo-morphological study was done for 181 cases of glioma which were received in Rajiv Gandhi Government General Hospital, which is a tertiary referral center, while immunohistochemical evaluation was done for 50 cases. An attempt was made to check the correlation between EGFR, Ki 67 and gliomas.

COMPARISON BETWEEN GENDER INCIDENCE OF GLIOMAS

In our study it was observed that the incidence of gliomas was increased in males, that is out of 181 cases, 105 cases (58%) were seen to occur in males, while 76 cases (42%) were seen to occur in females, this was seen to correlate with the study done by Vovorosa et al[129].

Table 23. Comparison between gender incidence of gliomas

	CURRENT STUDY	VOVOROSA ET AL
MALE	58%	55.6%
FEMALE	42%	45.4%

COMPARISON BETWEEN AGE INCIDENCE OF GLIOMAS-

In our study it was observed that the mean age of occurrence of gliomas was 37.7 years, this was in concurrence with the study conducted by X hu et al, where the mean age was 40.3 years. In this study 69% of the cases were within the age group 21-60 years, 21% of the cases were seen in <20 years of age, and the rest 10% were seen in the older age group. This was seen to concur with the study conducted by X Hu et al[118].

Table 24. Comparison between age incidence of gliomas

Age Distribution	X Hu et al	Our Study
<20	28%	21%
21-60	55%	69%
61-80	17%	10%

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COMPARISON OF HISTOPATHOLOGICAL SUBTYPES OF GLIOMAS-

In our study conducted for a period of two years, the distribution of the cases were as follows, the most commonly observed diagnosis was Glioblastoma, with a prevalence of 27%, followed by Diffuse Astrocytoma with a prevalence of 25.5%, Anaplastic Astrocytoma with a prevalence of 14.4%, Pilocytic Astrocytoma with 13.3% and Oligodendroglioma with 10.5%. Entities like Gliosarcoma, Myxopapillary Ependymoma, Clear cell ependymoma, Pleomorphic Xanthoastrocytoma are rare, and were associated with lower prevalence rates. These findings were in concordance with study of Larjavaara et al [130].

Table 25. Comparison between histopathological subtypes of gliomas

Diagnosis	Our study	Larjavaara et al
ANAPLASTIC ASTROCYTOMA	14.4%	9.4%
EPENDYMOMA	6.1%	3%
OLIGODENDROGLIOMA	10.5%	11%
PILOCYTIC ASTROCYTOMA	13.3%	5%
GLIOBLASTOMA	27.7%	47%
DIFFUSE ASTROCYTOMA	25.5%	14%

COMPARISON OF SIDE OF BRAIN INVOLVED IN GLIOMA-

In our study, the left hemisphere of brain was most commonly involved (45.3%), than the right side of the brain (29.3%). In the study conducted by Larjavaara et al, the right hemisphere was more commonly affected. This was probably because of variation in demographics of the mentioned study and our study[130].

Table 26. Comparison between the side of brain involved in gliomas

Side	Our study	Larjavaara et al
Left	45.3%	41%
Right	29.3%	50%
Midline	25.4%	9%

COMPARISON OF THE SITE OF OCCURENCE OF GLIOMA-

In our study, out of 181 cases, it was found that 24.3% of the cases had a predilection for frontal lobe. This was similar to the study by Larjavaara et al [130]. Temporal and parietal lobes are the next commonly affected lobes with 5.6% and 7.3% respectively. Occipital lobe was the least commonly affected lobe with only 0.6% of all the cases[130].

Table 27. Comparison between the site of occurrence of gliomas

Site	Our study	Larjavaara et al
Frontal	**24.3%**	**40%**
Temporal	5.6%	29%
Parietal	7.3%	14%
occipital	0.6%	3%
Cerebellum	11%	1.5%
Ventricle	1.7%	2.2%
Brainstem	1.1	4.1%

COMPARISON OF WHO GRADING OF GLIOMAS-

In our study, it was observed that WHO grade II tumors were of maximum incidence (43.1%), the second most common was Grade IV tumors with (28.2%), the third most common was Grade III with (14.9%), the fourth most common was Grade I tumors with (13.8%). In the study conducted by Larjavaara et al, Grade IV tumors showed the maximum incidence with 46.5%. The incidence of grade III and IV tumors was similar to our study [130].

Table 28. Comparison between WHO grading of gliomas

Grade	Our study	Larjavaara et al
Grade I	13.8%	5.13%
Grade II	43.1%	38.9%
Grade III	14.9%	9.36%
Grade IV	28.2%	46.5%

ANALYSIS OF IMMUNOHISTOCHEMICAL PARAMETERS

The expression of EGFR and Ki 67 was studied in different types of glioma. A subset of 50 cases constituting 25 cases of low grade (WHO Grade I and II) and 25 cases of high grade (WHO grade III and IV) representative of whole sample of 181 cases. The formalin fixed paraffin embedded sections of selected 50 cases was subjected to immunohistochemical analysis with EGFR and Ki 67.

In our study, the expression of Ki 67 was also seen to increase with increasing grades of glioma. In the scoring done for Ki 67, the scoring was assigned based on percentage of immunopositive cells per thousand cells. 26 out of 50 cases (52%) showed more than >5% positivity, with a significant p value of p=0.008.

The median value of Ki 67 positivity was 2 in Grade I, 2 in Grade II, 7.75 in Grade III and 23.5 in Grade IV in our study. On comparing the cumulative median values, it was observed that Grade III and IV showed higher Ki 67

positivity with a p value of 0.00011. This was in concordance with the study conducted by Skjulsvik AJ et al [131].

Table 29. Comparison between Ki 67 expression

Grade	Our Study			Skjulsvik AJ et al		
	Number of cases	**Median Ki67 value**	**Range**	**Number of cases**	**Average Median Ki67 value**	**Range**
I	9	2	2-47	21	1.45	0.7-8.5
II	16	2	2-20.2	110	3.9	0.5 -25.3
III	4	7.75	3-22.7	47	15.83	2.1-41.2
IV	21	23.5	2-74	89	19.4	2.2-80
Total	50			267		
p=0.00011				p<0.001		

COMPARISON OF EGFR POSITIVITY-

Among the 50 cases, EGFR expression was seen in 35 cases (70%), p value was found to be significant with (p=0.004) The expression was seen to be higher in high grade gliomas than in low grade gliomas and this was in correlation with the study conducted by X Hu et al and Richard Montogomery et al [118,132].

Table 30. Comparison between expression of EGFR

Grade	Our study	X Hu et al
Grade I	33.3%	22.2%
Grade II	56.2%	44.6%
Grade III	100%	71.2%
Grade IV	90.5%	88.6%

EGFR grading was done and the scoring was done based on percentage of immunopositive cells

- 0-25% - Score 1
- 26%-50% - Score 2
- 51%-75% - Score 3
- 76%-100% - Score 4

In our study, it was observed that EGFR staining increased with increasing grades of gliomas. In grade I gliomas it was seen that out of 9 cases, 6 cases (66.67%) were negative for EGFR staining, 2 cases (22.2%) showed Score 2. In Grade II, out of 16 cases, 7 cases (43.75%) were negative for EGFR and 5 cases (31.25%) showed score 1, 1 case (6.25%) showed score 2 and 3 cases (18.75%)showed score 4. In Grade III, out of 4 cases all showed positivity for EGFR, with all the cases showing strong positivity for EGFR with Score 4. In Grade IV, out of 21 cases, 2 cases (9.52%) showed Score 1, 3 cases (14.29%) showed score 3, and 12 cases (57.5%) showed strong positivity with score 4. The

above findings were in concurrence with the study of both X Hu et al and Richard Montogomery et al [118,132].

Table 31. Correlation between EGFR expression and WHO Grading of gliomas in our study

EGFR Result							
Grade	**Negative n (%)**	**Positive, n (%)**				**Total Positive, n (%)**	**Total Cases, N**
		EGFR Score					
		1+	**2+**	**3+**	**4+**		
I	6 (66.67)	0 (0)	2 (22.22)	0 (0)	1 (11.11)	3 (33.33)	9
II	7 (43.75)	5 (31.25)	1 (6.25)	0 (0)	3 (18.75)	9 (56.25)	16
III	0 (0)	0 (0)	0 (0)	0 (0)	4 (100)	4 (100)	4
IV	2 (9.52)	2 (9.52)	2 (9.52)	3 (14.29)	12 (57.15)	19 (90.48)	21

Table 32.Comparison between EGFR expression and WHO grading-study by x hu et al

GRADE	**IMMUNO NEGATIVE CASES**	**IMMUNOPOSITIVE CASES**			**TOTAL**
		+	++	+++	
I	7 (77.8%)	2 (22.2%)	0	0	2 (22.2%)
II	3 (55.4%)	15 (26.8%)	6 (10.7%)	4 (7.1%)	25 (44.6%)
III	15 (28.8%)	8 (15.4%)	17 (32.7%)	12 (23.1%)	37 (71.2%)
IV	4 (11.4%)	3 (8.6%)	10 (28.6%)	18 (51.4%)	31 (88.6%)

Table 33. Comparison between EGFR and Ki 67 positivity in our study

KI67_SCORE		EGFR		Total
		NEGATIVE	POSITIVE	
<5% NEGATIVE	Count	15	9	24
	% within EGFR	100.0%	25.7%	48.0%
>5% POSITIVE	Count	0	26	26
	% within EGFR	0.0%	74.3%	52.0%
Total	Count	15	35	50
	% within EGFR	100.0%	100.0%	100.0%

Pearson Chi-Square=23.214** P<0.001

In our study, a comparison and correlation between EGFR and Ki 67 was done, a total of 26 cases showed >5% positivity for Ki 67. All these 26 cases also showed positivity for EGFR (100%).

Of 35 cases that showed positivity for EGFR, 74.3% of the cases also showed positivity for Ki 67 and 25.7% of cases were negative for Ki 67.

This was found to be significant with a p value <0.001.

This correlation between EGFR and Ki 67 showed that in Grades III and IV, the expression of EGFR was strong and also associated with increase in the Ki 67 index, reflecting the mitotic activity of these higher-grade tumors. This correlation between these two markers was highly significant with a p value of<0.001.

Furthermore, a Sensitivity and Specificity analysis of EGFR was done. It showed a sensitivity of 74.29%(56.74-87.51%: -95% CI) and a Specificity of 100% (78.20%-100%).This showed a positive predictive value of 100%.

Summary

SUMMARY

- In the current study,181 cases were analysed histomorphologically while immunohistochemical evaluation was done for a subset of 50 cases with two markers, EGFR and Ki 67 respectively.
- The mean age of occurrence of gliomas was found to be 37.7 years, with peak incidence occurring in the age group of 21-60 years, while least incidence was seen in >80 years of age.
- There was seen to be a male preponderance with 58% of cases occurring in males as and when compared to females.
- Amongst the gliomas in our institute, the maximum number of cases were observed to be glioblastoma with 27.7%, followed by diffuse astrocytomas with 25%, the least commonly diagnosed gliomas were Ependymomas.
- The site of predilection for gliomas was found to be the Frontal lobe with 24.3% of cases occurring in this region, the occipital lobe showed the least occurrence of cases.
- The side of predilection was observed to be the left side with 45.3% of cases occurring on the left side.
- Maximum number of cases were falling under the category of WHO grade II, (43.1%) of cases, followed by WHO Grade IV, (28.1%)
- Immunohistochemical evaluation was done for a subset of 50 cases with EGFR and Ki 67. For EGFR,35 out of 50 cases (70%) showed positivity and it was also observed that the positivity increased with increasing grades of

glioma with the maximum positivity seen in Grades III and IV tumors. This association was found to be statistically significant.

- For Ki 67, 26 out of 50(52%) cases showed >5% labelling index and it was also observed that the labelling index increased as the grade of the tumor increased.
- This correlation between EGFR and Ki 67 showed that in Grades III and IV, the expression of EGFR was strong and also associated with increase in the Ki 67 index, reflecting the mitotic activity of these higher-grade tumors. This correlation between these two markers was highly significant with a p value of<0.001.
- Sensitivity and Specificity analysis of EGFR was done. It showed a sensitivity of 74.29%(56.74-87.51%: -95% CI) and a Specificity of 100%(78.20%-100%).This showed a positive predictive value of 100%.

Strengths of the study

STRENGTHS OF THE STUDY

- The study spans for a period of 2 years, done at a tertiary care hospital in Southern India
- The clinicopathological aspects of glioma-their incidence, age distribution, gender predilection, site of involvement has been studied extensively, which can add value to any future population based studies.
- There was seen to be a strong association of EGFR and Ki 67 with high grade gliomas, Grade III and IV in our study.
- Sensitivity and specificity analysis of EGFR was done, and it was found to be statistically significant.
- This study helps determine the quality of EGFR as a prognostic marker in glioma and is also useful in evaluation of prospective targeted therapy.

Limitations of the study

LIMITATIONS OF THE STUDY

- Study is hospital based, it does not reflect the true incidence and prevalence in the community.
- Few cases were lost to follow up.
- Immunohistochemical analysis could not be done for all cases due to economic restraints.

Conclusion

CONCLUSION

Th incidence of gliomas is on the rise and it is seen that the prognosis depends on numerous factors like age, site of occurrence. In our study it is seen that EGFR and Ki 67 expression increases with increasing grades of glioma. Thus, proving that these markers can be used to predict the behavior of these tumors and thereby the prognosis.

In this era of targeted therapy, EGFR inhibitors are being extensively researched upon and this study opens up realms on targeted therapy in high grade gliomas.

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Annexures

INFORMATION SHEET

We are conducting **"A STUDY ON EPIDERMAL GROWTH FACTOR RECEPTOR AND KI 67 EXPRESSION IN CORRELATION WITH GRADING OF GLIOMA"** among patients attending Government General Hospital, Chennai and for that your specimen may be valuable to us.

- The purpose of this study is to plan for additional treatment measures in certain cases of Brain tumors-Glioma easily with the help of certain special tests.
- We are selecting certain cases and if your specimen is found eligible, we may be using your specimen to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator

Signature of participant

Date:

ஆராய்ச்சி தகவல்தாள்

ஆராய்ச்சி தலைப்பு :

ஆய்வாளர் : மரு. **ஸ்நேஹா சுரேஷ்,**
நோய்குறியியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை-600003.

தங்களது மார்பக புற்றுநோய் கட்டி (அறுவை சிகிச்சை செய்யப்பட்டகட்டி) இங்கு பெற்றுக்கொள்ளப்பட்டது.

இராஜீவ்காந்தி அரசு பொதுமருத்துவமனைக்கு வரும் நோயாளிகளிடம் இருந்து பெறப்பட்டமார்பக புற்றுநோய் கட்டிகளைப் பற்றிய ஒரு ஆராய்ச்சி இங்கு நடைபெற்றுவருகின்றது.

இந்தமார்பகப் புற்றுநோய் கட்டிகளில் ER, PR வெளிப்பாட்டை FNAC முறையில் செய்த மெழுகுவிலும் திசுவில் செய்த மெழுகுவிலும் ஒப்பிட்டு ஆய்வு செய்தல். எனதுஆய்வின் நோக்கமாகும்.

நீங்களும் இந்தஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்தஆராய்ச்சியில் உங்களுடைய திசுக்களைஎடுத்து சில சிறப்புப் பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்குள்ளாகாது என்பதையும் தெரிவித்துக்கொள்கிறோம்.

முடிவுகளைஅல்லதுகருத்துகளைவெளியிடும் போதோஅல்லதுஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லதுஅடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்தஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான்இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்தஆராய்ச்சியில் இருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த சிறப்புப் பரிசோதனைகளின் முடிவுகளைஆராய்ச்சியின் போதுஅல்லதுஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்தஆய்வை பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர் : மரு. ஸ்நேகா சுரேஷ், செல் : 9790416762

பங்கேற்பாளர் கையொப்பம் .....இடம் : ..... தேதி : .....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் :

ஆராய்ச்சியாளர் கையொப்பம்.....இடம் : ..... தேதி : .....

INFORMED CONSENT FORM

Title of the study: **A STUDY ON EPIDERMAL GROWTH FACTOR RECEPTOR AND KI 67 EXPRESSION IN CORRELATION WITH GRADING OF GLIOMA**

Name of the Participant :
Name of the Principal(Co-Investigator) :
Name of the Institution : Madras Medical College
Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read tome). I was free to ask any questions and they have been answered. I am over 18 years of age and,exercising my free power of choice, hereby give my consent to be included as a participant in **"A STUDY ON EPIDERMAL GROWTH FACTOR RECEPTOR AND KI 67 EXPRESSION IN CORRELATION WITH GRADING OF GLIOMA".**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the resected breast tumors will be subjected to immunocytochemistry and immunohistochemistry examination.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understood that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:
Name and signature / thumb impression of the participant (or legal representative if participant incompetent)
Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

ஆராய்ச்சி ஒப்புதல்கடிதம்

ஆராய்ச்சி தலைப்பு :

சென்னை மருத்துவக் கல்லூரி நோய்குறியியல் துறையில் பயிலும் முதுகலை மருத்துவர் சு.கவிதா, அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில் பங்குகொள்ள ..... ஆகிய நான் முழு மனதுடன் சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்டவிஷயங்களை நான் புரிந்து கொண்டு நான்எனது சம்மதத்தைத்தெரிவிக்கிறேன்.

இந்தஆராய்ச்சியில் பிறரின் நிர்ப்பந்தமின்றிஎன் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான்இந்தஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால்எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

நான் மார்பகப்புற்றுநோய் கட்டி நோய்கள்குறித்தஇந்தஆராய்ச்சியின் விவரங்களைக்கொண்டதகவல்தாளைப் பெற்றுக்கொண்டேன்.

நான்என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன்இந்த மருத்துவஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்.

எனக்கு அறுவை சிகிச்சை செய்யப்பட்டு நோய்க்குறியியல் துறையில் சதைப் பரிசோதனைக்கு பயன்பட்டமெழுகுக்கட்டிகளைவைத்துஆராய்ச்சி மற்றும் சிறப்புப் பரிசோதனை செய்து கொள்ள சம்மதம் தெரிவிக்கிறேன்.

பங்கேற்பாளர் கையொப்பம் .....இடம் : ..... தேதி : .....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் :

ஆராய்ச்சியாளர் கையொப்பம்.....இடம் : ..... தேதி : .....

MASTER CHART – Statistics of Gliomas for the period of 2 years from Oct 2015 to Jun 2017

S No.	NP No.	AGE	GENDER	SIDE	SITE	MICROSCOPIC FEATURES						DIAGNOSIS	GRADE	IMMUNO-HISTO-CHEMISTRY			
						CELLULARITY	NP	MITOSIS	VP	NECROSIS	CALCIFICATION			EGFR	EGFR %	EGFR Score	Ki 67 %
1	006/17	25	F	R	F	3	2+	P	P	P		GBM	IV	P	77	4	3
2	008/17	14	M		BS	3	1+	A	A	A		ODG	II	P	10	1	<2
3	010/17	44	F		IDEM	3	1+	A	A	A		PA	I				
4	017/17	19	M	R	F	3	1+	A	A	A		GBM	IV				
5	100/16	65	M	L	F	3	2+	P	A	P		AA	III				
6	101/17	65	M	R	FP	3	2+	P	A	A		GBM	IV	P	87.5	4	10.5
7	104/16	12	M		Ce	3	1+	A	A	A	P	ODG	II	N	0	1	<2
8	105/16	30	F	L	TP	3	1+	A	A	A	P	DA	II				
9	106/17	46	M	L	FTP	3	2+	P	A	A		DA	II				
10	109/16	30	M		CC	3	1+	A	A	A		ODG	II				
11	110/16	45	F	L	P	3	2+	P	A	A		DA	II				
12	112/16	35	F	L	FT	3	2+	P	A	P		DA	II				
13	116/17	60	M	L	PO	3	2+	P	A	A		DA	II	P	88.7	4	22.7
14	119/16	59	M	R	F	3	1+	A	A	A	P	GBM	IV				
15	124/16	14	M		Ce	3	1+	A	A	A		ODG	II	N	0	1	<2
16	126/16	52	M		CC	3	1+	A	A	A		ODG	II				
17	126/17	15	M		Ce	3	1+	A	A	A	P	PA	I	P	60	1	20.2
18	130/16	12	F		Ce	3	1+	A	A	A	P	ODG	II	N	0	1	<2
19	131/17	30	M	R	F	3	2+	P	P	P		GBM	IV	P	71	3	63.75

S No.	NP No.	AGE	GENDER	SIDE	SITE	MICROSCOPIC FEATURES						DIAGNOSIS	GRADE	IMMUNO-HISTO-CHEMISTRY			
						CELLULARITY	NP	MITOSIS	VP	NECROSIS	CALCIFICATION			EGFR	EGFR %	EGFR Score	Ki 67 %
20	138/17	18	M	L	P	3	2+	P	A	A		DA	II	P	79.5	4	5
21	139/17	55	M	L	PO	3	2+	P	P	P		DA	II	P	86	4	39
22	141/17	48	M	L	TP	3	2+	P	P	P		DA	II	P	79.5	4	3
23	144/16	35	F	L	Ce	3	1+	A	A	A		AA	III	P	24	2	8
24	149/16	42	M	L	P	3	1+	A	A	A		DA	II				
25	150/17	29	M	L	PO	3	1+	A	A	A		DA	II				
26	154/16	22	F		Ce	3	1+	A	A	A		PA	I				
27	156/16	32	M	L	F	3	1+	A	A	A		AA	III				
28	157/16	36	F	R	F	3	1+	A	A	A		GBM	IV				
29	157/17	32	F	L	FP	3	1+	A	A	A		AA	III				
30	159/17	50	M		Su Se	3	2+	P	P	P		PAFA	II	P	73	3	7
31	167/16	60	F	L	FP	3	1+	A	A	A		AA	III				
32	169/17	67	M	R	TP	3	2+	P	P	P		GBM	IV				
33	170/16	46	M		F	3	1+	A	A	A		ODG	II				
34	172/16	43	M	L	F	3	1+	A	A	A	A	DA	II				
35	176/15	24	F	L	FP	3	2+	P	P	A		AA	III				
36	177/15	60	M	L	TP	3	1+	A	A	A		EPEN	II				
37	179/17	53	F	L	PO	3	1+	A	A	A		DA	II				
38	180/15	29	F	R	P	3	2+	P	P	P		GBM	IV				
39	183/17	48	M	L	FTP	3	2+	P	P	P		AA	III	P	81.5	4	73.5
40	185/15	51	F	R	F	3	2+	A	A	A		GBM	IV				

S No.	NP No.	AGE	GENDER	SIDE	SITE	MICROSCOPIC FEATURES						DIAGNOSIS	GRADE	IMMUNO-HISTO-CHEMISTRY			
						CELLULARITY	NP	MITOSIS	VP	NECROSIS	CALCIFICATION			EGFR	EGFR %	EGFR Score	Ki 67 %
41	187/17	48	M	R	TP	3	2+	P	P	P		GBM	IV				
42	199/15	45	F	L	Th	3	1+	A	A	A		AA	III				
43	199/16	60	F		CC	3	1+	A	A	A		ODG	II				
44	200/16	2	F		IV	3	1+	A	A	A		ODG	II	P	86	4	<2
45	201/15	17	F		IDEM	3	2+	A	A	A		PA	I				
46	203/17	18	M	L	TP	3	2+	P	P	P		DA	II				
47	205/15	48	M	L	Th	3	2+	P	P	P		DA	II				
48	212/15	33	F		DL	3	2+	P	P	A		PA	I				
49	215/16	10	F	R	BS	3	1+	A	A	A		GBM	IV				
50	219/16	65	M	R	T	3	1+	A	A	A		GBM	IV				
51	221/15	25	M	L	F	3	2+	P	P	A		DA	II				
52	221/16	47	M	L	F	3	2+	P	A	A		AA	III	P	88	4	74
53	222/15	45	F	R	Th	3	2+	A	A	A		GS	IV				
54	228/15	57	M		CC	3	2+	P	P	P		PA	I				
55	229/16	54	F	R	T	3	1+	P	P	A		GBM	IV				
56	230/17	12	M		Po Fo	3	1+	A	A	A		PA	I	P	13.77	1	<2
57	231/15	27	F		CC	3	2+	P	P	P		PA	I				
58	233/15	34	M	R	FP	3	2+	A	A	A		GBM	IV				
59	235/16	13	M		Po Fo	3	1+	A	A	A		PA	I	P	26	2	17.4
60	243/16	22	M	L	PO	3	2+	A	P	P		DA	II	P	86	4	49

S No.	NP No.	AGE	GENDER	SIDE	SITE	MICROSCOPIC FEATURES						DIAGNOSIS	GRADE	IMMUNO-HISTO-CHEMISTRY			
						CELLULARITY	NP	MITOSIS	VP	NECROSIS	CALCIFICATION			EGFR	EGFR %	EGFR Score	Ki 67 %
61	245/15	35	M	L	F	3	2+	A	A	A		EPEN	II				
62	245/16	58	F	L	P	3	2+	P	P	P		EPEN	II				
63	25/17	35	F	L	F	3	1+	A	A	A		AA	III				
64	250/16	14	M		Po Fo	3	1+	A	A	A		PA	I	P	88	4	15
65	258/16	16	M		T	3	1+	A	A	A		PA	I				
66	266/16	55	M	R	F	3	2+	A	A	A		GBM	IV				
67	269/15	30	M	L	F	3	2+	A	A	A		EPEN	II				
68	278/16	48	M		F	3	2+	A	P	P		ODG	II				
69	279/15	40	M	L	P	3	2+	P	P	A		DA	II				
70	28/17	57	F	L	T	3	2+	P	P	A		DA	II				
71	281/16	40	M	R	F	3	A	A	A	A		GBM	IV				
72	287/16	70	M	L	FT	3	2+	P	P	P		DA	II				
73	288/16	35	F	R	On	3	1+	A	A	A		GBM	IV				
74	290/16	53	F	L	F	3	2+	P	P	P		CCEP	III	P	93.5	4	61.5
75	295/15	25	F	L	TP	3	2+	A	A	A		EPEN	II				
76	303/16	24	F		Ce	3	1+	A	A	A		PA	I				
77	304/16	61	M	L	TP	3	2+	P	P	P		DA	II				
78	318/16	39	M	L	FP	3	1+	A	A	A		AA	III				
79	327/16	28	M	R	TP	3	2+	P	P	P		GBM	IV				
80	334/16	8	M	L	Ce	3	1+	A	A	A		AA	III				

S No.	NP No.	AGE	GENDER	SIDE	SITE	MICROSCOPIC FEATURES						DIAGNOSIS	GRADE	IMMUNO-HISTO-CHEMISTRY			
						CELLULARITY	NP	MITOSIS	VP	NECROSIS	CALCIFICATION			EGFR	EGFR %	EGFR Score	Ki 67 %
81	336/16	42	M	L	F	3	2+	P	P	P		EPEN	II				
82	339/15	60	M	L	P	3	2+	P	P	P		EPEN	II				
83	348/16	30	F	R	F	3	1+	A	A	A		GBM	IV				
84	35/17	55	F	R	T	3	2+	P	P	A		GBM	IV				
85	352/16	6	M	L	Th	3	1+	A	A	A		DA	II				
86	362/16	19	F	L	T	3	1+	A	A	A		DA	II	N	0	1	<2
87	364/15	21	F	R	FTP	3	2+	A	A	A		GBM	IV				
88	366/16	2	F	R	On	3	1+	A	A	A		GBM	IV				
89	369/15	27	M	R	PO	3	2+	P	P	A		GBM	IV				
90	380/16	30	M	R	F	3	1+	A	A	A		GBM	IV				
91	381/15	65	M	L	FP	3	2+	P	P	P		DA	II				
92	383/16	8	M	L	FP	3	1+	A	A	A		AA	III				
93	385/16	40	F	L	F	3	1+	A	A	A		DA	II				
94	387/15	40	M		FTP	3	2+	A	A	A		PA	I				
95	388/15	46	M		CC	3	2+	A	A	A		PA	I				
96	393/15	30	F		F	3	2+	P	P	P		ODG	II				
97	4 0 5	34	M		IV	3	1+	A	A	A		ODG	II				
98	402/16	14	M		Ce	3	1+	A	A	A	A	PA	I				
99	407/15	75	M		CC	3	2+	A	A	A		PA	I				
100	408/15	60	F	L	F	3	2+	P	P	P		EPEN	II				

S No.	NP No.	AGE	GENDER	SIDE	SITE	MICROSCOPIC FEATURES						DIAGNOSIS	GRADE	IMMUNO-HISTO-CHEMISTRY			
						CELLULARITY	NP	MITOSIS	VP	NECROSIS	CALCIFICATION			EGFR	EGFR %	EGFR Score	Ki 67 %
101	41/17	51	M		Th	3	2+	P	P	P		PXA	II	P	48	2	5
102	417/15	25	F	R	TP	3	2+	P	P	A		EPEN	II				
103	430/16	33	F	L	PaSe	3	1+	A	A	A		DA	II				
104	432/16	55	F	L	FT	3	2+	P	P	P		DA	II	P	76.3	4	54.7
105	434/16	62	M		CC	3	2+	P	P	P		ODG	II	N	0	1	<2
106	435/15	33	M	R	FP	3	2+	A	A	A		GBM	IV				
107	435/16	17	F		Ce	3	1+	A	A	A		PA	I				
108	437/15	40	F	R	PO	3	1+	A	A	A		GBM	IV				
109	438/15	25	M	L	F	3	2+	P	P	A		DA	II				
110	444/16	15	F	R	F	3	1+	A	A	A		GBM	IV				
111	450/15	67	F	R	FP	3	1+	A	A	A		GBM	IV				
112	46/17	60	M	R	T	3	1+	A	A	A		GBM	IV				
113	465/15	30	M	L	T	3	2+	A	A	A		DA	II				
114	466/16	52	F	L	P	3	1+	A	A	A		DA	II				
115	469/15	27	F	R	P	3	2+	P	P	A		GBM	IV				
116	471/15	60	M	L	Th	3	2+	A	A	A		DA	II				
117	477/15	40	M	R	TP	3	2+	P	P	P		ODG	II				
118	477/16	31	M	L	F	3	1+	A	A	A	P	DA	II	N	0	1	<2
119	48/17	40	M	R	F	3	1+	A	A	A		GBM	IV				
120	488/16	45	M	R	FP	3	2+	P	P	P		GBM	IV	P	83	4	7.4

S No.	NP No.	AGE	GENDER	SIDE	SITE	MICROSCOPIC FEATURES						DIAGNOSIS	GRADE	IMMUNO-HISTO-CHEMISTRY			
						CELLULARITY	NP	MITOSIS	VP	NECROSIS	CALCIFICATION			EGFR	EGFR %	EGFR Score	Ki 67 %
121	491/15	50	F	R	TP	3	2+	A	A	A		MPEP	I				
122	494/16	38	M	L	FTP	3	2+	A	A	A		DA	II	P	20	1	<2
123	497/16	40	M	L	T	3	1+	A	A	A		DA	II				
124	506/15	56	M	R	F	3	2+	P	P	A		GBM	IV				
125	507/15	40	M	L	TP	3	2+	P	P	A		AA	III				
126	507/16	23	M	L	F	3	1+	A	A	A		AA	III				
127	513/16	12	M	R	F	3	1+	A	A	A		GBM	IV				
128	523/16	33	M	L	TP	3	1+	A	A	A		EPEN	II				
129	527/15	62	M	L	FTP	3	2+	P	P	P		AA	III				
130	528/16	52	M	L	T	3	2+	P	P	A		EPEN	II	P	89.7	4	32
131	53/17	13	F		BS	3	1+	A	A	A		ODG	II	P	47	2	<2
132	534/16	50	F	L	TP	3	2+	P	P	P		DA	II				
133	535/16	67	M	L	F	3	2+	A	P	A		AA	III				
134	538/16	32	M	L	F	3	1+	A	A	A		AA	III				
135	539/16	47	F	R	F	3	2+	A	P	A		GBM	IV				
136	547/16	60	F	L	F	3	2+	P	P	P		DA	II	P	95%	4	49.5
137	548/16	61	F	R	P	3	2+	P	P	P		GBM	IV				
138	55/17	58	F	L	P	3	1+	A	A	A		DA	II				
139	56/17	38	M	R	F	3	2+	A	P	A		GBM	IV				
140	560/16	25	F		Po Fo	3	1+	A	A	A		PA	I	P	24	1	6.5

S No.	NP No.	AGE	GENDER	SIDE	SITE	MICROSCOPIC FEATURES						DIAGNOSIS	GRADE	IMMUNO-HISTO-CHEMISTRY			
						CELLULARITY	NP	MITOSIS	VP	NECROSIS	CALCIFICATION			EGFR	EGFR %	EGFR Score	Ki 67 %
141	565/16	7	F		Ce	3	1+	A	A	A		PA	I				
142	568/16	37	M	L	F	3	1+	A	A	A		AA	III				
143	569/16	45	M	L	F	3	2+	P	P	P		DA	II	P	17.8	1	<2
144	579/16	50	F	L	F	3	1+	A	A	A		AA	III				
145	58/17	60	F	R	T	3	2+	P	P	P		GBM	IV				
146	590/17	13	F	L	F	3	1+	A	A	A		AA	III				
147	597/16	13	M	R	Ce	3	1+	A	A	A		GBM	IV				
148	598/16	17	M	R	TP	3	1+	A	A	A	P	GBM	IV	N	0	1	<2
149	60/17	58	M	R	F	3	2+	P	A	A		GBM	IV				
150	601/16	47	F	L	FP	3	2+	P	A	A		AA	III				
151	612/16	35	M	L	PO	3	2+	P	P	P		DA	II	P	85	4	23.5
152	614/16	40	F	L	P	3	1+	A	A	A		DA	II				
153	616/16	14	F	L	Ce	3	1+	A	A	A	P	AA	III	N	0	1	<2
154	619/16	66	F	L	FP	3	2+	P	P	P		AA	III	P	58.5	3	16.3
155	625/16	37	F		Po Fo	3	1+	AA	A	A		PA	I	N	0	1	<2
156	630/16	7	M	L	F	3	1+	A	A	A		DA	II	N	0	1	<2
157	631/16	47	F	R	F	3	1+	A	A	A		GBM	IV				
158	633/16	15	F		Ce	3	1+	A	A	A		PA	I	N	0	1	<2
159	636/16	2	M	R	Ce	3	1+	A	A	A		GBM	IV	N	0	1	<2
160	645/16	9	M		Ce	3	2+	A	A	A		PA	I				

S No.	NP No.	AGE	GENDER	SIDE	SITE	MICROSCOPIC FEATURES						DIAGNOSIS	GRADE	IMMUNO-HISTO-CHEMISTRY			
						CELLULARITY	NP	MITOSIS	VP	NECROSIS	CALCIFICATION			EGFR	EGFR %	EGFR Score	Ki 67 %
161	650/16	10	M		Po Fo	3	1+	A	A	A		PA	I				
162	66/17	16	F		IV	3	1+	A	A	A		PA	I	N	0	1	<2
163	70/16	45	M	R	O	3	2+	P	A	A		GBM	IV				
164	71/16	42	F	L	TP	3	2+	A	A	A		DA	II				
165	72/16	65	M	R	TP	3	2+	P	P	P		GBM	IV				
166	73/16	28	F		Ce	3	1+	A	A	A		ODG	II	P	95.1	4	47
167	74/17	52	M	R	F	3	2+	P	A	A		GBM	IV				
168	75/17	50	F	R	F	3	2+	P	A	A		GBM	IV				
169	76/17	27	M	L	F	3	1+	A	A	A		DA	II	P	75	4	5.3
170	77/17	65	M	R	F	3	2+	P	P	P		GBM	IV	P	89	4	43
171	80/17	45	M		CC	3	1+	A	A	A		ODG	II				
172	82/16	55	F	L	TP	3	2+	A	A	P		DA	II				
173	87/16	50	M	L	FT	3	1+	A	A	A		DA	II				
174	87/17	60	M	R	PO	3	2+	P	P	P		GBM	IV	P	10	1	<2
175	90/16	12	F		Ce	3	1+	A	A	A		ODG	II	N	0	1	<2
176	92/16	43	F		CC	3	2+	P	P	P		ODG	II				
177	93/16	62	M	L	P	3	2+	A	A	P		DA	II				
178	94/17	47	F	L	CC	3	1+	A	A	A		DA	II				
179	96/16	27	M	L	Ce	3	2+	A	A	A		AA	III				
180	97/17	7	M	L	Ce	3	1+	A	A	A		AA	III				
181	98/17	72	F	R	FP	3	2+	P	P	P		GBM	IV	P	27	2	5

KEY TO THE MASTER CHART

- Age-in years
- Sex-M-male, F-female
- Side-Right-R,left-L
- Site -

F	Frontal
FP	Frontoparietal
FT	Frontotemporal
FTP	Frontotemporoparietal
T	Temporal
TP	Temporoparietal
P	Parietal
O	Occipital
PO	Parieto occipital
CC	Corpus callosum
On	Optic nerve sol
IDEM	Intradural extramedullary
IV	Intraventricular
Po Fo	Posterior fossa
Th	Thalamus
Ce	Cerebellum
BS	Brainstem
Pa Se	Parasellar

CELLULARITY-

1-LOW

2-MODERATE

3-HIGH

NP-NUCLEAR PLEOMORPHISM

1+-MILD NUCLEAR PLEOMORPHISM

2+-MARKED NUCLEAR PLEOMORPHISM

MITOSES-

A-ABSENT

P-PRESENT

VASCULAR PROLIFERATION-VP

A-ABSENT

P-PRESENT

NECROSIS-NEC

A-ABSENT

P-PRESENT

CALCIFICATION-CA

A-ABSENT

P-PRESENT

MICROSCOPIC DIAGNOSIS-

PA-Pilocytic Astrocytoma

PA with FA-Pilocytic Astrocytoma with focal anaplasia

EPEN-Ependymoma

CCEP-Clear cell ependymoma

MPEP-Myxopapillary Ependymoma

DA-Diffuse Astrocytoma

PXA-Pleomorphic Astrocytoma

ODG-Oligodendroglioma

AA-Anaplastic Astrocytoma

GBM-Glioblastoma multiforme

GS-Gliosarcoma

IHC-

EGFR-POS-positive

NEG-negative

EGFR Scoring-Percentage of stained cells

1-0-25%

2-26%-50%

3-51%-75%

4-76%-100% [132]