HISTOPATHOLOGICAL STUDY OF GLIOMAS AND EVALUATION OF IDH – 1 EXPRESSION IN GLIOBLASTOMA BY IMMUNOHISTOCHEMISTRY

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CERTIFICATE FROM THE DEAN

The is to certify that the dissertation entitled, "**HISTOPATHOLOGICAL STUDY OF GLIOMAS AND EVALUATION OF IDH – 1 EXPRESSION IN GLIOBLASTOMA BY IMMUNOHISTOCHEMISTRY"** submitted by Dr. J. Niveditha to the Faculty of Pathology, The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the reward of M. D. Degree in Pathology is a bonafide work carried out by her during the period 2017 – 2019.

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DECLARATION BY CANDIDATE

I, Dr.J.Niveditha, solemnly declare that the dissertation titled, "**HISTOPATHOLOGICAL STUDY OF GLIOMAS AND EVALUATION OF IDH–1 EXPRESSION IN GLIOBLASTOMA BY IMMUNOHISTOCHEMISTRY"** is a bonafide work done by me at Department of Pathology, Madurai Medical College & Government Rajaji Hospital, Madurai, during the period July 2017 – July 2019.

I also declare that this bonafide work or a part of this work was not submitted by me or any other for any reward, degree and diploma to any University, board either in India or abroad.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, towards partial fulfillment of the requirement for the reward of M. D. Degree in Pathology.

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INTRODUCTION

INTRODUCTION

The tumors of the central nervous system are an infrequent occurrence, amounting to less than 2% of all malignancies. They affect the young adult population predominantly, thus leading to increased global disease burden which causes a major effect on the disease adjusted life years, compared to other tumors. Gliomas are the most common malignant primary brain tumors, accounting for 80% of all primary malignant tumors of the brain.

The revamped WHO 2016 classification of CNS tumors is mainly a revised edition of the previous 2008 counterpart. This latest edition has incorporated a sea of change in the approach to CNS tumors, especially gliomas. A molecular signature, namely Isocitrate dehydrogenase (IDH) has been assigned to these gliomas that help in their diagnosis and prognostication. Accordingly all diffusely infiltrating gliomas (Grades II –IV) have been grouped together based on histopathological assessment and IDH – (Isocitrate dehydrogenase) mutation status.

Recent studies state that the presence of an IDH mutation is an indicator of better prognosis with evidence of increased overall and progression free survival. This is due to to the fact that, IDH mutation increases the chemotherapeutic and radiotherapeutic sensitivity of the gliomas. Also their accurate ability to detect even a single neoplastic astrocyte helps in establishing a specific diagnosis in morphologically ambiguous cases. This further helps in identifying infiltrating and non infiltrating gliomas as IDH is negative in the latter. The surgical margins in resection of gliomas are of utmost importance as recurrence spells doom in the

patients. IDH mutant gliomas can be resected almost completely compared to the IDH negative ones, as the infiltrating margins are accurately delineated in the former. Glioblastomas are of two types – primary (de novo) that arise as grade IV neoplasms and secondary, that arises from a low grade glioma. Presence of IDH mutation differentiates a primary from secondary glioblastoma as it is predominantly positive in secondary type. This further adds on to the prognostic value of IDH by specifically detecting secondary glioblastomas that typically occur in younger patients compared to IDH negative (wild type) primary glioblastomas.

Thus their preferential age, location and an unique molecular variable that serves as an independent diagnostic, prognostic and predictive marker makes the IDH mutant gliomas rich in research potential. This study therefore aims to determine the frequency of occurrence of gliomas ,glioblastomas and their types.

By analyzing the distribution of IDH mutant and wild type glioblastomas we aim to study and correlate their frequency, specific age and site dependence. This can provide ground for novel treatment strategies targeting IDH mutations like IDH inhibitors in the near future.

AIMS AND OBJECTIVES

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To study the frequency of occurrence of gliomas in specimens received from Government Rajaji hospital, Department of Neurosurgery Madurai to the Department of Pathology, Madurai Medical College.

To analyse the age,sex and location wise distribution of gliomas of patients studied.

To study the histopathological classification of gliomas.

To analyse the expression of immunohistochemical marker Isocitrate dehydrogenase (IDH -1) in glioblastomas

To study the frequency of occurrence of primary and secondary glioblastomas.

To study the frequency of occurrence of EGFR mutation in primary glioblastomas

REVIEW OF LITERATURE

REVIEW OF LITERATURE

FUNCTIONAL ANATOMY AND EMBRYOLOGY

The nervous system has two major divisions, the central nervous system (CNS) and the peripheral nervous system (PNS). Central nervous system is one of the organ systems known for its unparalleled structural and functional complexity. The histomorphology of the CNS is composed predominantly of grey matter and white matter.

The CNS consists of the brain, spinal cord, optic nerve and retina, and contains the majority of neuronal cell bodies[1]. The PNS consists of the cranial and spinal nerves, the peripheral autonomic nervous system (ANS) and the special senses (taste, olfaction, vision, hearing and balance). It is composed mainly of the axons of sensory and motor neurons that pass between the CNS and the body. The ANS is subdivided into sympathetic and parasympathetic components[1].

The brain resides insides the cranium and is composed of two cerebral hemispheres, midbrain, cerebellum, pons and the medulla oblongata[1]. Each cerebral hemisphere is divided into six lobes: frontal, parietal, occipital, temporal, insular and limbic lobes with varying depths of sulci and gyri.

Nervous system develops from neural plate, which is derived from the ectoderm. The brain weighs 800g at birth and about 1400 g in the adult[1]. The primary brain vesicles namely forebrain (prosencephalon), midbrain (mesencephelon) and hindbrain (rhombencephalon) give rise to the various parts of brain-Telencephalon (cerebral hemispheres, corpus striatum), Diencephalon (Thalamus, hypothalamus),

Mesencephalon (Cerebral peduncles, Tegmentum, Tectum), Rhombencephalon - Myelencephalon (Medulla oblongata) and Metencephalon (Pons, Cerebellum).The fetal cerebral hemispheres with emerging sulci and gyri begin their development at 21 weeks of gestation and attain the surface topography of an adult brain by 40 weeks[1].

HISTOLOGY OF BRAIN:

Grey matter is made up of neuronal cells in a neuropil background which is an intimately interwoven textured eosinophilic material containing neuronal and glial processes[2]. White matter is composed of myelinated axons and oligodendroglial cells that are required for production and maintenance of myelin sheaths. The four principle types of supporting cells of CNS *i.e*, glial cells are – astrocytes, oligodendrogliocytes, ependymal cells and microglia[2].

Astrocytes – fibroblasts of CNS

These are highly branched cells that provide mechanical support, mediate metabolite exchange between neurons and blood vessels and also help in repair of the neural tissue. Their increase in size (hypertrophy) and number (hyperplasia) termed as reactive astrocytosis in cases of CNS injuries, is a close mimic to many low grade diffuse astrocytomas requiring the help of immunohistochemistry for distinction.

Oligodendrocytes

These are cells with small round nuclei and a perinuclear halo (fried egg appearance) that is a distinctive and diagnostically useful feature. Their affinity to neuronal perikarya as they aggregate around myelinated axons is called perineuronal satellitosis .

Ependymal cells:

These are ciliated cuboido – columnar epithelial cells lining the ventricular system and choroid plexus.

Microglia:

They form the monocyte – macrophage system of CNS, small and inconspicuous with rod shaped nuclei.

Historic background:

The diagnosis of brain tumors delve deep into the psyche of the person receiving and delivering the diagnosis. The various types of brain tumors display a bewildering array of histological variants that has led to incorporation of molecular signatures into their classification. Historically, the first classification of brain tumors was based on the early writings of the nineteenth century by Bailey and Cushing (1926) who highlighted the histology, morphology, location and prognosis after surgery of the tumors described[3]. Their descriptions of oligodendroglioma and medulloblastoma still holds good. They proposed the medullary epithelium to be the origin of all tumors and further classified tumors according to the lineage of differentiation. Later, Pío Del Río Hortega (1933) pioneered the most exhaustive and expansive classification of central nervous tumors with the aid of silver staining techniques[3]. His classification was based on cytomorphology and embryologic characteristics of tumors rather than their anatomic location. His description of the oligodendrocyte nucleus as 'round' and the perinuclear halo of oligodendroglioma is still relevant. After Rio Hortega, Zurich (1965) proposed a classification that incorporated clinical history and prognosis

followed by the first WHO classification in 1979[3]. This broadly divided the brain tumors under the headings –

(1) Tumors of neuroepithelial tissues

(2) Meningeal tumors

(3) Tumors of nerve sheath cells

(4) Primary cerebral lymphoma.

(5) Tumors arising in blood vessels

(6) Germ cell tumors

(7) Metastatic tumors.

(8) Malformative tumors and tumor-like lesions

(9) Local extensions from regional tumors

(10) Tumors of the anterior pituitary

(11) Unclassified tumors.

Later three more editions succeeded this classification, all of them incorporating novel techniques like immune histochemistry, in situ hybridization and molecular genetics methods. The WHO 2016 classification of CNS tumors is the latest successor and path breaker that employs molecular techniques in diagnosis and prognosis of gliomas.

Gliomas – nomenclature and origin:

Gliomas with their gross resemblance to normal brain tissue were known by several names. The English termed it “medullary sarcoma” (Abernethy, 1804), while the French called it “encephaloide” and the German authors knew it as "fungus

medullare."[4] The name “glioma” originated in 1865 by virtue of Virchow who was the first to study it macroscopically and microscopically[5]. He divided the brain tumors as glioma and sarcoma. He described glioma – as which grossly resembled a hypertrophied brain and microscopically was composed of proliferation of glial fibers which points to the present day –astrocytoma and sarcoma as a grossly spherical tumor with definite boundaries that microscopically had increased cellularity, hemorrhage leading to apoplectic clinical evolution which inexplicably points to Glioblastoma.Thus the descriptions of Virchow done in 1865[6] although crippled by lack of histological techniques paved the way for glioma research 50 years from then on.

Classification of gliomas:

Gliomas are classified based on the differentiation they exhibit – astrocyte, oligodendrocyte or ependymal cell. Astrocytic tumors are graded based on the degree of malignancy into four grades – (I-IV),oligodendroglial and ependymal tumors are of grades (II –III).The **St. Anne-Mayo grading system**, also referred to as the **Daumas-Duport grading system**, introduced by **Catherine Daumas-Duport**, a French pathologist in 1988 was a popular system for grading diffuse astrocytomas [7,8]. It was primarily based on the presence of nuclear atypia, mitosis, endothelial proliferation and necrosis[7]

The accumulation of the criteria was taken up in the grading as follows:

grade 1: 0 criteria

grade 2: 1 (nuclear atypia)

grade 3: 2 (nuclear atypia and mitosis)

grade 4: 3 and 4 (endothelial proliferation and necrosis)

The grade I astrocytomas are genetically distinct tumors like pilocytic astrocytoma, pleomorphic xanthoastrocytoma (PXA) and the subependymal giant cell astrocytoma which are non-infiltrating, circumscribed and clinically indolent [9].

The WHO classification of CNS tumors 1979 categorised astrocytic tumors as astrocytoma, anaplastic astrocytoma, astroblastoma, pilocytic astrocytoma and subependymal giant cell astrocytoma,excluding glioblastoma [10]. Glioblastoma, gliosarcoma, giant cell glioblastoma also known as monstrocellular sarcoma and gliomatosis were categorized under "poorly differentiated and embryonal tumors".Giant cell glioblastoma was considered an entity among tumours of blood vessel and a glioblastoma variant

The ependymoma variants – myxopapillary and papillary ependymoma as well as subependymoma were added to them while the oligoastrocytoma variants -- oligodendroglioma and mixed oligo-astrocytoma and anaplastic oligodendroglioma were included later. [10]

The WHO classification of CNS tumors – 1993 saw major changes in glioblastoma classification with its reinstation in Astrocytic tumors and subsequent deletion from "poorly differentiated and embryonal tumors". Other notable changes were the adoption of St. Anne Mayo method of classifying astrocytic tumors[7, 8], recognition of anaplastic oligoastrocytoma as a separate entity, inclusion of pleomorphic xanthoastrocytoma and addition of clear cell variant under ependymomas[11]. Polar spongioblastoma, gliomatosis cerebri, astroblastoma were

classified under neuroepithelial tumors of uncertain origin (glial tumors of uncertain origin).[11]

The WHO classification of 2000 was rather an uneventful “blue book” with no substantial changes done except for addition of tanycytic variant to ependymomas, and addition of chordoid glioma with deletion of polar spongioblastoma from the neuroepithelial tumors of uncertain origin [12].The WHO classification of CNS tumors – 2007 had classified gliomas based on their histological patterns and clinic pathological correlation. Accordingly astrocytic tumors with cytological atypia alone were grade II (diffuse astrocytoma), those with anaplasia and mitotic activity were grade III(anaplastic astrocytoma) and tumors additionally showing microvascular proliferation with or without necrosis were grade IV(glioblastoma). Other notable changes in this classification were pilomyxoid astrocytoma being recognised as a subset of pilocytic astrocytoma and glioneuronal tumor with neurophil-like islands being added in anaplastic astrocytoma group [13,14]. Under the oligoastrocytomas and mixed gliomas category - high-grade oligo-astrocytomas with necrosis were placed under this designation based on the pattern “glioblastoma with oligodendroglial component.”[14]Neuroepithelial tumors of uncertain origin welcomed angiocentric glioma as a new entitiy.[13]

Salient features of the 2016 WHO Classification of Tumors of the CNS:

The new classification has brought upon major restructuring of diffuse gliomas,embryonal tumors and medulloblastomas. The century old principle of diagnosis of tumors based on microscopy alone has been broken and the integration of phenotypic and genotypic parameters into the classification has added a dose of

objectivity that has been missing in certain diagnostics. This has led to more narrowly defined entities thereby increasing the accuracy of diagnosis.

Now according to the new WHO classification of 2016, the presence of certain mutations like IDH in astrocytic tumors, co deletion of 1p 19q in oligodendroglial tumors are considered the major deciding factors of grading and prognosis.

EPIDEMIOLOGY

Incidence:

Tracking the incidence of gliomas can be done through various organizations that collect data from government cancer surveillance programs or health system records.

According to the Central Brain Tumor Registry of the United States (CBTRUS), the incidence rate of primary malignant brain tumor and other CNS tumors in 2012 was 3.4 per 100,000 after age-adjustment using the world standard population [15].

Approximately 60% of all intracranial tumors are neuroepithelial in origin and gliomas are the most common type of malignant brain tumors that account for 27% of all brain tumors and 80% of all malignant brain tumors [16]. The most common glioma histology is glioblastoma accounting for 56%. Low grade gliomas are the second most common accounting for 30% of gliomas.

The prevalence of glioblastoma is 30.3% distributed among the age group of 14yrs-73yrs[17]. The peak incidence of glioblastoma was seen in patients above 50 years of age[17, 18].

Age:

The analysis of various studies and literature reviews reveal that gliomas have an increasing incidence with age. The infiltrative astrocytomas- the diffuse and anaplastic astrocytoma shares a similar median age group of 38 years[19]. The primary glioblastomas occur in patients aged 55-85 years [19]. The secondary glioblastoma (IDH mutant) patients are significantly younger than their IDH wild type counterparts with a mean age of 42 years [19]. Oligodendrogliomas and oligoastrocytomas are more prevalent in the 35–44 age group[8].Ependymomas on the other hand have a wide age range that most often depends on the localization of the tumor.

Ethnicity:

The role of ethnicity is evidenced by the fact that, gliomas occur in whites more than in blacks, Asians and American Indians[15]. Further,it has been stated that there is comparatively a poor prognosis in Hispanic patients[20].

Sex:

The incidence rate is higher in males in every subtype of gliomas and the male/female ratio is markedly higher for glioblastoma (1.6) than for non-glioblastoma (1.4)[21] .With few exceptions, gliomas generally occur more commonly in males.The numbers of sex ratios taken from 15 independent studies of common primary brain tumors show a definite male preponderance [22].

The purpose to discuss the four molecular subtypes of glioblastoma is due to their gender disparity [23, 24]. The greatest and the most consistent disparity in male to female ratio (2:1) are exhibited by the mesenchymal subtype [25]. In contrast, the *Classical* subtype occurs equally in males and females. The possible explanation

for this can be due to the fact that there is a considerable degree of overlap between the mechanisms of sexual differentiation and oncogenesis.[25]

Localization:

The anatomic location of gliomas has a say in the prognosis. Larjavaara S et al (2006) studied and concluded that the densest occurrence of gliomas was in the frontal lobe followed by the temporal lobe with the right cerebral hemisphere being the most affected[26]. The location also appears distinctly different among children where the commonest site of pilocytic astrocytoma is brainstem/cerebellum. A study by Steed TC et al (2016) reported that IDH-mutant glioblastomas (46.0%) were predominantly distributed in frontal lobe, while those with IDH-wildtype (42.0%) were mainly in temporal lobe[27].

AETIOLOGY AND RISK FACTORS

Inherited genetic mutations:

Certain monogenic Mendelian disorders that occur in families like Turcot syndrome associated with non-polyposis colorectal carcinoma, Li-Fraumeni syndrome, Neurofibromatosis type 1, Ollier-type multiple enchondromatosis, L-2-hydroxyglutaric aciduria are associated with increased incidence of glioblastoma[18].

Allergies and antihistamines:

Various studies show a consistent correlation between the presence of allergy,asthma,hay fever, eczema, and food allergies with decreased glioma risk[28]. A study by Scheurer M E (2011) reported that an inverse association occurred between atopy and glioma, specifically, the risk of glioma was 40% lower in patients with allergy, 30% lower in eczema patients, and 30% lower among asthmatics.[29] A putative

risk of anaplastic glioma in patients using antihistamines for >10 years has been identified [29]. Also the use of NSAIDS is found to have a strong protective effect over glioblastomas[29]

Ionizing radiation:

Data from studies on atomic bomb survivors and patients receiving therapeutic radiation suggest that there is an increased risk of leukemias and gliomas. Davis F *et al* (2011) suggests that an increased risk of adult gliomas may be present with exposure to three or more CT scans to the head and neck region due to a cumulative effect.[30] While the data of these literature is persuasive, still quantification and clarification is needed.

Non ionizing radiation:

Cellular phones:

In 2011, IARC conducted a study to compare the risk of glioma in heavy users of cellular phones and concluded it to be a possible carcinogen not only for glioma but also for vestibular schwannoma. Further studies in the particular field fail to evoke a notable response in terms of association between glioma and mobile phone exposure as the latency of usage is unknown.

Extremely Low Frequency Magnetic Fields (ELFs):

Several studies have assessed the risk of gliomas in those with occupational exposure to ELFs and found that there is no association between the two.[31]

Occupational Chemical Exposures:

Several occupations have been linked to the risk of developing gliomas and a multitude of studies conducted have shown an increase in some and a decrease in

some with no definitive associations in any. Physicians, industrial & chemical workers were found to have increased risk [32]. Engineers, architects, butchers, textile workers also had high risk[33-35]. Fishermen, forestry workers had low risk[36]

Pesticides and solvents:

While studies like Jenkins R B et al (2011) indicate that the cumulative exposure to pesticides has no increased risk of developing gliomas,certain French studies like Viel J F(1998) have reported a positive association between risk of glioma and exposure to pesticide[37-40]. One study by UMHS observed decreased glioma risk to cumulative exposure to chlorinated solvents[40].

Traumatic brain injury:

Experimental studies carried out so far provide equivocal evidence of the development of a post traumatic glioma[41]. The proposed theory in all these studies suggests that many factors play a role other than the trauma itself. Unlike in the physiological conditions where only microglia participate in the brain injury and repair processes, in trauma other immune cells enter the parenchyma along with the blood. Other arenas explored in relation to the post traumatic gliomas are the role of IL -6 that is produced by reactive glial cells,the role of eosinophils,the disruption of blood brain barrier and the inflow of stem cells.IL 6 activates STAT3, a protein responsible for cell growth, differentiation,proliferation and apoptosis.[42,43] *In vitro* and *in vivo* studies have shown that inhibition of STAT 3 not only suppresses the growth and proliferation of glioblastoma cells but also mediates their apoptosis. In case of the eosinophils, the eosinophil peroxidase synthesized by them during intense proliferation, recruits ROS that induces mutations leading to development of cancer

transformed cell-lines .Though in flow of stem cells is seen in ischemia and demyelination, they have the increased risk of neoplastic transformation only in trauma[41]. Moreover decreased activity of tumor suppressor genes further compounds the situation.

The following criteria are highly suggestive of a causal relationship between trauma and glioblastoma [44]:

1. The injury must be severe to cause a tissue repair process
2. The area of the traumatic injury must exactly correlate with the location of the glioblastoma
3. There must be a latency of at least 1 year between the injury to the brain and the development of the tumor. A longer gap more strongly correlates with tumor development.

Diet:

A combined analytical study comparing three large prospective studies has concluded that diet has no association whatsoever with the risk of developing gliomas[45].

Viruses:

The most common viruses that are being studied in conjunction with gliomas are CMV and EBV. Studies have yielded discordant results with a recent study of viral association with the glioblastoma genome data set using NGS showing no association[46].

A study of 45 glioma tissue samples done in Slovenia, has found RNA/DNA, microRNA and proteins of certain neurotropic viruses like EBV, CMV, HSV in plasma of glioblastoma patients.[47]

Socioeconomic status:

Two SEER studies conducted in America have established a strong positive correlation between higher socioeconomic status and development of glioma/glioblastoma[48]

Pathogenesis of Glioma:

Gliomagenesis is pivotal to understanding the current stratum of classification of gliomas.

To understand more about gliomagenesis, the study of glioblastoma that takes up two distinct routes helps us. Though it is impossible to distinguish an established glioblastoma histopathologically into primary and secondary, the differences in clinical and molecular profile of these entities are striking. First, the primary glioblastomas occur in older individuals with much aggression but the secondary glioblastomas occur in younger patients after transformation of a low grade glioma, regardless of therapy. Second, the frequency of specific genetic mutations varies in gliomas but the same genetic pathway appears to be targeted in glioblastoma.[49] Therefore to dissect the molecular pathways of gliomagenesis, the knowledge of cell of origin becomes imperative.

A clue to the cell of origin: The existence of a gliosarcoma or an oligoastrocytoma with biphasic tissue pattern suggests the presence of either an independent mutation of two terminally differentiated cells or an individual mutation of a glial progenitor that retains its ability to differentiate into both components.[50]

Neoplastic transformation:

P53, encoded by TP53 is an important tumor suppressor gene involved in neoplastic transformation of astrocytes accounting for inactivating mutations of TP 53 in more than 50% grade II astrocytomas[51]. In higher-grade astrocytomas such as glioblastoma, dysregulation of the p53 pathway occurs by amplification of MDM2 or MDM4[52, 53].The growth factors over expressed are- PDGFR, FGF, VEGF, EGFR [54]. Loss of chromosome arm 22q and gain of 7q are less commonly occurring genomic alterations [55, 56]

Differentiation and tumor phenotype:

The factors that govern differentiation of glioma cell are unclear. For example, while overexpression of Ras and Akt in progenitor cells yields astrocytic tumours like glioblastomas, overexpression of PDGF-B in the same progenitor cell produces tumours like oligodendrogliomas[57]. Therefore the gliomas are hetrerogenous tumors especially those that have developed from pluripotent cells.

Invasion:

All gliomas have a tendency to invade the surrounding structures regardless of their grading, suggesting that invasion is an early event. The secondary structures of Scherer require a special mention here – namely predilection for white matter,

perineuronal satellitosis, perivascular spread and subpial spread. Gliomas have the ability to migrate to modulate the extracellular space. Few proteases elaborated by gliomas like - cysteine, serine and metalloproteinases are helpful in abrogating the extracellular matrix[58]. The glioma cells produce tenascin and vitronectin that are involved in complex cell to extracellular matrix interactions[59]

Focal adhesion kinase (FAK) is a significant intermediate signaling molecule in glioma migration wherein it signals through different pathways that alter proliferation, migration and survival[60].Migration is also stimulated by many growth factors like FGF, EGF, VEGF that are expressed in astrocytomas [61]

Over expression of the vIII EGFR mutant results in up regulated expression of multiple genes associated with invasion, like metalloproteinases (MMP1 and MMP13) and collagens[58].

Other converging pathways that influence invasion are also seen in glioblastoma, namely the activation of IGFBP2[62].

Tumor progression:

The enroute to glioblastoma progression is a well documented one with primary glioblastoma developing from a de novo phenomenon and secondary glioblastoma arising from progression of a low grade glioma[63]. The progression of a low grade glioma to glioblastoma may take just 1-2 years or as long as decade[64]. Cell cycle regulatory genes are targeted by many genetic alterations. P16, Rb, cyclin D1 form the crux of cell cycle regulatory point where most of these abnormalities converge. Over 50 % grade III & IV astrocytomas have loss of chromosome 9p that affects the gene CDKN2A encoding p16. This leads to progression of low grade

glioma to high grade[65, 66]. Inactivation of Rb in mouse astrocytes has been shown to lead to anaplastic astrocytomas[67]. Alterations in CDK4, CDK6, cyclin D1 appear to subvert the cell cycle control leading to progression of 15 % malignant gliomas to glioblastomas[68, 69]. PTEN gene is also one of the important implicated genes identified in approximately 20% glioblastomas[57].EGFR is a transmembrane receptor tyrosine kinase and is the most commonly amplified oncogene in gliomas. Particularly glioblastomas with EGFR gene amplifications occur in 40% of cases. Transgenic overexpression of erbB in glial cells and downstream Ras in progenitor cells cause a significant increase in glioma formation and progression[57, 66].

Necrosis and hypoxia:

It is a hallmark of increased aggressiveness and higher grade of the glioma. Factors implicated in occurrence of necrosis in gliomas are:

1. Metabolic demand outweighs supply
2. Vascular thrombosis due to deranged coagulation
3. Tissue factor expression by tumor cells that acts as a pro coagulant
4. HIFα expression in pseudopalisading tumors cells of necrosis that might cause rapid cell migration and apoptosis[70]
5. Expression of angiopoietin 2 by the small endothelial cells to use apoptosis and vascular regression[71, 72]

The sequelae of hypoxia are leads to emergence of apoptosis resistant, highly malignant clones [73]

Angiogenesis:

Two pathological forms of angiogenesis occur: increased vascular density and microvascular proliferation.Increased capillaries are made out by CD31 immunohistochemistry staining. The hallmark of a distinctive form of angiogenesis is the 'glomeruloid' vessel proliferation due to its resemblance to the renal glomeruli. Glomeruloid vessels are composed of both proliferating endothelial and smooth muscle cells.

These vessels are proliferative to the extent that even mitotic figures are made out in the glomeruloid vessels. They resemble semicircular glands abutting areas of necrosis. Microvascular proliferations are also found in the infiltrating edge of glioblastomas. PDGFRα amplification is seen in 7% of oligodendrogliomas[74].

HISTOPATHOLOGY OF GLIOMAS

Diffuse astrocytoma – IDH mutant:

Macroscopy.

Diffuse astrocytomas are ill defined grey white homogenous masses with microcysts that sometimes may be extensive, leading to a gelatinous appearance[74].

Microscopy

It exhibits a high degree of differentiation of the fibrillary astrocytes with nuclear atypia and increased cellular density. The nuclei are irregular, hyperchromatic, cigar shaped with 'hand poured' quality showing uneven edges against the 'cookie cutter' appearance of an oligodendroglioma nuclei[74].Cytoplasm is scanty. Microcysts, intercellular edema and calcifications are also seen.'Secondary structures of

Scherer'that include subpial tumor spread, perineuronal satellitosis, perivascular tumor spread are present.

Genetics:

This diffusely infiltrative glioma has IDH 1/2 mutation along with the presence of TP53, ATRX genes. Loss of heterozygosity (LOH) of 17p13.1 or Tp53 mutation is present in almost 50% of these cases.

Immunohistochemistry:

GFAP is positive in majority of cases with dense staining of the processes. S100 - positive in the nucleus of the neoplastic astrocyte. Ki 67 shows a proliferative index of less than 4%[8] .The recent marker that identifies even a single neoplastic astrocyte against a backdrop of reactive astrocytes is IDH1/2. It shows strong cytoplasmic and weak nuclear staining.

Differential diagnosis:

Reactive astrocytes in gliosis closely resemble diffuse astrocytoma. A clue to diagnosis in H&E is that the neoplastic astrocytes show uniform morphology while the reactive astrocytes are in different stages showing polymorphic population. Besides, they differ in IDH 1/2 staining. While neoplastic astrocytes show "oak board"pattern of uniform light staining, each individual reactive astrocyte takes up strong staining.

Gemistocytic astrocytoma:

It is a variant of diffuse astrocytoma and is composed of plump gemistocytes which have abundant, glassy eosinophilic cytoplasm with eccentric nuclei and small nucleoli. Their presence of more than 20% is required for a diagnosis of gemistocytic

astrocytoma[75]. Studies indicate that these tumors progress to a higher grade with greater mitotic activity and they have greater number of p53 mutations [76]

Diffuse astrocytoma IDH wild type:

This is a rare tumor corresponding to grade II diffuse astrocytoma but lacking the IDH 1/2 mutation. They are similar in all aspects to their IDH mutant counterpart.

Diffuse astrocytoma NOS: It includes those diffuse astrocytomas in which genetic testing have not been done.

Pediatric diffuse astrocytomas:

Patients less than 20 years at diagnosis are considered under pediatric astrocytoma category. The annual incidence of pediatric diffuse astrocytoma is 0.27 cases per 100 000 population which is considerably lower than that of adult diffuse astrocytoma, which is 0.58 per 100 000 [77]. The site of pediatric astrocytomas is not only the cerebral hemispheres but also thalamus in a significant proportion of cases. Amplification MYB is seen in approximately 25% of pediatric diffuse astrocytomas[78].

Anaplastic astrocytoma, IDH-mutant

Macroscopy:

The tumor appears more friable, granular with areas of discoloration and hemorrhage – a stark contrast to the smooth rubbery texture of diffuse astrocytoma.

Microscopy::

This tumor shows increased cellularity with moderate nuclear pleomorphism. Gemistocytes are more common. Microvascular proliferation and necrosis are absent but mitotic activity is present.

Genetics:

Allelic loss of chromosome 19q, 7q gain and 10q loss, EGFR amplification are usually seen.

Immunohistochemistry:

Positive for GFAP. p53 shows strong and diffuse nuclear expression. The majority expresses IDH1 and show negative immune staining for nuclear ATRX. Ki-67 proliferation index is usually in the range of 5-10%.

Anaplastic astrocytoma – wild type:

This tumor with focal and dispersed anaplasia accounts for about 20% of all anaplastic astrocytomas. It has an aggressive behavior compared to IDH counterpart, as it behaves similar to glioblastoma .

Anaplastic astrocytoma NOS: –

Here the IDH status of the tumor has not been assessed.

Glioblastoma – wild type:

IDH-wild type (negative) glioblastoma is the most common and also the most malignant astrocytic glioma, accounting for almost 90% of all glioblastomas.

Macroscopy::

Usually unilateral, but those in the brain stem and corpus callosum are bilaterally symmetrical, the latter being called ‘butterfly glioblastoma’. Glioblastomas are poorly delineated; the cut surface is composed of central areas of yellowish necrosis due to myelin breakdown and grayish tumor masses in the periphery thereby imparting variability in color.The central necrosis can sometimes be present as much as 80% of the total tumour mass. Glioblastomas are typically stippled with foci of recent and remote hemorrhage in red and brown. Macroscopic cysts, containing a

turbid fluid and constituting a liquefied necrotic tumour tissue, are a feature of glioblastoma in contrast to the well-delineated retention cysts present in diffuse astrocytomas.

Microscopy:

Very few human neoplasms are as hetrerogenous as glioblastoma, hence the now obsolete term "glioblastoma multiforme" was used to describe it. The diagnosis is made based on tissue pattern rather that individual cell morphology. Increased cellularity, mitosis, necrosis, microvascular proliferation are hallmarks of diagnosis. Transition from one cell type to another rather abruptly indicates addition of certain genetic alterations.

Variants of glioblastoma:

Small cell glioblastoma:

This tumor is a variant of primary wild type glioblastoma. It is composed of monomorphic, small,round hyperchromatic nucleus with minimal cytological atypia and scanty cytoplasm. Nuclear regularity, clear haloes, micro calcifications and chicken wire-like microvasculature, if present may lead to a diagnostic dilemma with anaplastic oligodendroglioma[79].EGFR amplification and loss of chromosome 10 are the most common genetic alteration seen. IDH mutations are absent [79]

Primitive neuronal cells and glioblastoma with a primitive neuronal component;

This tumor is composed of a combination of diffuse astrocytic tumors and clusters of primitive nodules showing neuronal differentiation. The neuronal differentiation foci is sharply demarcated from the adjacent glioma and is composed of increased cellularity, mitosis, Homer-Wright rosettes, cell wrapping and anaplastic

cytology of tumor cells resembling medulloblastoma. It shows loss of GFAP and increased Ki67 compared to adjacent glioma.

Distinctive feature is increased CSF dissemination and about 40% MYC expression of gene amplification[80].

Oligodendroglioma components:

It is composed of focal areas of oligodendroglioma along with glioblastoma with the presence of necrosis reducing the survival rate of patients[81].It is reported as glioblastoma with oligodendroglioma.

Gemistocytes and gemistocytic astrocytic neoplasms:

Gemistocytes are astrocytes with copious eosinophilic glassy cytoplasm and eccentric angulated nuclei. Perivascular lymphocytosis is a distinct feature in gemistocytic variant. It has been proposed that presence of gemistocytes leads to faster progression to high grade gliomas than non-gemistocytic variant[82].

Granular cells and granular cell astrocytoma/glioblastoma:

The presence of granular PAS positive cytoplasm is the distinct feature of granular cells scattered in a glioblastoma. Certain grade II astrocytomas with granular cells have a poor prognosis with glioblastoma like aggressiveness[83].

Lipidized cells and heavily lipidized glioblastoma:

The presence of grossly large cells with foamy cytoplasm admixed with glioma cells are designated as malignant gliomas that are heavily lipidized [84]

Metaplasia and gliosarcoma:

The various metaplastic components seen in glioblastoma and particularly more common in gliosarcoma are squamous epithelial cells , glandular and ribbon-like epithelial structures called adenoid glioblastomas, formation of bone and cartilage[85- 87]

Pediatric high-grade diffuse astrocytic tumours:

The grade III/IV astrocytomas are considered together as their genetic alterations are similar and distinct from their adult counterparts[88] .These tumors usually occur in the midline of the neuro axis especially in pons, midbrain. Some genetic alterations seen in young patients include TP53 (present in 30-50% of cases), ATRX (present in 25%), CDKN2A (deletion in 30%) and PDGFRA (present in 30%) [89]IDH and EGFR mutations are not present.

Giant cell glioblastoma:

The glioblastomas with giant cells can be considered as giant cell glioblastoma .[90] Giant cell glioblastomas account for < 1% of all glioblastomas more common in pediatric populations [91, 92]

Macroscopy:

Firm, well circumscribed mass, due to increase in connective tissue content.

Microscopy:

This tumor is composed of numerous multinucleated giant cells, small fusiform syncytial cells, and a reticulin network[93]. The giant cells are bizarre and angulated with more than 20 nuclei. Also perivascular tumor cell aggregation leads to pseudorosette like structures[94].

Immunohistochemistry:

GFAP is positive and p53 shows more than 80% nuclear positivity[95]

Genetics:

IDH mutation is absent. Presence of p53 mutations and PTEN mutations with absence of EGFR amplification indicates that this tumor is a hybrid between primary and secondary glioblastoma[96, 97].

Gliosarcoma:

A variant of wild type glioblastoma with biphasic tissue pattern composed of glial and mesenchymal components.

Microscopy:

It is composed of an anaplastic glial component resembling a glioblastoma. It can also have squamous, adenoid and gland like metaplasia. The other sarcomatous component is composed of spindle shaped tumor cells arranged in fascicles with nuclear atypia, mitotic activity and necrosis. They may show mesenchymal differentiation composed of cartilage,bone and adipose tissue[98, 99]

Immunohistochemistry:

The glial component is positive for GFAP and negative for IDH1/2[100].The mesenchymal component is best stained with reticulin and it is also positive for vimentin [101, 102]

Genetics:

Gliosarcoma contains PTEN mutations, CDKN2A deletions, and TP53 mutations with infrequent EGFR amplification [103]

Differential diagnosis:

Glioblastoma with meningeal invasion shows desmoplastic reaction due to fibroblastic proliferation that can mimic sarcomatous proliferation.

Epithelioid glioblastoma:

Macroscopy:

These are unifocal tumors with areas of hemorrhage and necrosis. Leptomeningeal spread and cyst formation also occurs

Microscopy:

This variant is composed of epithelioid cell, rhabdoid cells in addition to microvascular proliferation, mitosis and necrosis. It is composed of monomorphic, dyscohesive epithelioid cells with a distinct cell membrane, eosinophilic cytoplasm and laterally placed nucleus. Necrosis is present.

Immunohistochemistry:

Tumor cells are vimentin and S100 positive with patchy positivity of GFAP. V600E-mutant BRAF shows reactivity in about 50% of epithelioid glioblastomas[104].

Genetics:

BRAF V600E mutation is detected in about half of all epithelioid glioblastomas[105, 106]. They lack IDH1/2 mutations.

Glioblastoma IDH –mutant:

It is a high grade glioma with astrocytic differentiation, cellular atypia and necrosis showing IDH 1/2 mutation. It accounts for 10% of all glioblastomas.

Macroscopy:

It is diffusely infiltrating but areas of hemorrhage and necrosis are usually absent.

Microscopy:

The IDH mutant and wild type glioblastomas differ in that the presence of palisading necrosis is more common in wild type and oligodendroglioma like components more common in glioblastomas with IDH mutation[107] .

Immunohistochemistry:

IDH 1/2 is commonly positive. Loss of expression of ATRX gene is present along with TP53 and IDH mutations [108].

Genetics:

The secondary glioblastomas that lack IDH mutations exist and they in turn develop from grade III astrocytomas instead of grade II astrocytomas. They show a shorter clinical history and a poorer prognosis [107]. The primary glioblastomas with IDH mutations show an age predilection of a decade younger patients than those with secondary glioblastomas,though they show similar genetic profiles[107].

The mutations in TP53 are usually seen. The other genetic variations include the presence of CPG hypermethylation phenotype [109]

Expression profile:

More than 90% of IDH-mutant glioblastomas have a proneural expression signature [110].IDH-mutant diffuse astrocytoma and IDH-mutant and 1p/19q-codeleted oligodendroglioma too have the typical proneural signature[111].This supports the assumption that these neoplasms share a common neural progenitor.

Glioblastoma, NOS:

A high grade glioma with astrocytic differentiation showing all the features of glioblastoma but the IDH status has not been fully assessed.

Diffuse midline glioma, H3 K27M variant:

This grade IV glioma is an infiltrative tumor with mutation in H3 K27M. It occurs predominantly in children and is located mostly in brain stem, thalamus, and spinal cord. Prognosis is very poor.

Macroscopy:

Diffuse infiltration by the tumor causes marked distortion and enlargement of anatomical structures such as the fusiform enlargement of the pons.

Microscopy:

Diffuse glioma infiltrates both the white matter and grey matter. The tumor cells can be monomorphic or pleomorphic and with astrocyte or oligodendroglial morphology.

Immunohistochemistry:

NCAM1, S100, and OLIG2 are positive in almost all cases. Nuclear p53 is positive in 50% of cases.

Genetics:

H3F3A, HIST1H3B, and HIST1H3C are the histone encoding genes mutated in position K27M in these gliomas. [112,113]

Oligodendroglioma, IDH-mutant and 1p/19q-codeleted:

By definition, this tumor is composed of cells with IDH1 or IDH2 mutation and co deletion of chromosomal arms 1p and 19q.

Macroscopy:

It is composed of well defined mass with soft grey, pinkish areas. Calcification is common. Areas of cystic degeneration and hemorrhages are common.

Microscopy:

They are diffusely infiltrating gliomas composed of monomorphic cells with round nucleus ,perinuclear halo in H&E sections called 'honeycomb' or 'fried egg' appearance. Stroma shows chickenwire vasculature and microcalcifications. Cells with abundant eosinophilic cytoplasm called mini gemistocytes are also seen. Some oligodendrogliomas have signet ring like cells and are called signet ring oligodendrogliomas[114].

Immunohistochemistry:

Till date no specific immune histochemical marker has been found out for oligodendroglioma. IDH mutant tumors help to distinguish it from other clear cell tumors of CNS. Nuclear TP 53 is mutually exclusive with IDH mutant oligodendrogliomas. Loss of ATRX is absent. Oligodendrogliomas are almost always immune positive for MAP2, S100 protein, and LEU7 [115]. OLIG1, OLIG2, and SOX10 are also expressed in oligodendrogliomas[116]. Vimentin is positive in anaplastic oligodendrogliomas[117]. Ki 67 proliferation index is less than 5%.

Genetics:

Oligodendrogliomas express an unbalanced translocation between chromosomes 1 and 19 leading to deletion of arms of 1p and 19q. IDH mutation with co deletion of 1p and 19q are always coexistent in almost all of the cases. IDH2 mutation is present in a higher frequency than IDH1 mutant astrocytomas in

oligodendrogliomas which can be identified by immuno histochemistry in over 90% of cases[118]

Majority of oligodendrogliomas harbor CIC mutations (the *Drosophila capicua* gene) [119]. Epigenetic silencing of the pH regulator gene *SLC9A1* located on 1p has been linked to the low intracellular pH that contributes to the distinct biology of these gliomas and astrocytomas[120]. TERT mutations are present virtually in all oligodendrogliomas though they lack *ATRX* mutation[121, 122]

G-CIMP phenomenon:

This is the occurrence of concurrent hypermethylation of many CpG islands due to extensive changes in DNA methylation[123]. The DNA methylation profiles in IDH-mutant and 1p/19q-codeleted oligodendrogliomas are different from those in IDH-mutant but 1p/19q-intact astrocytomas [124-126], a distinction that has been called as G-CIMP type A versus G-CIMP type B. MGMT promoter hypermethylation and reduced expression is very common [127]. Even though EGFR amplification is absent, almost all oligodendrogliomas have strong expression of EGFR protein and mRNA. PDGFA and PDGFB are commonly coexpressed in oligodendrogliomas [128].

Differential diagnosis:

Demyelinating diseases or cerebral infarcts, diffuse astrocytoma, clear cell ependymoma, neurocytoma and dysembryoplastic neuroepithelial tumour all have oligodendroglial like cells. Oligodendroglioma lacking IDH mutation and 1p/19q codeletion (pediatric-type oligodendroglioma).

These tumors are distinct from their adult counterparts as they lack IDH mutation and 1p/19q co deletion and show presence of BRAF mutation like pilocytic

astrocytoma[131]. The additional presence of rearrangements of MYB in more than 50% of cases also fuels the theory of their uniqueness from their adult counterpart[130].

Oligodendroglioma, NOS:

A diffusely infiltrating glioma in which molecular testing for combined IDH mutation and 1p/19q codeletion could not becompleted or was inconclusive but showing classic oligodendroglial histology.

Pilomyxoid astrocytoma:

It is a variant of pilocytic astrocytoma composed of bipolar monomorphous tumor cells with angiocentric arrangement in a prominent myxoid background [131]. Most commonly located in hypothalamic/chiasmatic region[132]. It commonly affects infants and children.

Macroscopy:

It is composed of solid gelatinous mass.

Microscopy:

The tumor predominantly shows a myxoid matrix with angiocentric arrangement of tumor cells. This perivascular arrangement leads to pseudorosette formation. There are strictly no Rosenthal fibers, but mitosis and vascular proliferation with glomeruloid tufts are seen. These tumours are genetically similar to WHO grade I pilocytic astrocytomas [132]

Immunohistochemistry:

It shows strong and diffuse reactivity for GFAP, S100 protein, and vimentin. Staining for V600E-mutant BRAF is negative. Ki -67 proliferation index is between 2-20 %[133]

Subependymal giant cell astrocytoma (SEGA):

These are composed of large ganglionic astrocytes arising from wall of lateral ventricle. It corresponds to Grade I. It rarely occurs after the age of 20-25 years[134]. It has a strong association with tuberous sclerosis.

Macroscopy

It is located over the basal ganglia from the wall of the lateral ventricle .It is well circumscribed and multinodular with cystic areas.

Microscopy:

It is circumscribed and calcified with large, plump cells arranged in sweeping fascicles. Clustering of tumour cells and perivascular palisading are common features. The tumor cells are heterogeneous and giant pyramidal-like cells resembling ganglions are seen. Nuclear pleomorphism and multinucleated cells with rich vascular stroma and hyalinized vessels showing infiltration of mast cells and lymphocytes is very consistent [135].

Immunohistochemistry:

GFAP and synaptophysin are variably positive, with intense S100 positivity[136].

Genetics:

Similar to other circumscribed astrocytomas, SEGA also has BRAF mutations. LOH in the *TSC2* gene indicates its occurrence in tuberous sclerosis[137].

Pleomorphic xanthoastrocytoma:

This tumor is composed of neoplastic astrocytes which are large and pleomorphic admixed with spindle cells, lipidized cells and multinucleated cells. It has eosinophilic granular bodies and a dense pericellular reticulin network. It corresponds to WHO grade II.

Macroscopy:

These are superficially located tumors with cystic areas and mural nodule.

Microscopy:

Histological hallmarks of PXA

1. Pleomorphism-it has mononucleated and multinucleated giant astrocytes with nuclei of varying sizes and intranuclear inclusions.
2. Xanthoastrocytoma – indicating the presence of xanthomatous cells with intracellular accumulation of lipids.
3. Reticulin network – best stained by reticulin highlighting individual tumor cells

Mitotic activity is < 5 mitoses per 10 high-power fields.

Immunohistochemistry:

It has BRAF V600e mutation with lack of IDH mutation. GFAP and S100 protein is invariably positive [138-140].CD34 is positive and Ki 67 is less than 1 %[141.]

Genetics:

It shows gains of chromosomes 3 and 7 and alterations of the long arm of chromosome 1 loss of CDKN2A are seen in 61% of cases[142, 143]. BRAF point mutations occur in approximately 50-78% of cases[144, 145] IDH mutations are lacking in these gliomas[146].

Differential diagnosis:

Diffuse astrocytomas, gangliogliomas with glial component, pilocytic astrocytomas and certain mesenchymal tumors may be in the differentials, all ruled out by respective markers.

Anaplastic pleomorphic xanthoastrocytoma:

This tumor has mitotic activity more than 5 mitosis per 10 high power fields. The presence of necrosis confirms the worst prognosis. It corresponds to WHO grade III

Macroscopy:

The tumor is well-circumscribed and supratentorial with areas of cystic degeneration

Microscopy:

The histological features are similar to the pleomorphic xanthoastrocytoma except for the presence of mitosis and necrosis.

Genetics:

BRAF mutations are comparatively lower in this type of tumor[146]

Differential diagnosis:

Epithelioid glioblastoma forms a close differential as both commonly exhibit BRAF mutations [147]. Anaplastic pleomorphic xanthoastrocytoma has eosinophilic granular bodies and lacks the cytological uniformity of epithelioid glioblastoma [148]

Sub ependymoma:

These are slow growing intraventricular glial tumors with bland nucleus minimal mitosis and a predominant fibrillary matrix with microcystic changes. It corresponds to WHO grade I.

Macroscopy:

Most common location is the fourth ventricle and the lateral ventricle. They form nodules of size 1-2 cm and are well circumscribed.

Microscopy:

These tumors are composed of clusters of bland uniform cells with isomorphic nuclei embedded in a dense fibrillary matrix with calcification and hemorrhage. Focal areas show ependymal pseudorossettes .Occasionally it forms a part of a classic ependymoma or tanycytic ependymoma, being classified as mixed ependymoma-subependymal tumours[149].

Immunohistochemistry:

These tumors are positive for GFAP and neuron specific enolase. EMA is rarely positive [150]

Genetics:

Nine molecular groups of ependymoma have been identified based on DNA methylation profiles dividing them into three groups each, based on their anatomical location namely supratentorial, posterior fossa and spinal compartment.

Myxopapillary ependymoma:

This tumor exclusively arises in the conus medullaris, cauda equina and filum terminale and it is characterized by fibrillary processes arranged radially around mucoid fibrovascular cores. It corresponds to WHO grade I.

Macroscopy:

These are soft grey and lobulated with gelatinous and cystic areas with encapsulation

Microscopy:

These tumors are composed of cuboidal cells radially arranged around fibrovascular cores with a radial pattern. Myxoid material positive for alcian blue is present in microcysts and in between the tumor cells. PAS positive rounded eosinophilic structures (so-called balloons) are also seen.

Immunohistochemistry:

These tumors consistently express GFAP which distinguishes it from other metastatic carcinomas, chordoma and myxoid chondrosarcoma. S100, vimentin and CD99 are also frequently positive [151]

Ependymoma:

Ependymomas occur in patients from birth to 81 years. These are intracranial tumors in both adults and children. They are located along the ventricular system, spinal canal and cerebral hemispheres. Ependymomas occurring in children predominate in the infratentorial location, the most common site being posterior fossa[152]. These ependymomas are located in the fourth ventricle, whereas the

supratentorial ependymomas occur in the lateral or third ventricle. Three distinct phenotypes called ependymoma variants namely papillary, clear cell and tanycytic ependymoma are recognized. Classic ependymoma corresponds to WHO grade I

Macroscopy:

They are well circumscribed and are soft, spongy with gritty calcium deposits."Plastic ependymomas" are those which arise in the caudal fourth ventricle and wrap around the brainstem, cranial nerves and muscles[153]

Microscopy:

A classic ependymoma is composed of tumor cells with round to oval nuclei and stippled nuclear chromatin. The hallmark features are perivascular anuclear zones and true ependymal rosettes. Pseudo rosettes can be seen in all ependymomas whereas the true ependymal rosettes are present only in a few. Pseudo rosettes results are composed of tumor cells arranged around blood vessel whereas true ependymal roses are arranged around a central lumen.

Immunohistochemistry:

These tumors are positive for S100 and vimentin [154]. EMA is found in most ependymomas with ring like cytoplasmic structures and dot like perinuclear staining[155] .OLIG2 positivity is particularly lacking in ependymomas [156]

Genetics:

Ependymomas display a broad range of aberrations namely gain of chromosomes 1q,5,7 ,9 and loss of chromosome 1p ,3,6[157] .One reproducible prognostic marker is the gain of chromosome 1q especially associated with poor prognosis in posterior fossa tumors [158]. NF2 gene is involved in tumorigenesis of

spinal ependymomas [159]. The methylation and transcription studies have divided ependymomas into nine distinct groups, three in each location namely – supratentorial, posterior fossa and spinal locations.

Variants of ependymoma:

Papillary:

This is characterized by well formed papillae which are epithelial like surfaces forming finger like projections lined by a single layer of cuboidal cells. The surfaces of tumor cells are smooth.

Clear cell ependymoma:

This is characterized by appearance of oligodendroglioma like tumor cells with perinuclear haloes and frequently seen in the supratentorial compartment. The differential diagnoses are oligodendroglioma, central neurocytoma, renal cell carcinoma (clear cell) and hemangioblastoma. It is positive for GFAP and EMA with the characteristic rosettes.

Tanycytic ependymomas:

This phenotype is commonly found in spinal cord. The elongated tumor cells are arranged in irregular fascicles. The ependymal rosettes and pseudo rosettes are less common in this variety[160].

Ependymomas – RELA fusion positive:

These ependymomas comprise about 70% of the cases occurring in the supratentorial region in children [161]. They are positive for RELA fusion gene and correspond to WHO grade I or II depending on their histological features.

Immunohistochemistry:

It is positive for GFAP and EMA. L1CAM correlates well with the presence of a RELA fusion in supratentorial ependymomas [161]

Genetics:

The C11or f95-RELA fusion is the commonest structural abnormality in ependymomas . It is a result of chromothripsis – a process of shattering and reassembly of the genome resulting in production of oncogenes by rearrangement of genes.

Anaplastic ependymoma:

It corresponds to WHO grade III .The grade doesn't correlate with the prognosis rather the extent of surgical resection and molecular profile [162]

Microscopy:

Increased cellularity with high nuclear cytoplasmic ratio is the characteristic feature. Their cellularity is too high that they can be mistaken for embryonal tumors. Pseudorossettes are also seen.

Immunohistochemistry:

This tumor has the same profile as that of the grade II ependymoma but Ki 67 index is higher.

Other miscellaneous gliomas:

Chordoid glioma of the third ventricle:

This slow growing glioma of the third ventricle is well circumscribed and non infiltrating SOL of adults. It corresponds to WHO grade II

Microscopy:

This tumor is composed of solid nests and clusters of polygonal epithelioid cells in a myxoid bubbly matrix with dense lymphoplasmacytic infiltration. A fusiform pattern with collagen and fibrous pattern with fibrosis are also identified. The nucleus is uniform and ovoid. The stroma is composed of intense lymphoplasmacytic infiltrate, composed of Russell bodies which are a consistent feature. Mitosis is nil to minimal.MRI shows show strong, homogeneous contrast enhancement[163].

Immunohistochemistry:

They are characterized by the strong, diffuse reactivity for GFAP [164].These tumors show consistent nuclear positivity for TTF-1 [165] .Ki-67 proliferation index < 5% [166]

Genetics:

FISH studies indicate losses at 11q13 and 9p21 [167]. No EGFR amplifications or *TP53* mutations were noted. Similarly they also lack IDH1/2 and BRAF mutations [168].

Angiocentric glioma:

This glioma is a chronic epilepsy causing tumor of children and young adults. It corresponds to WHO grade I.

Microscopy:

It is composed of monomorphous spindled out bipolar cells arranged around blood vessels of all calibers or resembling the ependymal pseudo rosettes. Tumors can form perpendicular palisading arrays in the pia- arachnoid complex composed of nodules reminiscent of schwannoma. The nuclei are elongated and spindle shaped with stippled granular chromatin. Few ovoid eosinophilic structures in the paranuclear areas of the epithelioid tumor cells correspond to the EMA positive microlumina found in classic ependymomas.

Immunohistochemistry:

The spindled cells are GFAP positive. There is also cytoplasmic EMA positivity. IDH mutation is negative with Ki 67 proliferation index less than 1-5%.

Genetics:

Limited studies on angiocentric gliomas indicate the presence of MYB rearrangements on locus 6q23 [169]. Angiocentric gliomas lack IDH1/2, and BRAF V600 mutations [170]

Astroblastoma:

These are rare gliomas that are composed of centrally located sclerosed blood vessels with the tumor cells showing broad non tapering processes radiating towards them. These are called astroblastic pseudorosettes. They are common in children and young adults and WHO grade is not established now as it is a very premature stage.

Microscopy:

Apart from the astroblastic pseudorosettes, the presence of the ‘stout processes’ form the hallmark of astroblastoma. These are distinct broad columnar processes extending to the centre of the vessel. Literature has classified this tumor into well

differentiated (presence of low mitosis and Ki 67 of 3 %[171]) and malignant (anaplasia, increased cellularity, necrosis, microvascular proliferation, more than 5 mitoses per 10 high-power fields and Ki 67 more than 10%).Prominent vascular hyalinization is another distinct feature.

Immunohistochemistry:

Cytoplasmic positivity for vimentin, S100, and GFAP is characteristic. IDH 1/2 is negative[172, 173]

Role of IDH in gliomas:

IDH 1/2 genes have become the foci of research since their discovery, first in 2008 mostly in low grade gliomas and secondary glioblastomas.IDH (Isocitrate dehydrogenases) enzymes play an important role in cancer and cellular metabolism. There are five genes that encode IDH leading to formation of three isoforms – IDH1, IDH2, and IDH3. The IDH1 enzyme is located in the cytoplasm and peroxisomes, catalyzing the oxidative decarboxylation of isocitrate (ICT) to 2-ketoglutarate (2KG) which results in the generation of NADPH from NADP+. The reverse reaction of reductive carboxylation is also done by the same enzyme. The IDH2 is a homodimer with structural similarity and similar action in the mitochondria [174].

Their main function is prevention of oxidative damage and generation of NADPH, for cellular defense. They also regulate dioxygenases that require 2KG as substrate. IDH3 is a NAD dependent enzyme with a role in TCA cycle, which catalyses 2KG formation by the irreversible conversion of isocitrate[175]

Role in cell physiology:

Whenever the cell energy level is high, IDH 1 & IDH2 participate in synthetic pathways – fatty acid and cholesterol synthesis. During that period, IDH3 is inhibited by its product – NADH thus leading to shuttling out of citrate from mitochondria and preventing TCA cycle participation.

Role in tumor:

In case of a tumor, the mitochondrial respiratory chain is disrupted; leading to high intramitochondrial NADH/NAD which disrupts the TCA cycle and shuttling of citrate to cytosol for synthetic mechanisms takes place. Thus it is hence proven that not only normal cells but also the tumor cells require IDH1 and IDH2.

Mutant isocitrate dehydrogenases:

IDH 1 and IDH 2 are somatic mutations that involve a single amino acid- the arginine residue at codon 132 in IDH1 and at codon 172 in IDH2.

The IDH3 mutation does not cause cancer for the following reasons:

It is biallelic and mutation will cause the entire TCA cycle to stop causing apoptosis of the cell.

It is unidirectional, meaning it catalyses only ICT to 2KG (oxidative decarboxylation) and not the reverse reaction (like IDH1, IDH2).

There has been a debate regarding the mechanism of action of IDH 1 and 2 as to whether they are oncogenes or tumor suppressor genes. However now it is more plausible that they are oncogenes as various studies indicate them to be gain of function mutations – as the neomorphic allele expression caused cell proliferation[176].

Mechanism of carcinogenesis:

Recent studies have proven that the mutated IDH1 and 2 genes produce a new oncometabolite called (D) – 2 hydroxyglutarate and its accumulation is the cause of tumorigenecity [177].This is proved by the addition of IDH inhibitors that caused reduced 2HG levels and inhibited the growth of glioma cells. The 2 HG metabolite competitively inhibits 2KG which is the major substrate for dioxygenases. The dioxygenases are involved in regulation of cell division and also alter the nucleic acid and protein methylation[178]

Another mechanism is that mutant IDH 1 and IDH 2 cause a hypermethylation phenotype that silences genes involved in cellular differentiation and death. The oncometabolite 2HG causes reduction in hypoxia inducible factor 1α (HIF 1α) levels thereby increasing the production of reactive oxygen species causing DNA damage and mutation. The other tumors showing IDH 1/2 mutation are – AML,MDS,MPN [179] .Solid tumors with mutations in IDH 1/2 are chondroblastomas especially in ollier's/mafucci's syndrome ,cholangiocarcinomas , colon and certain lung carcinomas.

The relevance of IDH mutation in the latest WHO classification of CNS tumors (2016):

The WHO classification of CNS tumors – 2007 was primarily dependent on microscopy, thereby grouping all tumors with same phenotype separately. Now with advent of detection of a distinct molecular signature for the tumors, namely IDH, the diagnosing method has revolutionized. Hence the use of genotype to define an entity not only improves diagnostic accuracy but also caters to better patient management.

IDH mutant indicates detection of presence of IDH protein by immunohistochemistry and IDH 1 codon 132, IDH 2 codon 172 by sequencing. IDH-wild type indicates absence of the IDH mutant gene. In settings where IDH testing is unavailable, a diagnosis of NOS is made.

The best example of this can be an entity – oligoastrocytoma –a diagnostic category that has suffered a lot due to high intraobserver variation. By taking into account both genotype and phenotype of the oligoastrocytoma, WHO 2016 classification is able to place the tumor either in the astrocytomas(IDH mutant) or the oligodendrogliomas (1p 19q co deletion)with the exception of molecularly 'true' oligoastrocytomas(IDH mutant and 1p 19q co deletion –both absent).

Accordingly WHO grade II, III astrocytic tumors, grade IV – glioblastomas and grade II, III oligodendrogliomas are the diffuse gliomas. Hence this major restructuring has resulted in grade II, III astrocytomas – i.e., that is diffuse and anaplastic astrocytomas respectively,being divided into IDH mutant,IDH wildtype and NOS categories.

The role of IDH in prognosis:

The role of IDH1/2 mutation in prognostication has been studied elaborately all over the world by many studies. The prognostic value of IDH1/2 in gliomas has been studied by Chen JR et al 2016 through meta-analysis[180]. They observed the overall survival (OS) and progression free survival (PFS) of glioblastomas and concluded that patients having IDH mutation had better OS and PFS. According to the study by Yan H et al (2009) which sequenced IDH1/2 gene in 445 CNS tumors, patients with IDH 1/2 mutations had better outcome than wild type IDH genes[181]. Two other meta

analytical studies carried out in 2013 namely Cheng H B et al[182], Zou P et al[183] also conclude and provide strong evidence that IDH 1/2 mutation is of paramount importance in the prognosis of gliomas.

Hence extensive review of all recent literature provides solid evidence for the utility in IDH testing in gliomas which are as follows:

- To identify reactive gliosis from gliomas in difficult cases
- To differentiate infiltrating and non infiltrating gliomas in adults
- To identify the IDH mutant gliomas associated with better prognosis
- To provide ground for novel treatment strategies targeting IDH mutations

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN

The present prospective study was conducted at the Department of Pathology, Madurai medical college from July 2017 to July 2019. Ethical clearance for the study was obtained from ethical committee of Madurai Medical College, Madurai.

A total sample of 96 space occupying lesions of brain were collected, out of which 50 gliomas were analyzed during the study period.

INCLUSION CRITERIA

- Biopsy specimens of space occupying lesions (SOL) of brain

EXCLUSION CRITERIA

- Benign tumors
- Neoplastic lesions other than gliomas

METHODOLOGY AND TECHNIQUES

The study material included 50 gliomas (Annexure - VI)

Clinical and morphological details were recorded according to the proforma (Annexure - III)

Operated resection/stereotactic biopsy specimens were collected and fixed in 10 % neutral buffered formalin.

After fixation, the specimens were fully embedded and processed routinely. Multiple 4 to 6 micron thin paraffin sections were obtained.

Staining was done by Hematoxylin and Eosin staining technique. (Annexure - IV)

HISTOMORPHOLOGICAL EVALUVATION

Stained slides were evaluated under light microscopic examination. Tumors were classified and categorized according to their pattern of differentiation. Tumors were graded based on cellularity,nuclear atypia,mitotic activity and necrosis.

IMMUNOHISTOCHEMICAL EVALULATION

Paraffin blocks with 4 micron thick serial sections from the biopsy specimens were used for IDH1 mutation and EGFR protein expression. All the cases of glioblastomas diagnosed were subjected to immunohistochemical evaluvation with monoclonal antibody IDH1 R132H. The presence of strong cytoplasmic staining and weaker nuclear staining in the tumor cells with R132H mutated peptide was taken as positive.

For analysis of EGFR, all IDH1 negative glioblastomas were selected. In case of EGFR, the tumor cell membrane staining is considered to be specific for interpretation of the result.

Score 0 was given for tumors that had no staining of the tumor cell membrane.

Score 1 was given for tumors that had weak membrane staining of more than 10% of the tumor cells

Score 2 was given for tumors that had moderate membrane staining of more than 10 % of the tumor cells.

Score 3 was given for tumors that had intense membrane staining of more than 10% of the tumor cells,

For two cases of gliosarcoma that were studied, immunohistochemical evalulation with markers glial fibrillary acidic protein (GFAP), vimentin and special stain study with reticulin were done. In case of GFAP and vimentin, intense cytoplasmic staining of the respective tumor cells was taken as positive. The presence of black reticular fibers with red nuclei was taken as reticulin positivity.

STATISTICAL ANALYSIS

Data obtained was entered into the Microsoft excel spread sheet. The data was analysed using ratios and percentage. Spearman's Rho and Pearman's Coefficient correlation studies were done and p value was derived to determine the statistical significance of the study. Observations and results were compared with other studies and inferences drawn.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

This prospective study included 92 specimens of various space occupying lesions of the central nervous system diagnosed at the Department of Pathology, Madurai Medical College.

The SOLs composed of both neoplastic and non neoplastic lesions. The non neoplastic lesions diagnosed were cerebral and cerebellar abscesses and cysts – arachnoid cyst, colloid cyst and epidermal inclusion cyst.

The neoplastic SOL of the central nervous system had both primary and secondary brain tumors. The primary brain tumors diagnosed were broadly divided by the researcher for convenience of the statistical analysis into – gliomas and non-gliomas.

Of these gliomas of all the WHO grades – I, II, III, IV namely Pilocytic astrocytoma,Diffuse astrocytoma, Anaplastic astrocytoma and Glioblastoma respectively were diagnosed. Ependymomas of grade II and grade III were also present.

The other primary CNS tumors excluding gliomas were- Meningiomas and its variants namely – meningothelial,psammomatous, fibroblastic,transitional along with grade II –Atypical and grade III – Anaplastic meningiomas. The ventricular SOLs that were subject to histopathological analysis were – central neurocytoma and choroid plexus papilloma. The cerbellopontine angle tumors were – schwannoma and mature cystic teratoma. Very rare Pituitary tumors – pituitary adenomas were also diagnosed. One case of an embryonal tumor with multilayered rosettes was also present. The secondary brain tumors included metastatic carcinomatous deposits .

Table 1: Various Types of Brain SOL

Brain SOLs	Frequency	Percentage
Neoplastic Lesions	88	92
Non Neoplastic Lesions	8	8
Total Brain SOL	96	100

Chart 1: Various Types of Brain SOL

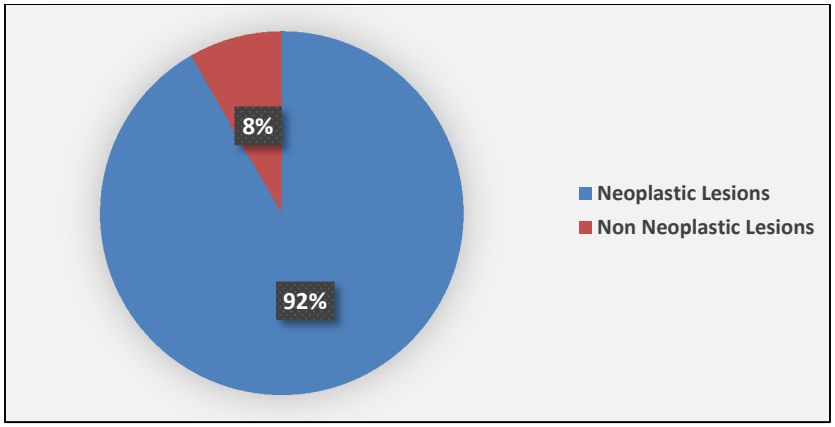
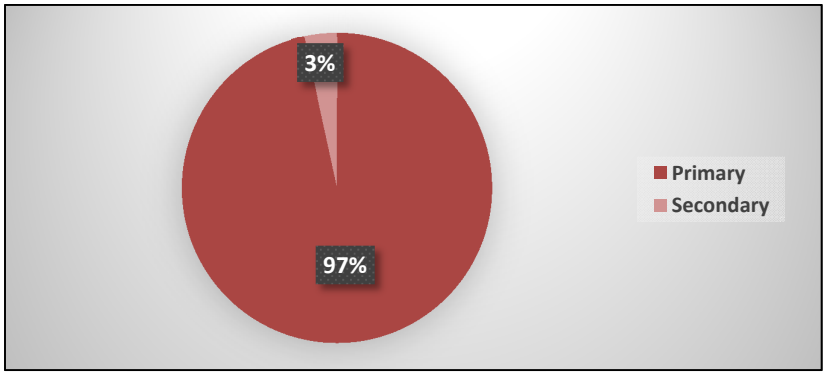


Table 2: Various Types of Neoplastic Brain Tumors

Types	Frequency	Percentage
Primary	85	97
Secondary	3	3
Neoplastic Lesions	88	100

Chart 2: Various Types of Neoplastic Brain Tumors



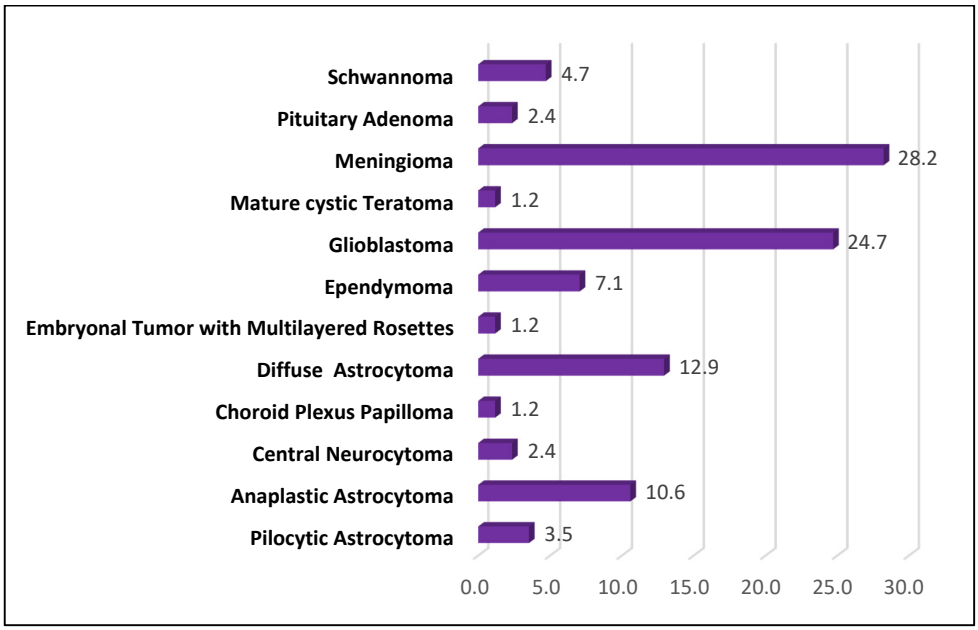
Of the brain SOLs, 92% were neoplastic and 8% were non neoplastic. Among the neoplastic lesions, 97% were primary CNS tumors and 3% were secondary tumors.

A brief analysis of the non neoplastic lesions of brain was done which showed cysts of brain to be the predominant lesion. Abscesses of cerebrum and cerebellum accounted for 3.1% of all brain SOLs whereas benign cysts of brain including arachnoid cyst, colloid cyst and epidermal inclusion cyst comprised of 5.2%.

Table 3: Distribution of Primary CNS tumors

Primary CNS tumors	Frequency	Percentage
Pilocytic Astrocytoma	3	3.5
Anaplastic Astrocytoma	9	10.6
Central Neurocytoma	2	2.4
Choroid Plexus Papilloma	1	1.2
Diffuse Astrocytoma	11	12.9
Embryonal Tumor with Multilayered Rosettes	1	1.2
Ependymoma	6	7.1
Glioblastoma	21	24.7
Mature cystic Teratoma	1	1.2
Meningioma	24	28.2
Pituitary Adenoma	2	2.4
Schwannoma	4	4.7
Total	85	100.0

Chart 3: Distribution of Primary CNS tumors



The analysis of histological types of the primary CNS tumors among the neoplastic brain lesions revealed the meningiomas to be the commonest accounting for 28.2% , followed by the glioblastomas as a close second - 24.7% Embryonal tumor with multilayered rosettes , choroid plexus tumor and mature cystic teratoma were the least common (1.2% each).

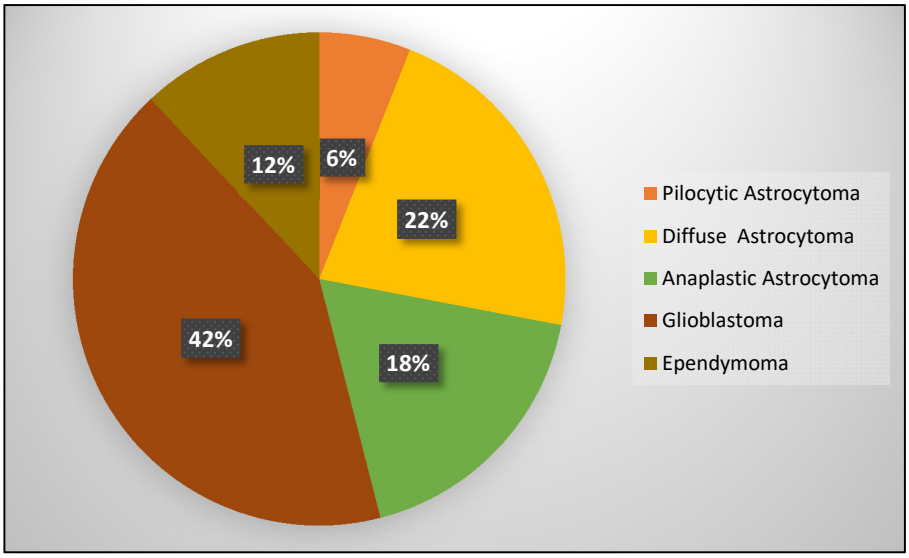
Among the 89 neoplastic brain lesions, 3 were secondary brain tumors - metastatic deposits accounting for 3.4%

Table 5: Distribution of Histological types of Gliomas

Gliomas	Frequency	Percentage
Pilocytic Astrocytoma	3	6
Diffuse Astrocytoma	11	22
Anaplastic Astrocytoma	9	18
Glioblastoma	21	42
Ependymoma	6	12

Total	50	100

Chart 5: Distribution of Histological types of Gliomas



The most common malignant CNS tumor was glioblastoma comprising of 42% of all gliomas. The next common was diffuse astrocytoma accounting for 22% of gliomas.

Table 6: Distribution of Histological types of tumors other than Gliomas

Tumors other than gliomas	Frequency	Percentage
Central Neurocytoma	**2**	**2.4**
Choroid Plexus Papilloma	**1**	**1.2**
Embryonal Tumor with Multilayered Rosettes	**1**	**1.2**
Mature cystic Teratoma	**1**	**1.2**
Meningioma	**24**	**28.2**
Pituitary Adenoma	**2**	**2.4**
Schwannoma	**4**	**4.7**
Total	**35**	**41.2**

Of the non-glial tumors, meningeal tumors were the commonest accounting for 28.2% of the primary CNS tumors.

Chart 6: Distribution of Histological types of tumors other than Gliomas

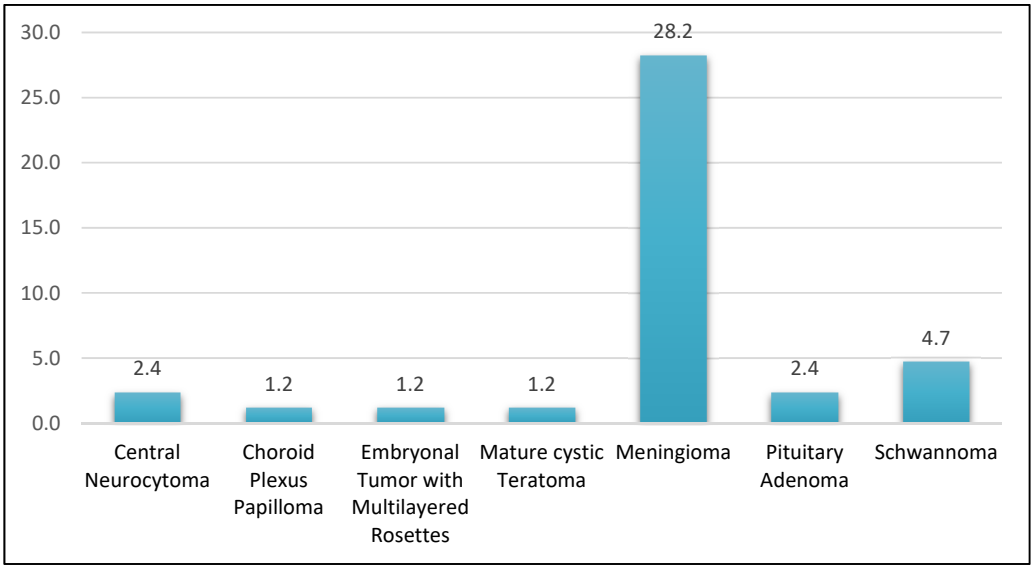
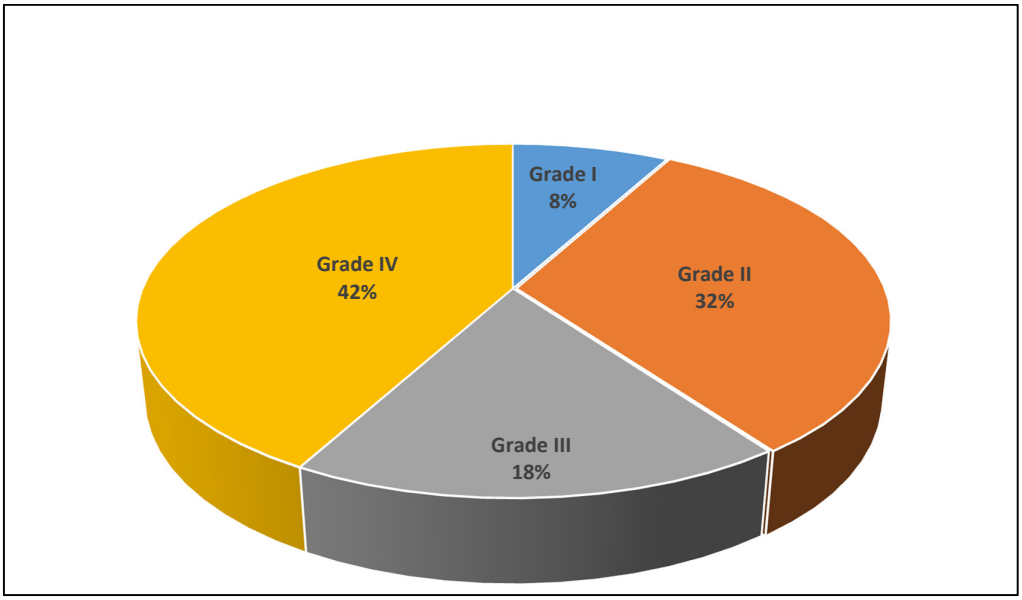


Table 7: Distribution of Gliomas according to the WHO grade

Gliomas	Frequency	Percentage
Grade I	4	8
Grade II	16	32
Grade III	9	18
Grade IV	21	42

Chart 7: Distribution of Gliomas according to the WHO grade



The gliomas were divided on their WHO grades I - IV. It was seen that amongst them grade IV was the commonest accounting for 42%.

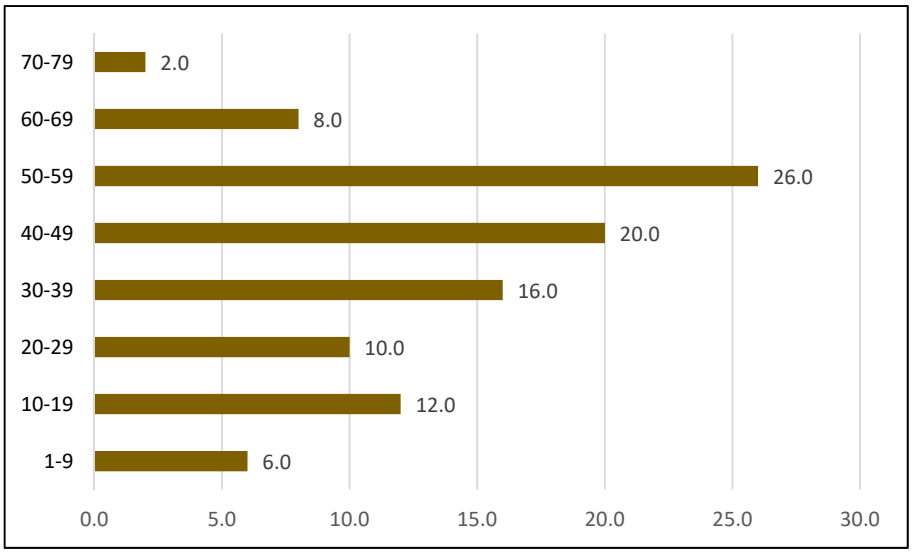
Age wise Distribution of Gliomas:

The age group analysis of gliomas done showed that the older age group of 50-59 years had the highest predilection of occurrence of gliomas accounting for 26%. This was closely followed by the age bracket of 40- 49 year old patients -20%. Overall frequency was highest in the adult age group of 40-59 years accounting for 46%.The least affected population were the patients of the seventh decade-2%.

Table 8: Age wise distribution of Gliomas

Age Group	Frequency	Percentage
1-9	3	6.0
10-19	6	12.0
20-29	5	10.0
30-39	8	16.0
40-49	10	20.0
50-59	13	26.0
60-69	4	8.0
70-79	1	2.0

Chart 8: Age wise distribution of Gliomas



Individual assessment of occurrence of every grade glioma in each age group was done .The pilocytic astrocytomas were predominant equally in first and the second decades -33.3% each.

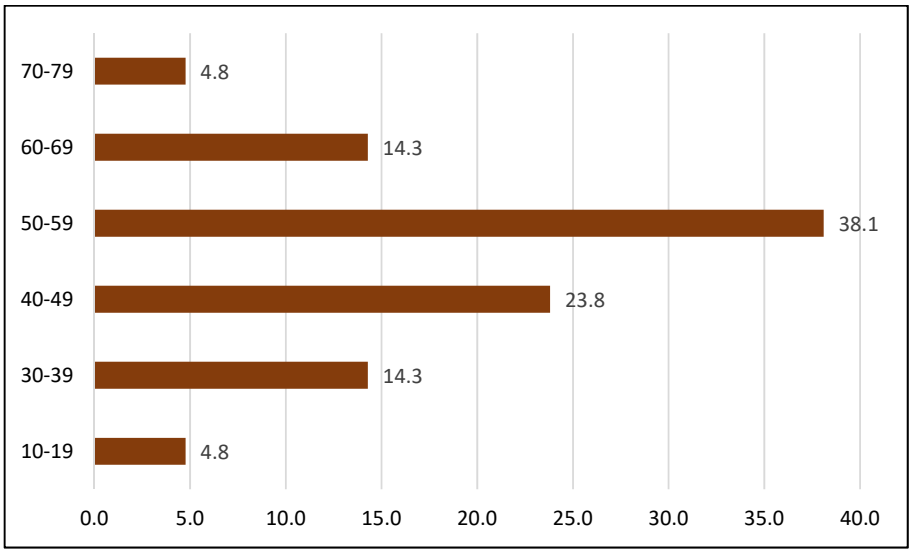
The diffuse astrocytomas commonly occurred in predominantly in the age group of 20-29 years accounting for 27.3%. Children and adolescents were the least affected by these tumors (9.1%).

Among the anaplastic astocytomas, the major group affected appeared to be third and fifth decade patients comprising of 33.3%

Table 9: Age wise distribution of glioblastomas

Age	Frequency	Percentage
10-19	**1**	**4.8**
30-39	**3**	**14.3**
40-49	**5**	**23.8**
50-59	**8**	**38.1**
60-69	**3**	**14.3**
70-79	**1**	**4.8**

Chart 9: Age wise distribution of glioblastomas



The analysis of age wise distribution of glioblastomas revealed a striking predilection for the fifth and sixth decade with a combined preponderance of 52.4%, with the age group of 50-59 years accounting for 38.1%.Regarding the ependymomas, the most common age group was the adolescent age group of 10-19 years, accounting for 50%.

A separate analysis was done on the primary CNS tumors affecting the pediatric age group. Based on the data collected, pilocytic astrocytomas were the commonest tumors in the pediatric CNS tumors accounting for 33.3%.

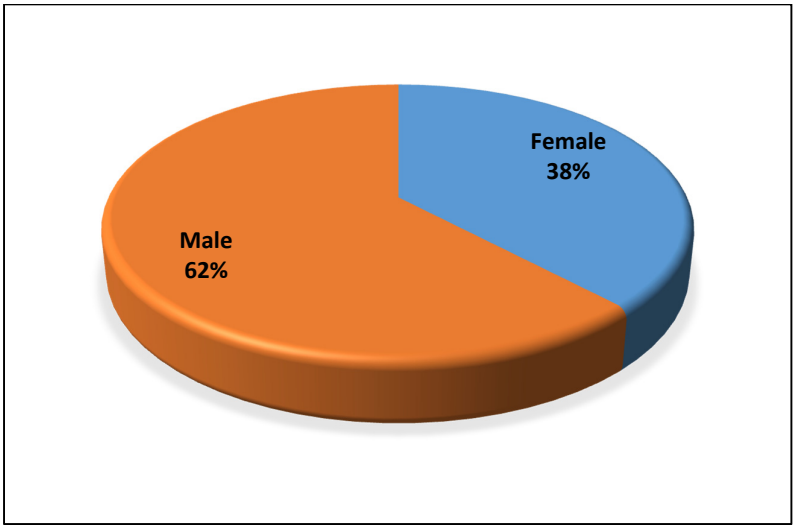
Gender wise distribution of gliomas:

The analysis of sex wise distribution of gliomas revealed that there was a definite male preponderance with 62 % of gliomas in males. The male: female ratio was 1.63:1

Table 10: Gender wise distribution of Gliomas

Gender	Frequency	Percentage
Female	**19**	**38**
Male	**31**	**62**

Chart 10: Gender wise distribution of Gliomas



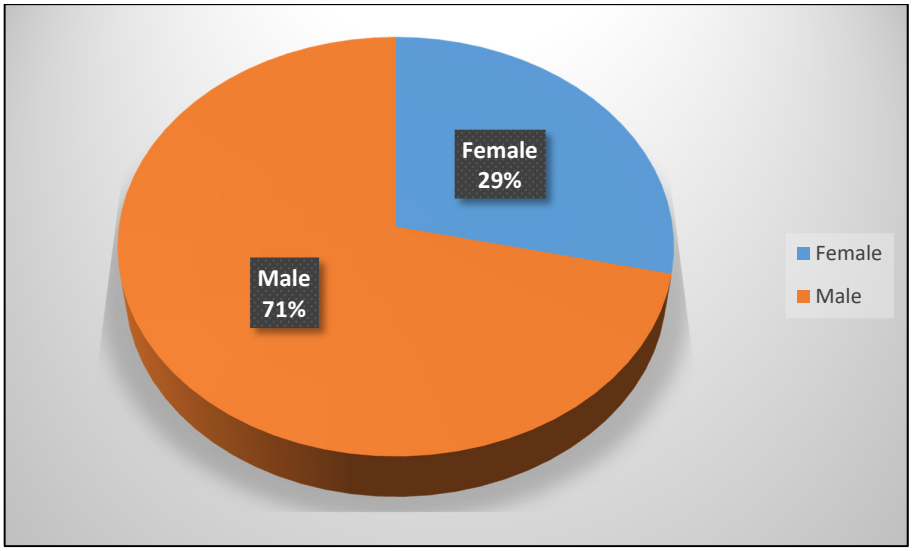
In the four cases of pilocytic astrocytoma received, the male preponderance appeared to be increased accounting for 66.7% similar to the diffuse astrocytomas which were also slightly more preponderant in males. The sex ratio (M: F) of anaplastic astrocytomas also was nearly equal – 1:1.2 with a slightly increased female preponderance.

The glioblastomas had a definite male preponderance of 71.4% with a male: female ratio 2.5:1

Table 11: Gender wise distribution of Glioblastoma

Gender	Frequency	Percentage
Female	**6**	**28.6**
Male	**15**	**71.4**

Chart 11: Gender wise distribution of Glioblastoma



The ependymomas had a male preponderance of 66.7% with a male: female ratio 2:1.

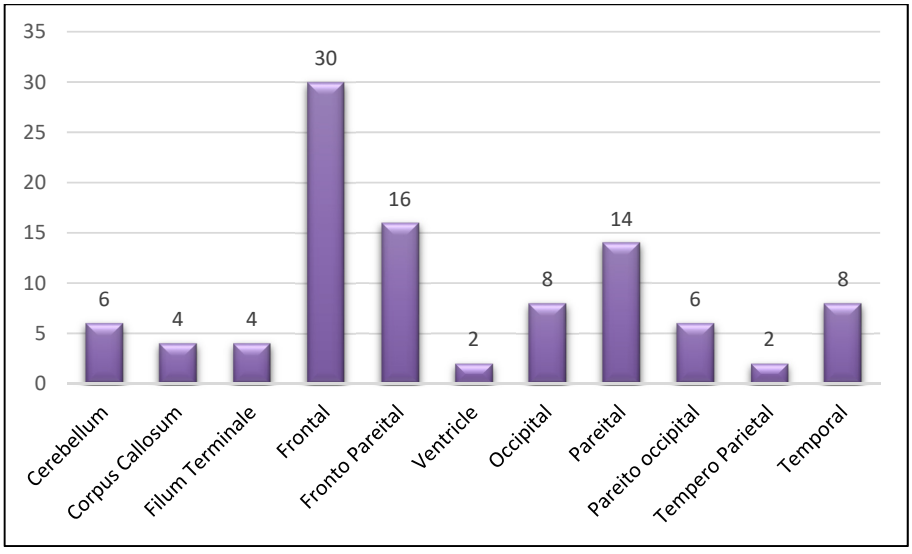
Distribution of gliomas according to their location:

Regarding the localization of gliomas, the commonest site of their occurrence was the frontal lobe accounting for 30% followed by the frontopareital lobe-16% and the parietal lobe – 14%.The least common site appeared to be the ventricles and temperopareital lobes – 2% each

Table 12: Distribution of gliomas according to their location

Location wise	Frequency	Percentage
Cerebellum	**3**	**6**
Corpus Callosum	**2**	**4**
Filum Terminale	**2**	**4**
Frontal	**15**	**30**
FrontoPareital	**8**	**16**
Ventricle	**1**	**2**
Occipital	**4**	**8**
Pareital	**7**	**14**
Pareito occipital	**3**	**6**
Tempero Parietal	**1**	**2**
Temporal	**4**	**8**
Total	**50**	**100**

Chart 12: Distribution of gliomas according to their location



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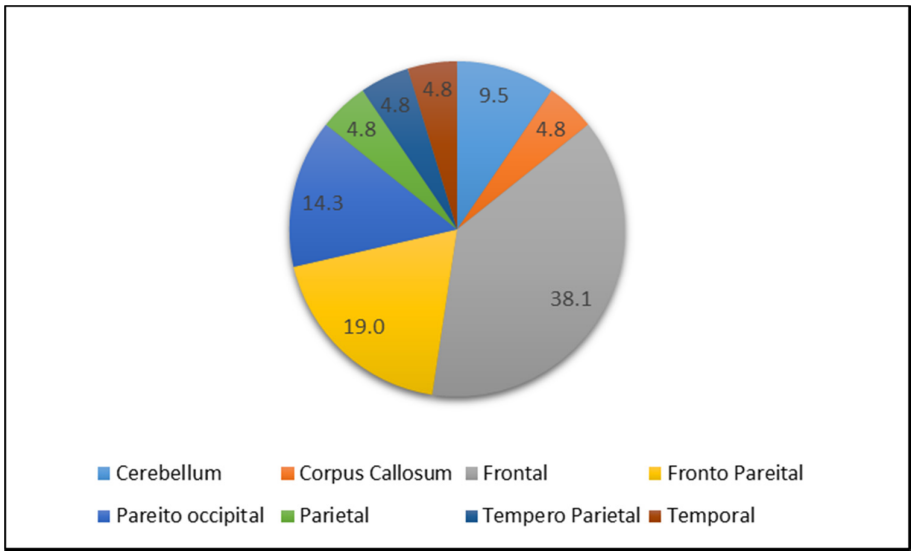
The pilocytic astrocytomas had a predilection for frontal and occipital lobes with the latter being slightly more common accounting for 66.7%.The diffuse astrocytomas were most commonly localized in almost all the lobes - frontal, parietal and temporal lobes – 27.3% each. The frontopareital region and parietal lobes appeared to have an equal predilection for anaplastic astrocytomas- 33.3% each.

Table 13: Location wise distribution of Glioblastoma

Location	Frequency	Percentage
Cerebellum	**2**	**9.5**
Corpus Callosum	**1**	**4.8**
Frontal	**8**	**38.1**
FrontoPareital	**4**	**19.0**
Pareito occipital	**3**	**14.3**
Parietal	**1**	**4.8**
Tempero Parietal	**1**	**4.8**
Temporal	**1**	**4.8**

Chart 13: Location wise distribution of Glioblastoma



The glioblastomas most commonly occurred in the frontal region accounting for about 38.1% whereas the next common site was frontopareital -19 %.

Ependymomas had a slightly increased predilection for Filum terminale with 33.3% cases occurring there, compared to other sites – cerebellum,ventricles and cerebrum

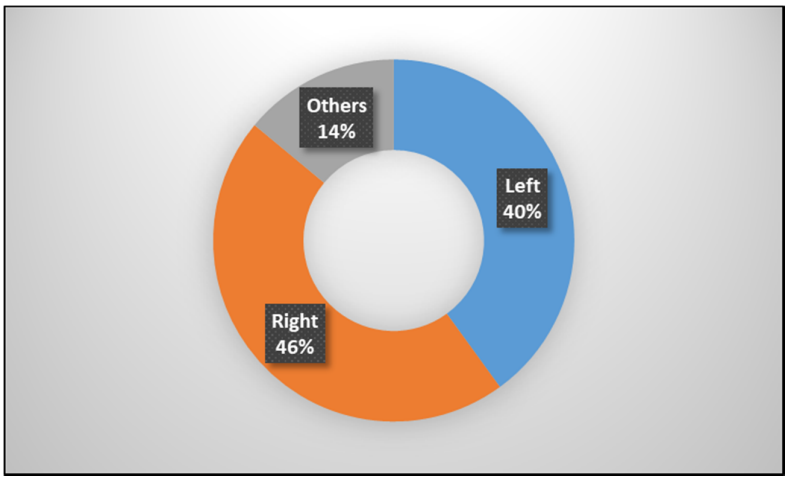
Laterality of gliomas:

The commonest side of occurrence of gliomas was the right lobe of cerebrum – 46% compared to the left side – 40%. Other sites were – corpus callosum, cerebellum, ventricles and filum terminale.

Table 14: Distribution of gliomas according to laterality

Side of cerebrum	Frequency	Percentage
Left	**20**	**40.0**
Right	**23**	**46.0**
Others	**7**	**14.0**

Chart 14: Distribution of gliomas according to laterality



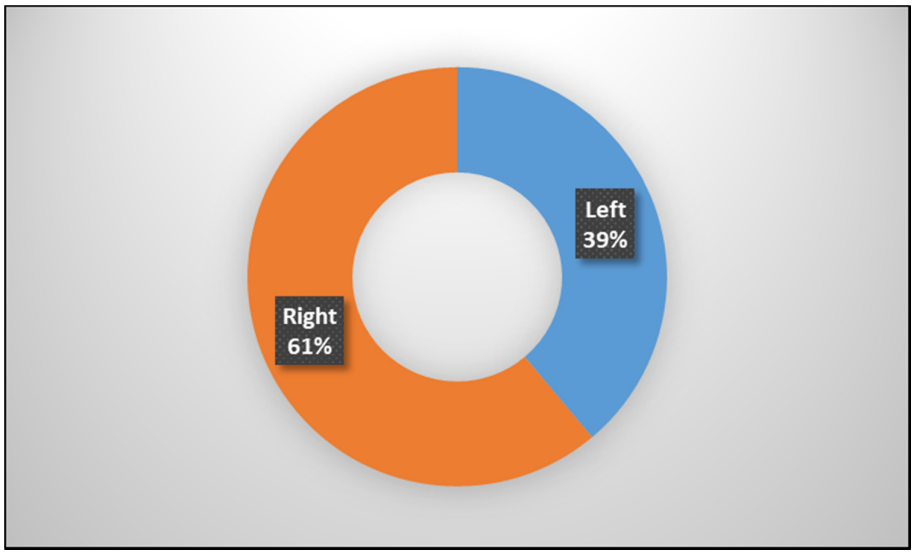
The left cerebrum was the predilected site for the pilocytic astrocytoma – 66.7% while the diffuse astrocytomas had a slight right preponderance of 54.5% Anaplastic astrocytomas had an equal distribution in both right and left cerebrum and one case occurred bilaterally with spread via the corpus callosum. One other case

occurred in a rather unusual site – cerebellum. Glioblastomas had a definite predilection for the right side of brain with an occurrence of 61.1%.

Table 15: Distribution of glioblastomas according to laterality

Side of cerebrum	Frequency	Percentage
Left	**7**	**38.9**
Right	**11**	**61.1**

Chart 15: Distribution of glioblastomas according to laterality



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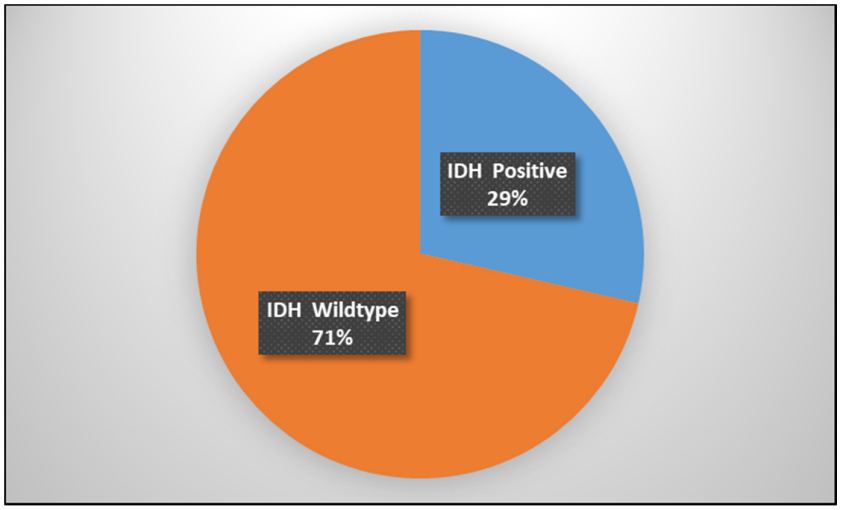
Analysis of frequency of occurrence of IDH mutant and wild type glioblastomas:

The aim of our study included the study of frequency of occurrence of IDH mutant and wild type glioblastomas. So we studied the expression of IDH1 (R132H) antibody expression, in the 21 glioblastomas diagnosed.

Table 16: Frequency of IDH Mutant Glioblastomas

Glioblastomas	Frequency	Percentage
IDH Positive	6	28.6
IDH Wildtype	15	71.4
Total	21	100

Chart 16: Frequency of IDH Mutant Glioblastomas



The IDH mutant glioblastomas accounted for 28.6% wheareas the IDH wild type glioblastomas were more prevalent, comprising of 71.4 %.

Table 17: Age wise Distribution of IDH Mutant Glioblastomas

Age Group	Frequency	Percentage
30-39	1	16.7
40-49	2	33.3
50-59	3	50.0
Total	6	100.0

Chart 17: Age wise Distribution of IDH Mutant Glioblastomas

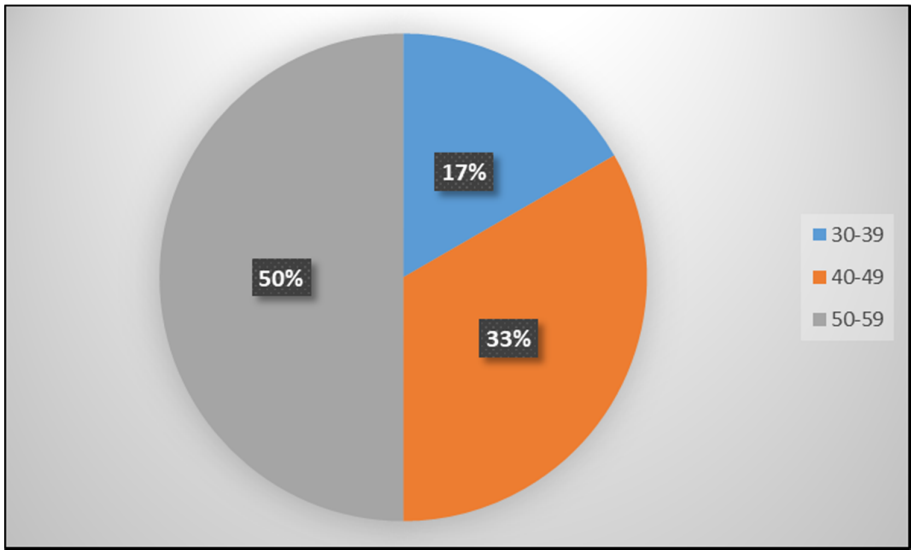
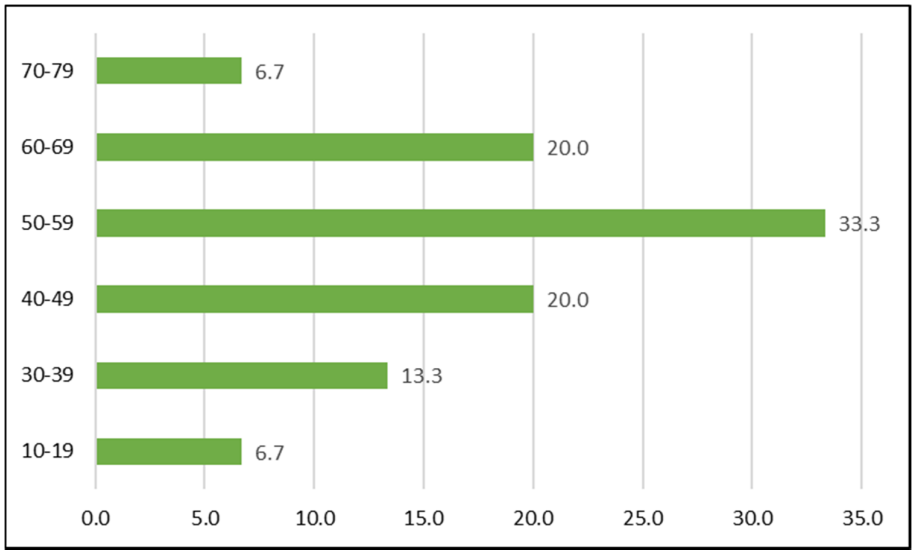


Table 18: Age wise Distribution of IDH Wild type Glioblastomas

Age Group	Frequency	Percentage
10-19	1	6.7
30-39	2	13.3
40-49	3	20.0
50-59	5	33.3
60-69	3	20.0
70-79	1	6.7
Total	15	100.0

Chart 18: Age wise Distribution of IDH Wild type Glioblastomas



Of the six IDH mutant glioblastomas we studied, majority (50%) were in the age group of 50-60 years. Among the IDH wild type glioblastomas, also the same age group accounted for 33%. However, about 26.7% of elderly population (60 -79 years) was also present in IDH wild type glioblastomas.

Table 19: Location wise Distribution of IDH Mutant Glioblastomas

Location	Frequency	Percentage
Frontal	**3**	**50.0**
FrontoPareital	**1**	**16.7**
Pareito occipital	**1**	**16.7**
Temporal	**1**	**16.7**
Total	**6**	**100.0**

Chart 19: Location wise Distribution of IDH Mutant Glioblastomas

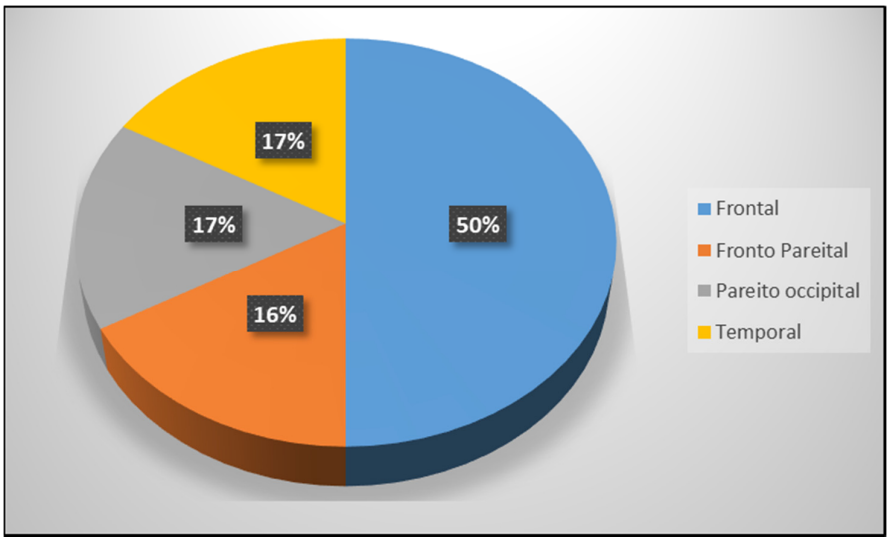


Table 20: Location wise Distribution of IDH Wild type Glioblastomas

Location	Frequency	Percentage
Cerebellum	**2**	**13.3**
Corpus Callosum	**1**	**6.7**
Frontal	**5**	**33.3**
FrontoPareital	**3**	**20.0**
Pareito occipital	**2**	**13.3**
Parietal	**1**	**6.7**
Tempero Parietal	**1**	**6.7**
Total	**15**	**100**

The most common location in both IDH mutant and wild type was the frontal lobe, accounting for 50% and 33% respectively.

Table 21: Gender wise Distribution of IDH Mutant Glioblastomas

Gender	Frequency	Percentage
Female	**1**	**16.7**
Male	**5**	**83.3**
Total	**6**	**100.0**

Chart 20: Gender wise Distribution of IDH Mutant Glioblastomas

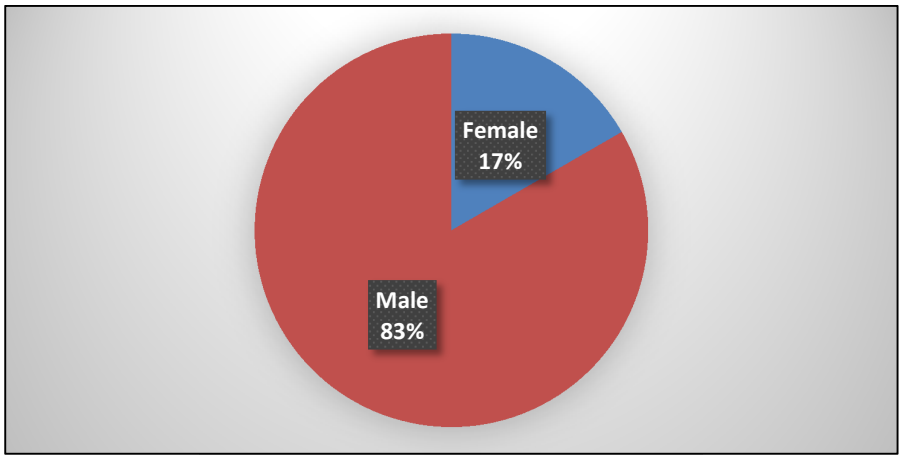


Table 22: Gender wise Distribution of IDH Wild type Glioblastomas

Gender	Frequency	Percentage
Female	**5**	**33.3**
Male	**10**	**66.7**
Total	**15**	**100**

The IDH mutant and IDH wild type glioblastomas, both showed a definitive male preponderance with 83.3% and 66.7% respectively.

Analysis of EGFR status in IDH wild type glioblastoma :

As a part of our study, we wanted to observe the occurrence of primary and secondary glioblastomas. Hence in all IDH negative (wild type) cases, an analysis of

EGFR expression was done. Out of the 15 IDH negative cases, 6 were positive for EGFR. This accounted for 40% of IDH wild type glioblastomas. Among the 6 EGFR positive cases, 3 cases had intense (3+) staining.

Table 23: Analysis of EGFR status in IDH wild type Glioblastoma

EGFR status	Frequency	Percentage
EGFR Negative	**9**	**60**
EGFR Positive	**6**	**40**
Grand Total	**15**	**100**

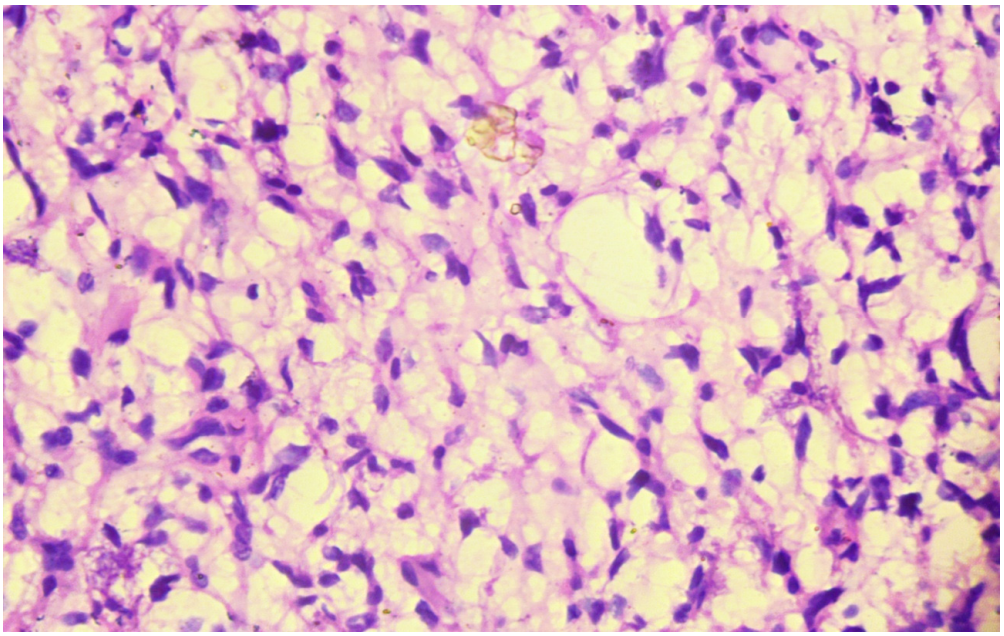


Figure 1: S1135/18 -Pilocytic astrocytoma – microcysts (H & E 400X)

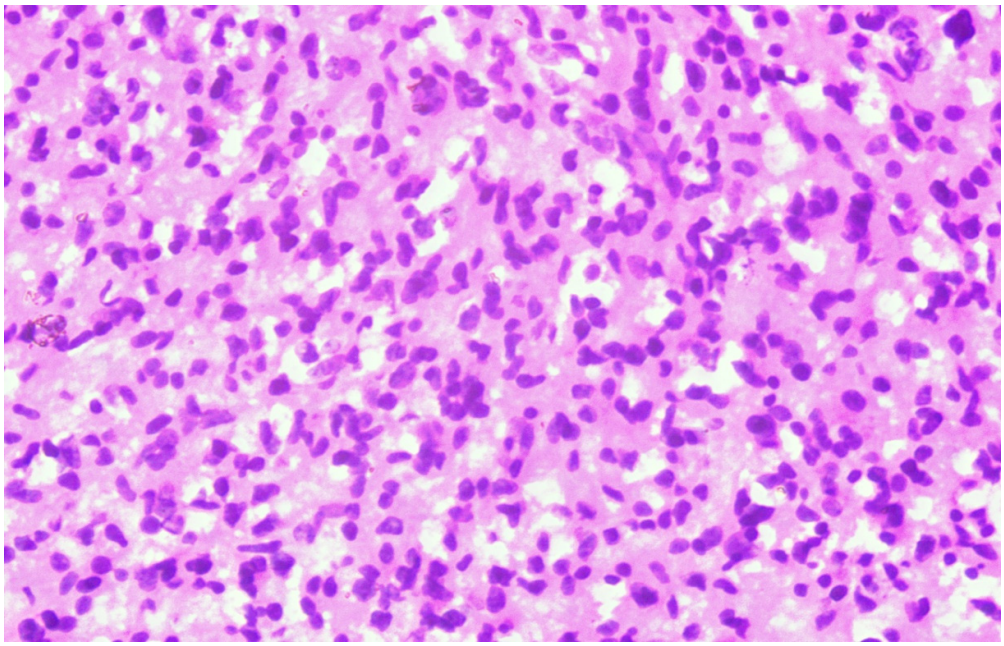


Figure 2: S2329/18- Diffuse astrocytoma with increased cellularity(H & E 400X)

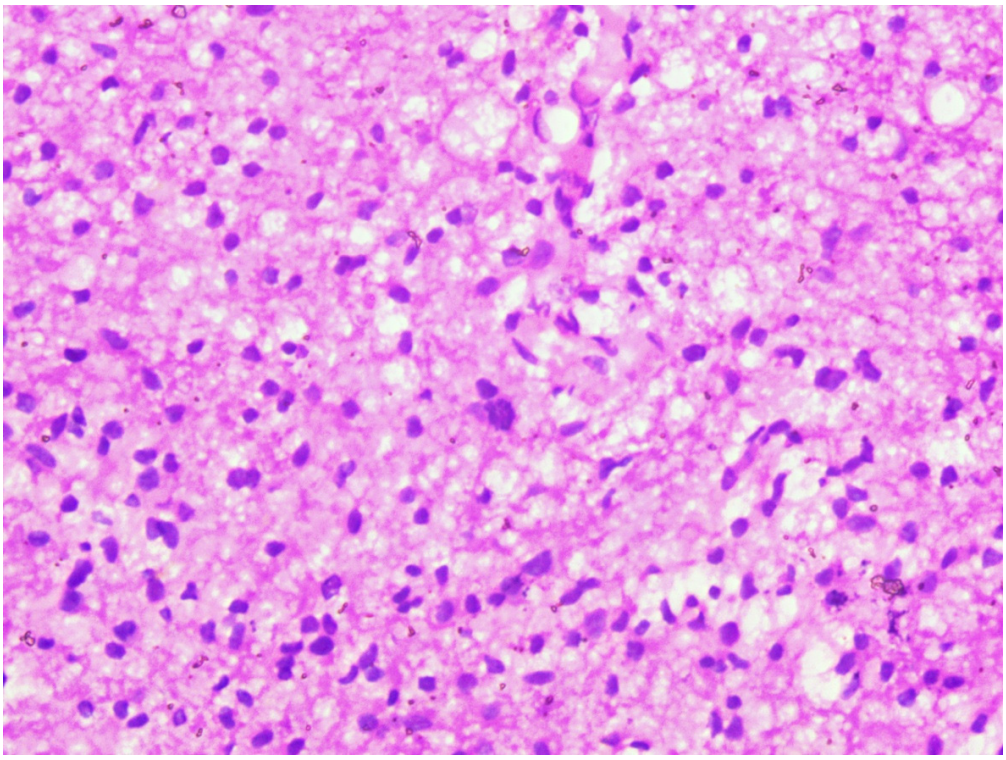


Figure 3: S1385/18-Anaplastic astrocytoma with mitosis (H&E 400X)

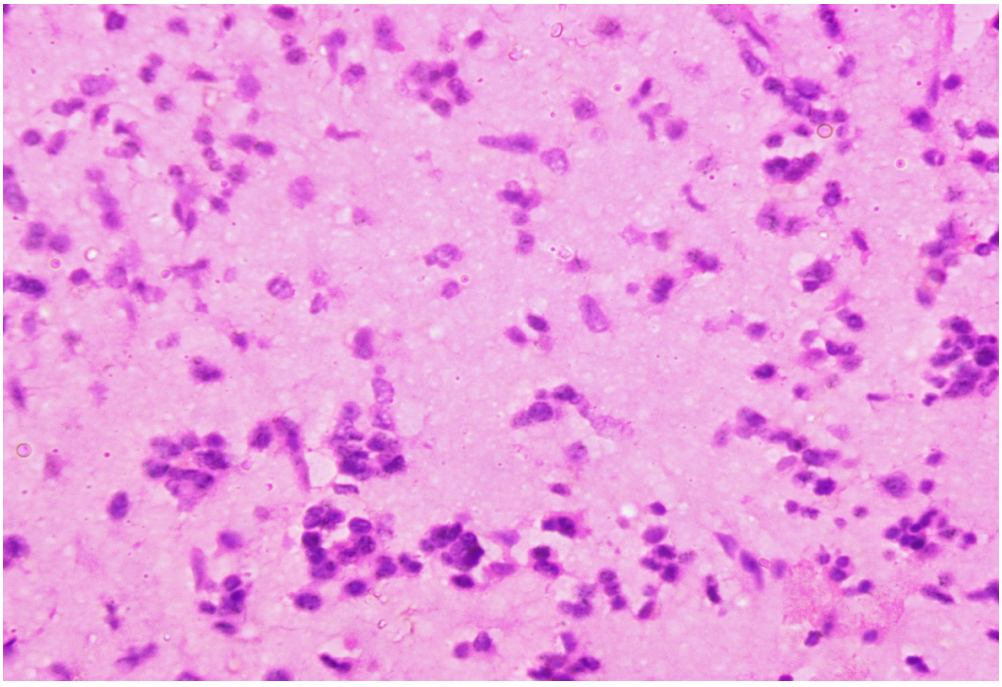


Figure 4: S768/18 Perineuronal satellitosis (H & E 400X)

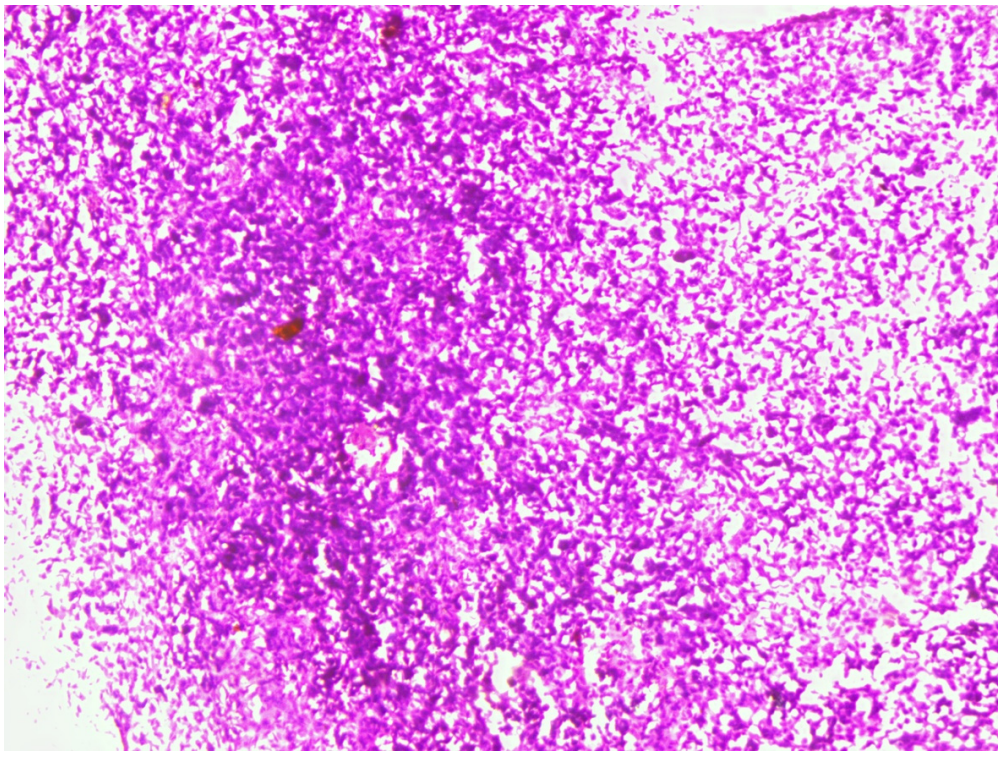


Figure 5: S387/18 Glioblastoma (H & E 200X)

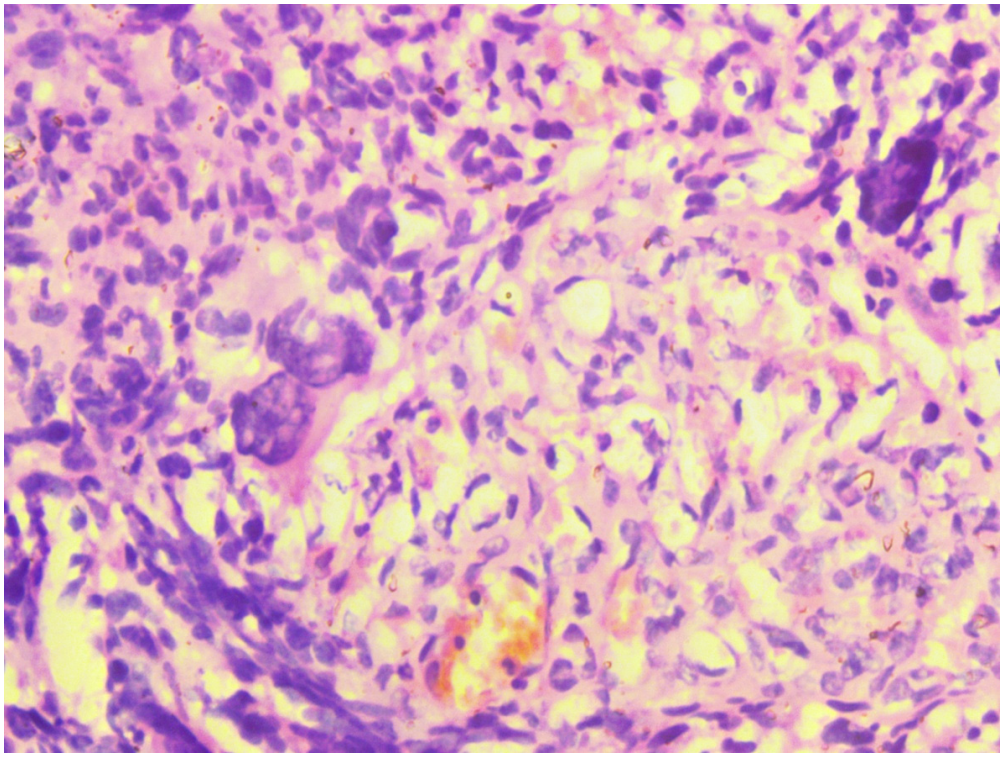


Figure 6: S423/19 Giant cells in GBM (H & E 400X)

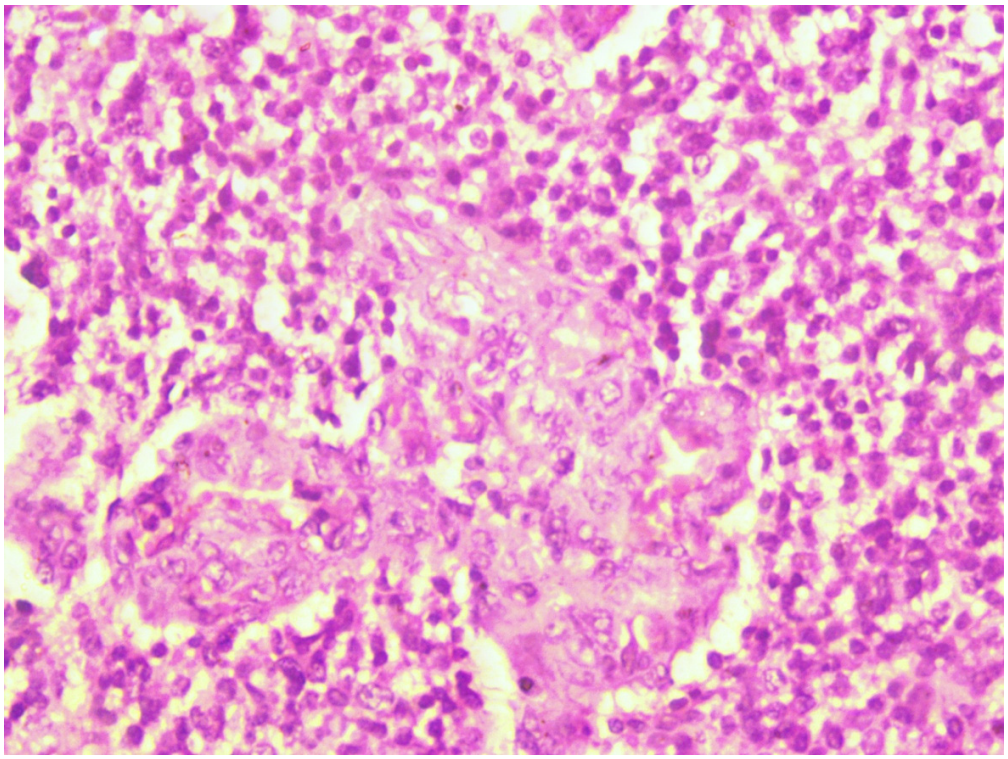


Figure 7: S355/17 Glomeruloid vessel in glioblastoma(H & E 400X)

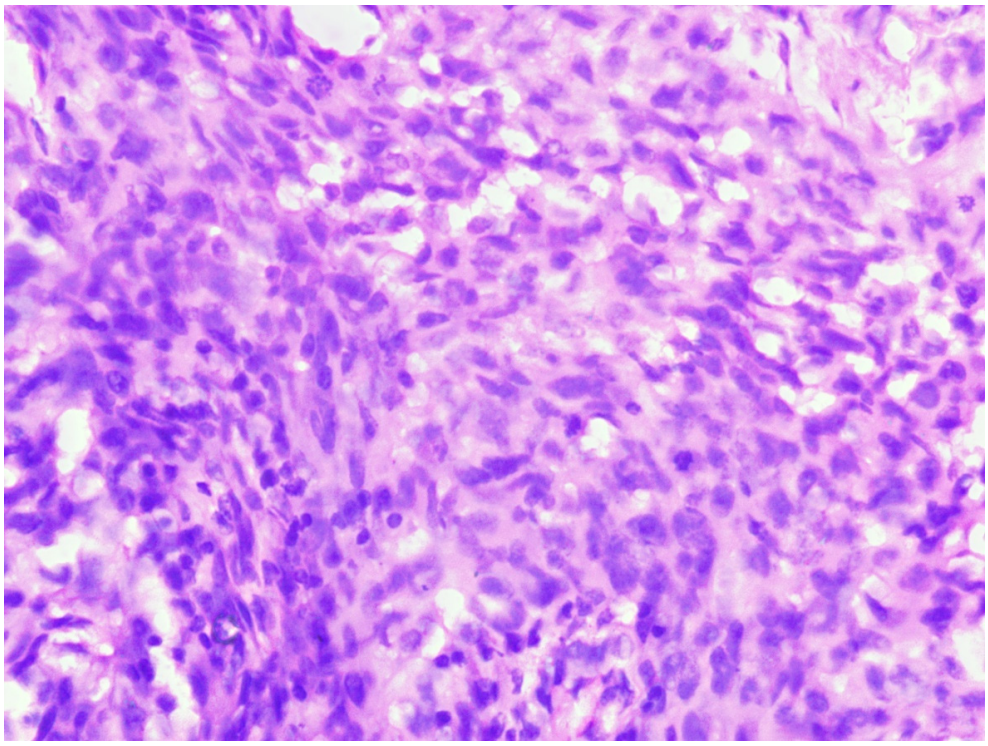


Figure 8: S1198/18 Gliosarcoma – spindled sarcomatoid areas (H & E 400X)

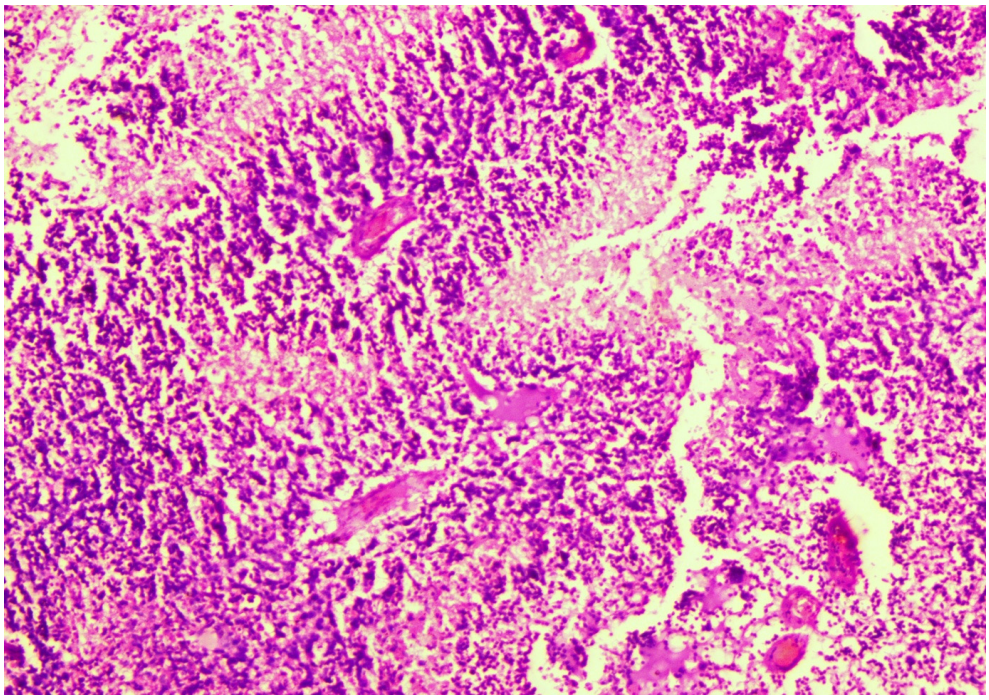


Figure 9: S87/18 Serpingenous necrosis of glioblastoma (H & E 200X)

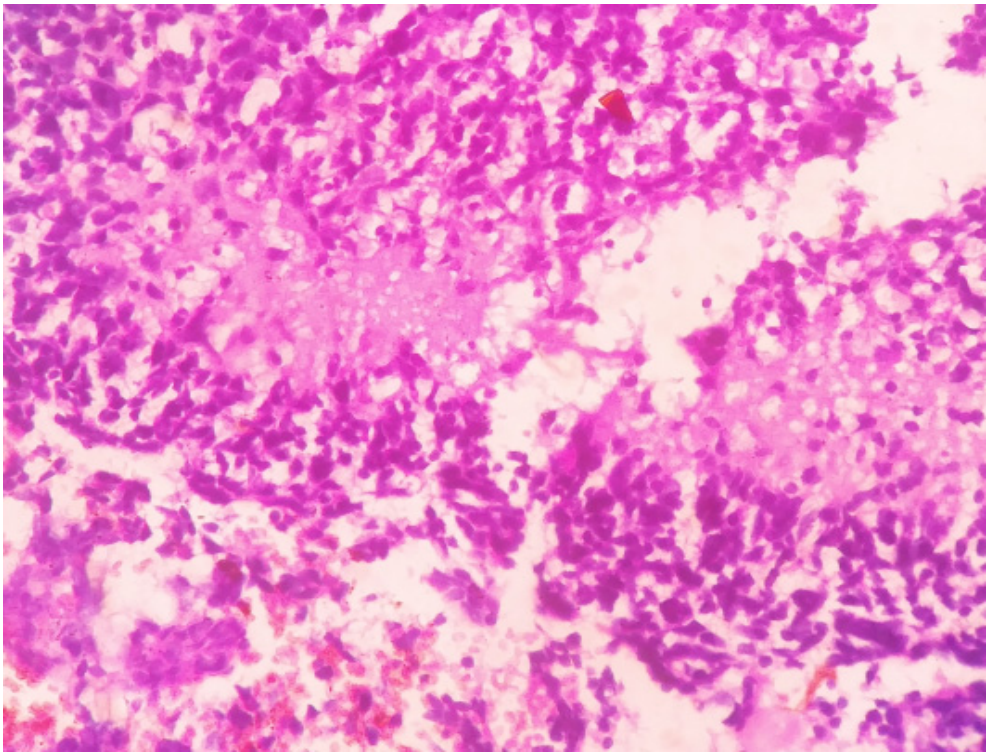


Figure 10: S87/18 Pseudopallisading necrosis of glioblastoma (H & E 400X)

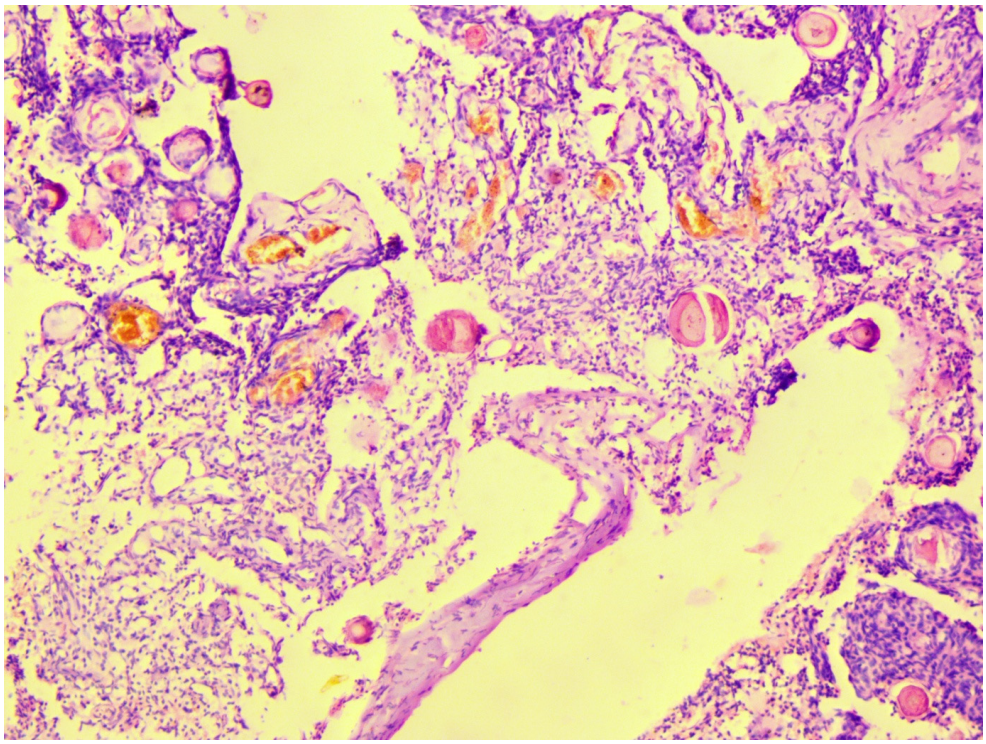


Figure 11: 1743/18 Psammamatous meningioma with psammoma bodies (H & E 200X)

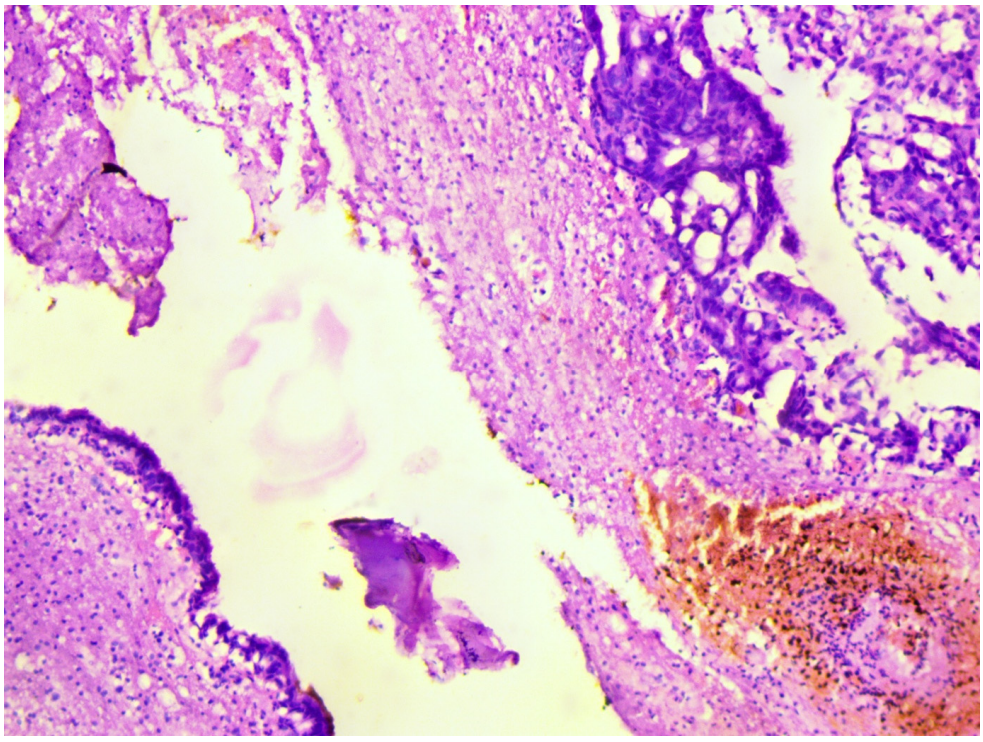


Figure 12: S303/19 Colloid cyst lined by columnar epithelium (H & E 200X)

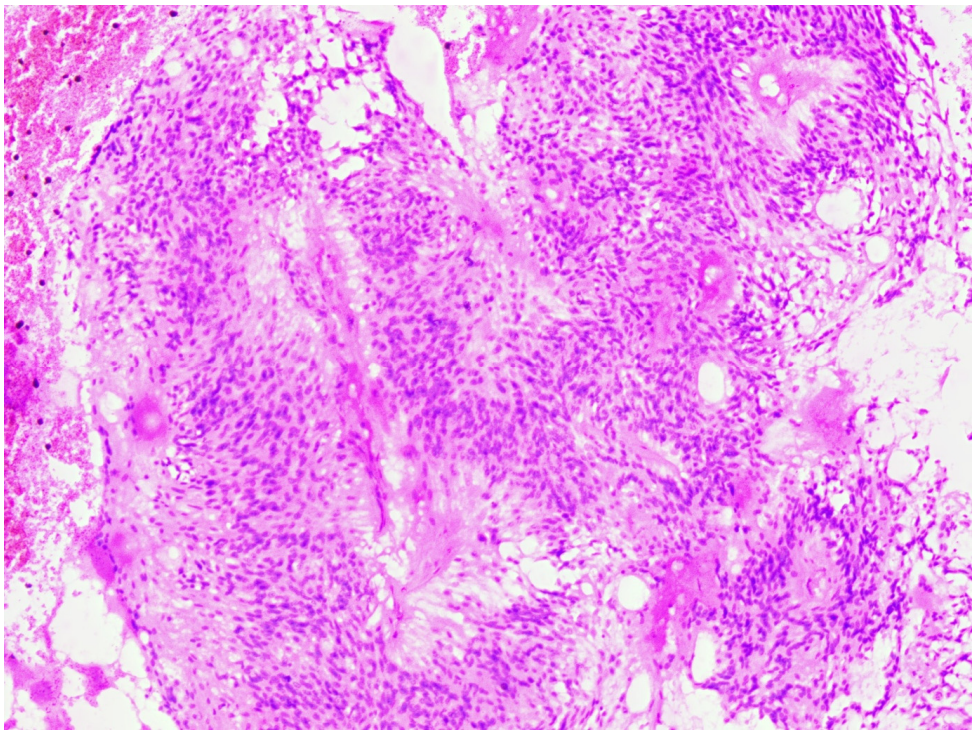


Figure 13: 975/19 Ependymoma grade II with true and pseudo rosettes (H &E 200X)

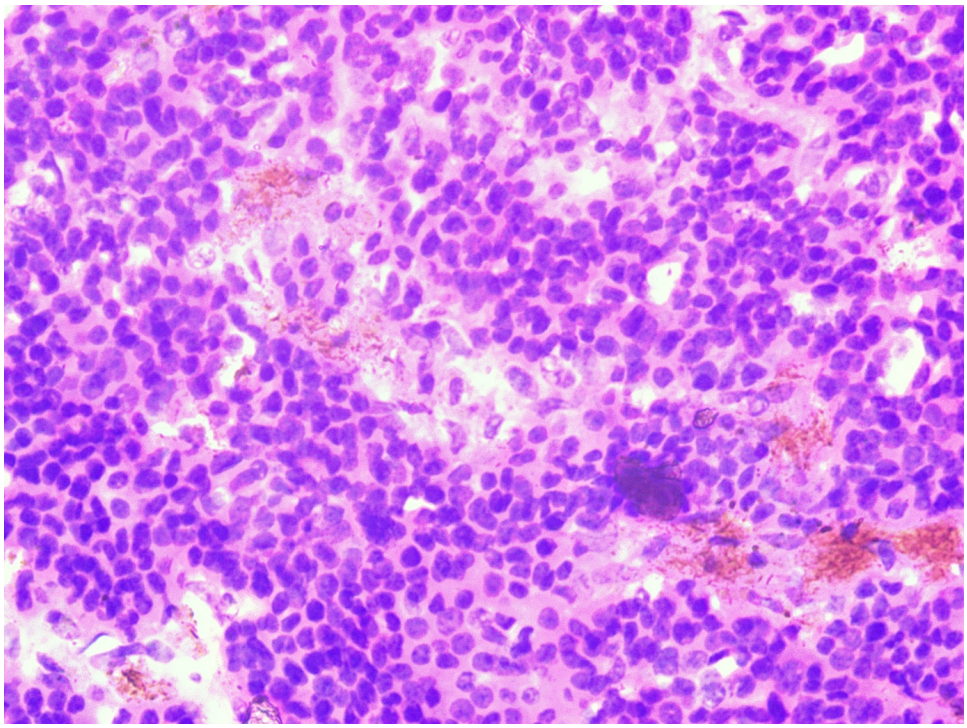


Figure 14: S1299/18 Pituitary adenoma – sheets of monomorphous cells lacking acinar architecture(H & E 400X)

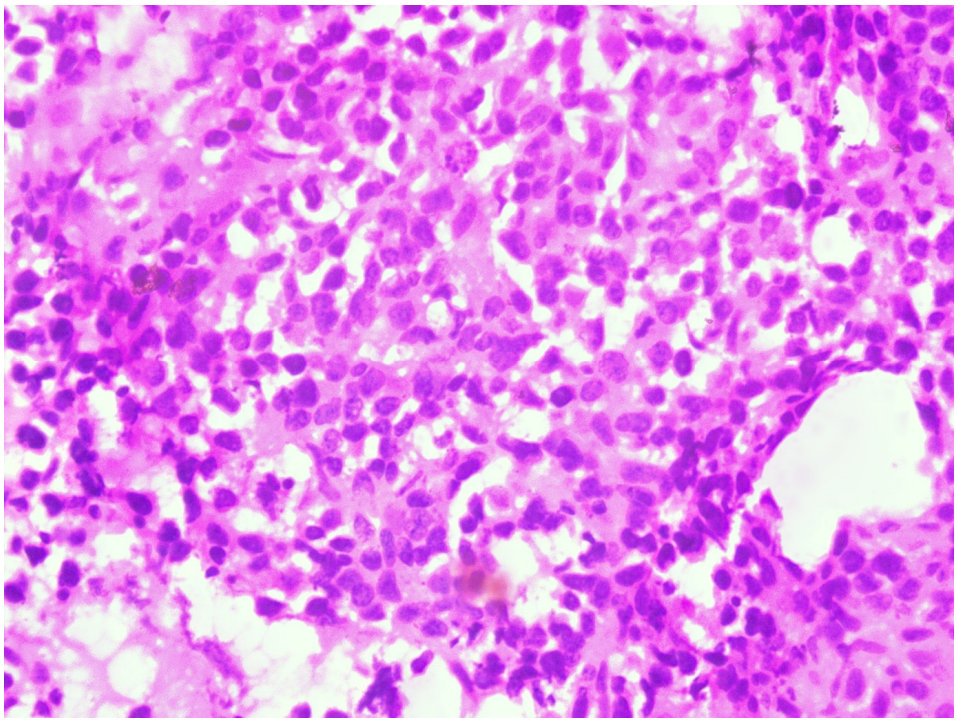


Figure 15: S 994/19 Central neurocytoma – recurrence with sheets of monomorphous round cells (H & E 400X)

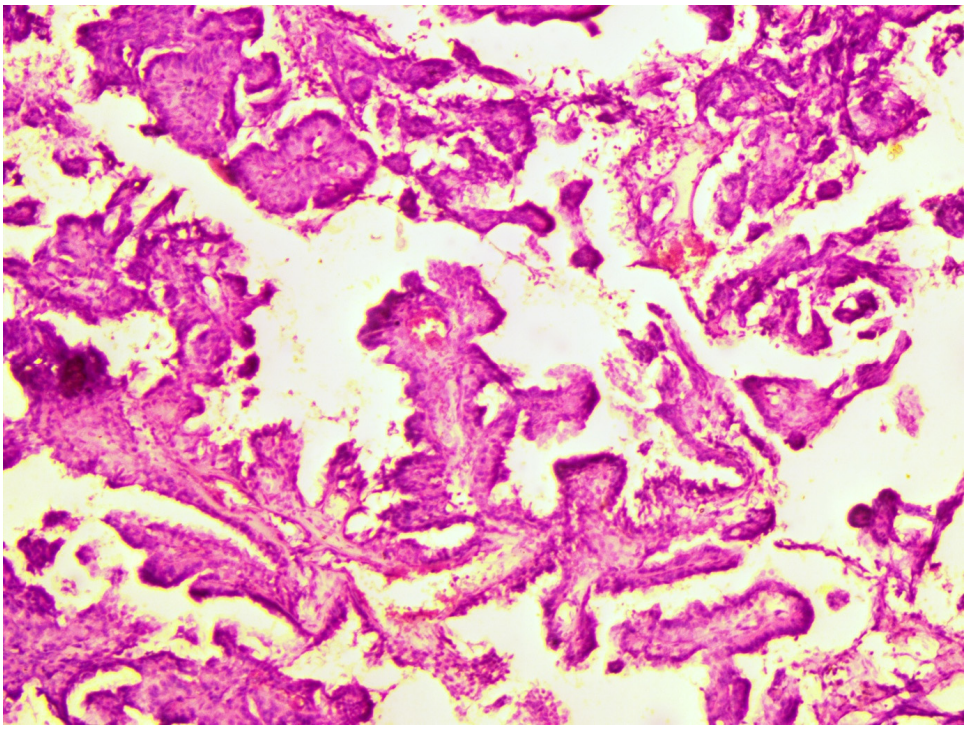


Figure 16: S 919/18 Choroid plexus papilloma with single layer of epithelial cells overlying a fibrovascular core (H & E 200X)

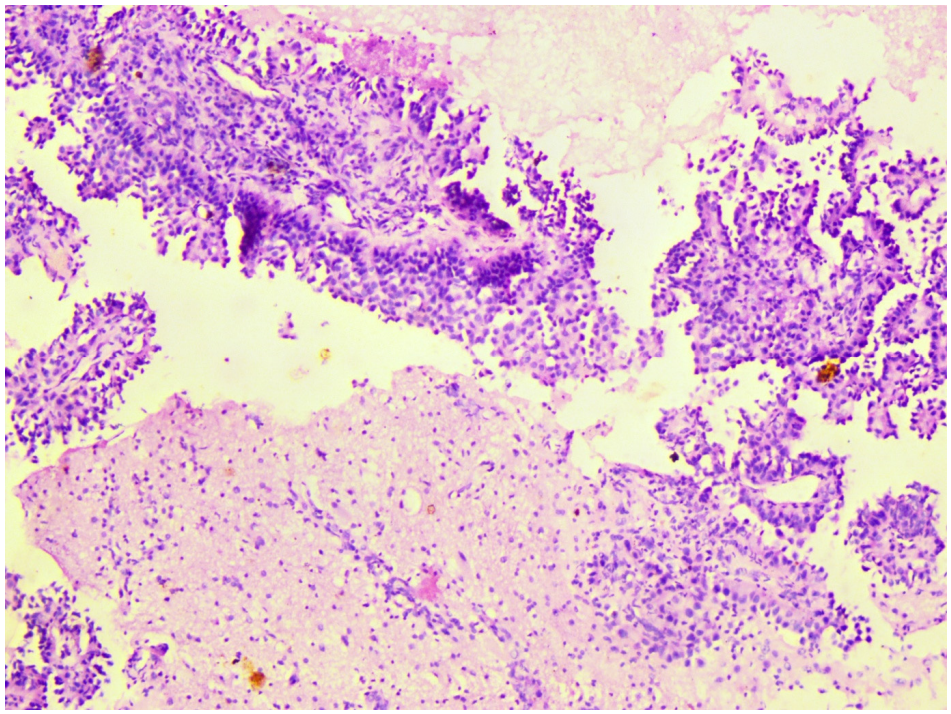


Figure 17 - S1847-18 Adenocarcinoma deposits (H & E 200X)

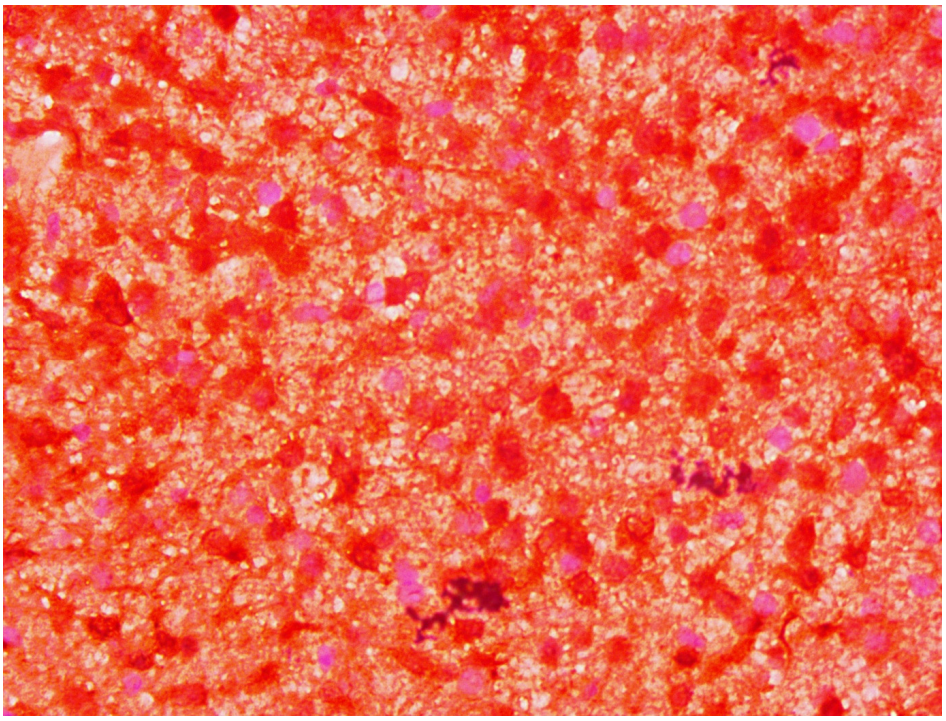


Figure 18 - S2226/18 glioblastoma -IDH1 positive

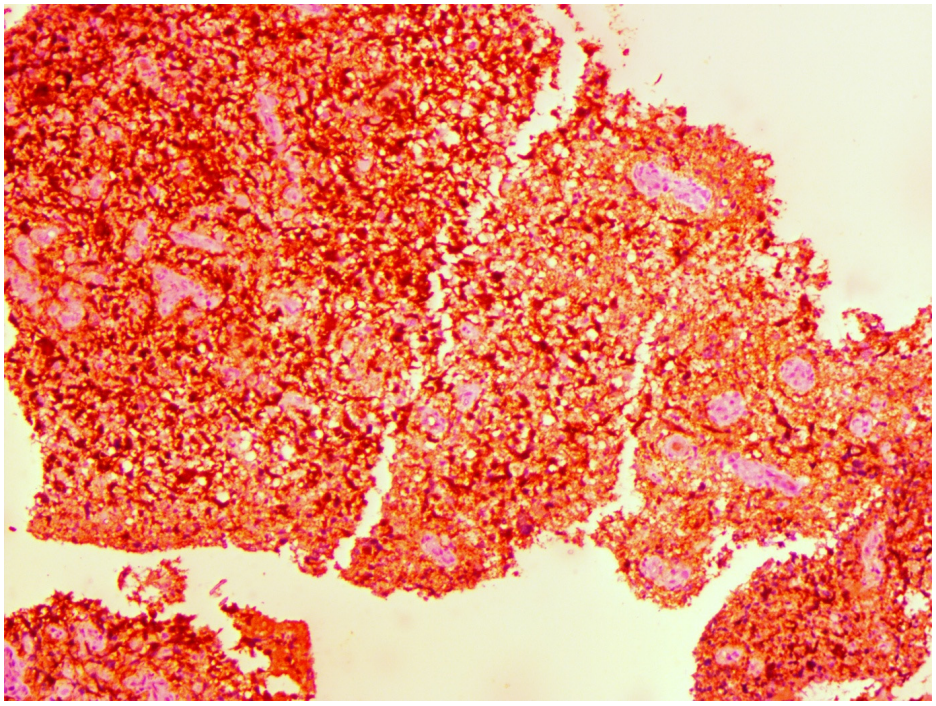


Figure 19 - 3704/18 glioblastoma -IDH 1 POSITIVE

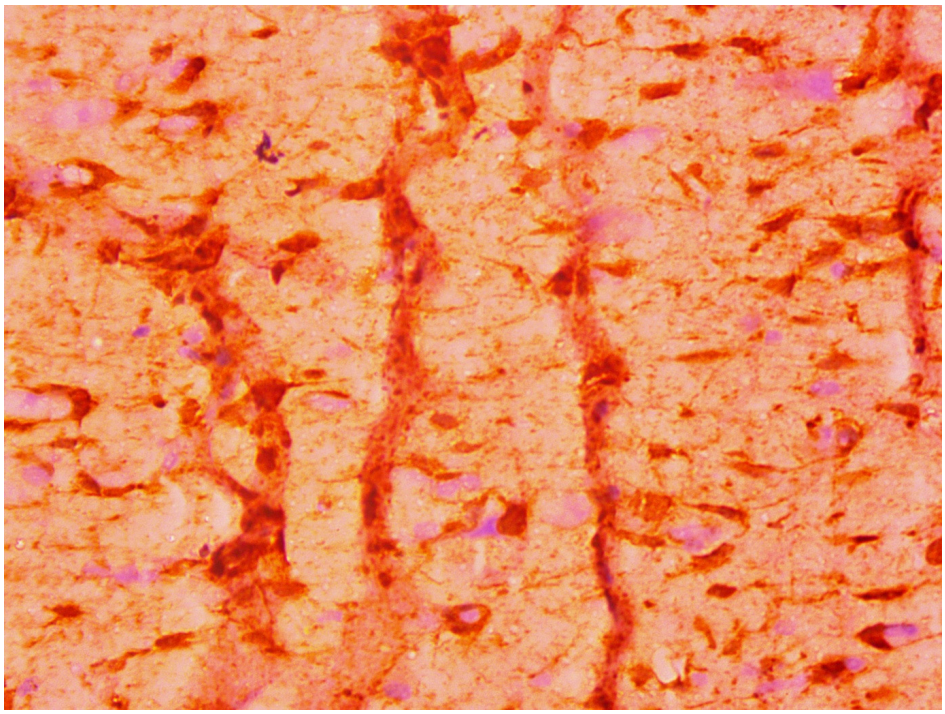


Figure 20 -S2226/18 glioblastoma -IDH1 POSITIVE

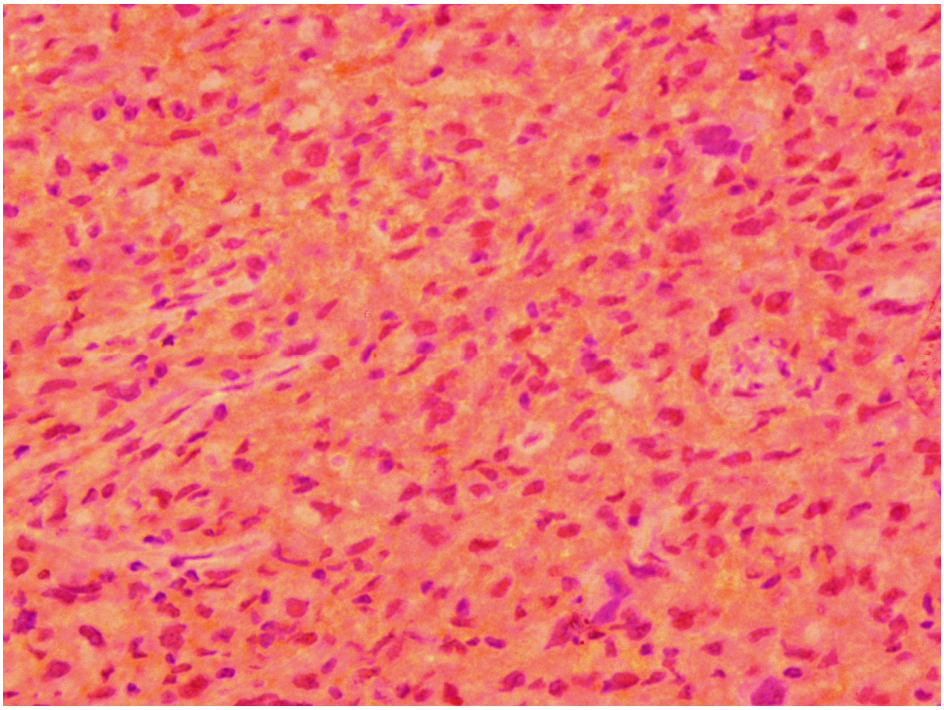


Figure 21 -S1291/18 glioblastoma -IDH1 POSITIVE

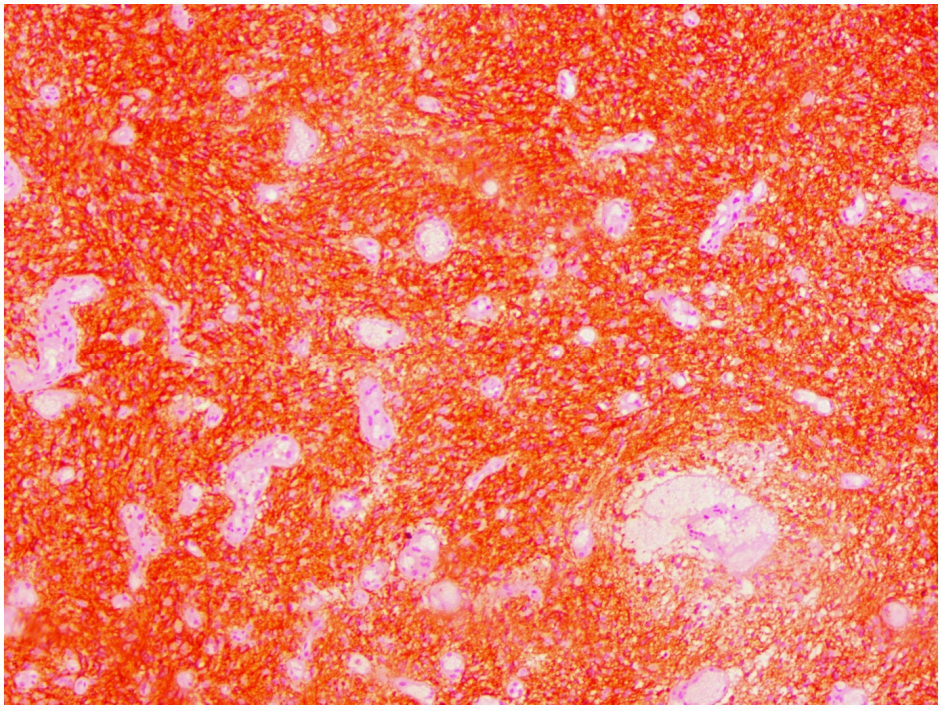


Figure 22 -S 387/18 glioblastoma IDH1 POSITIVE

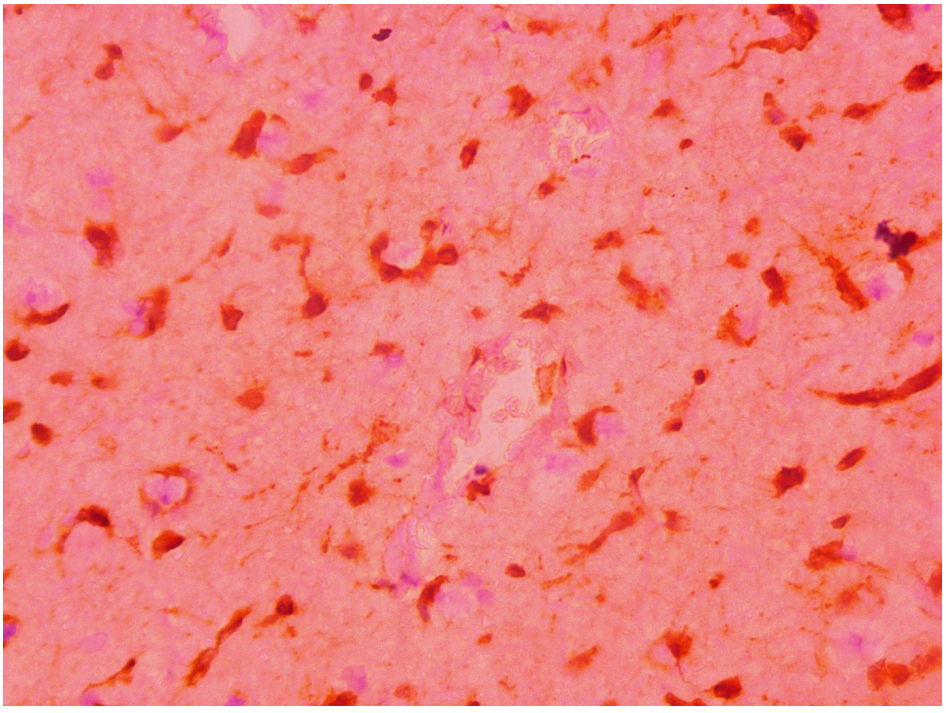


Figure 23 -S691/18 – glioblastoma -IDH1 POSITIVE

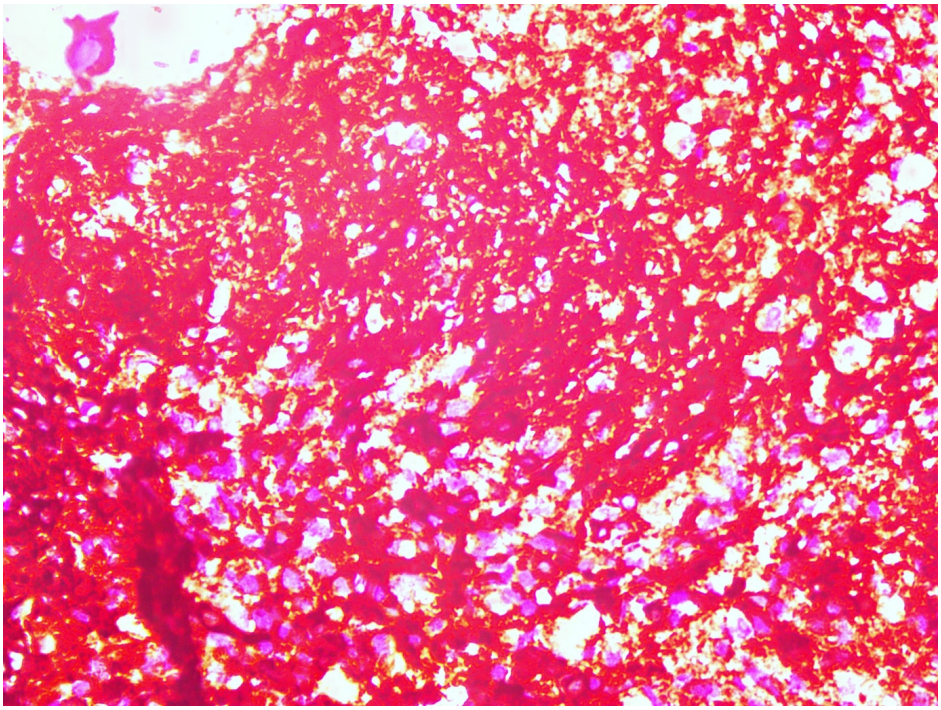


Figure 24 -S 239/19 glioblastoma EGFR STRONG POSITIVE (3+)

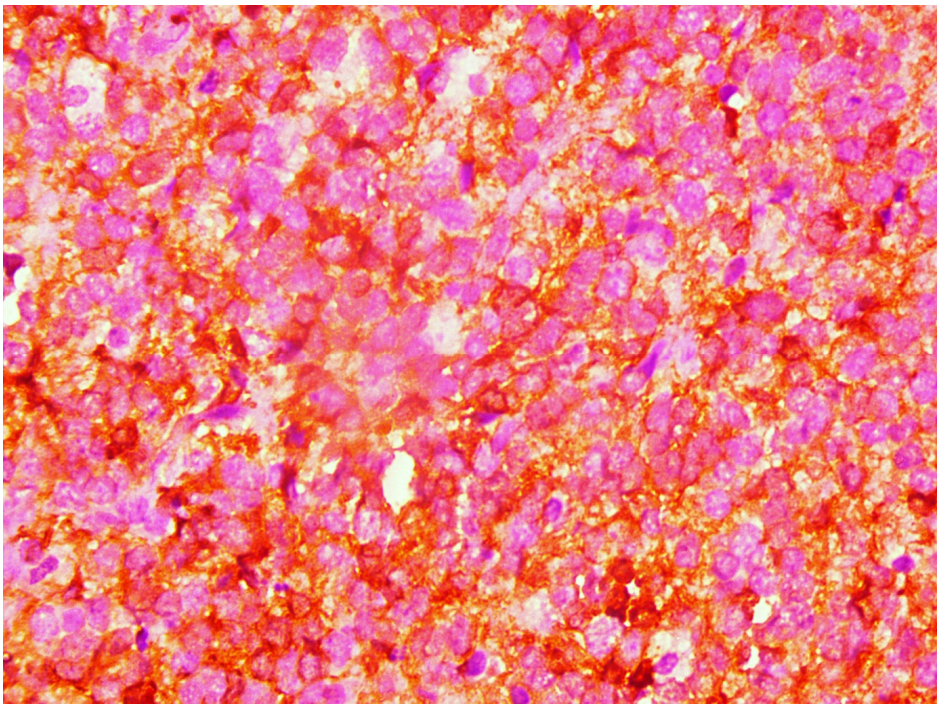


Figure 25 -S376/19 glioblastoma EGFR STRONG POSITIVE (3+)

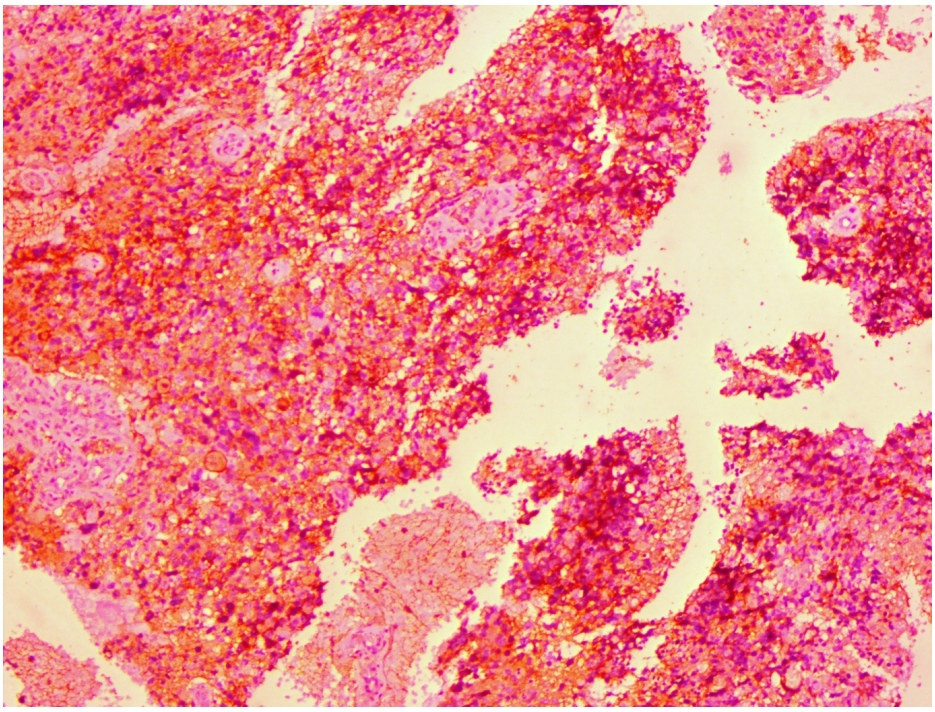


Figure 26 -S423/19 glioblastoma EGFR STRONG POSITIVE (3+)

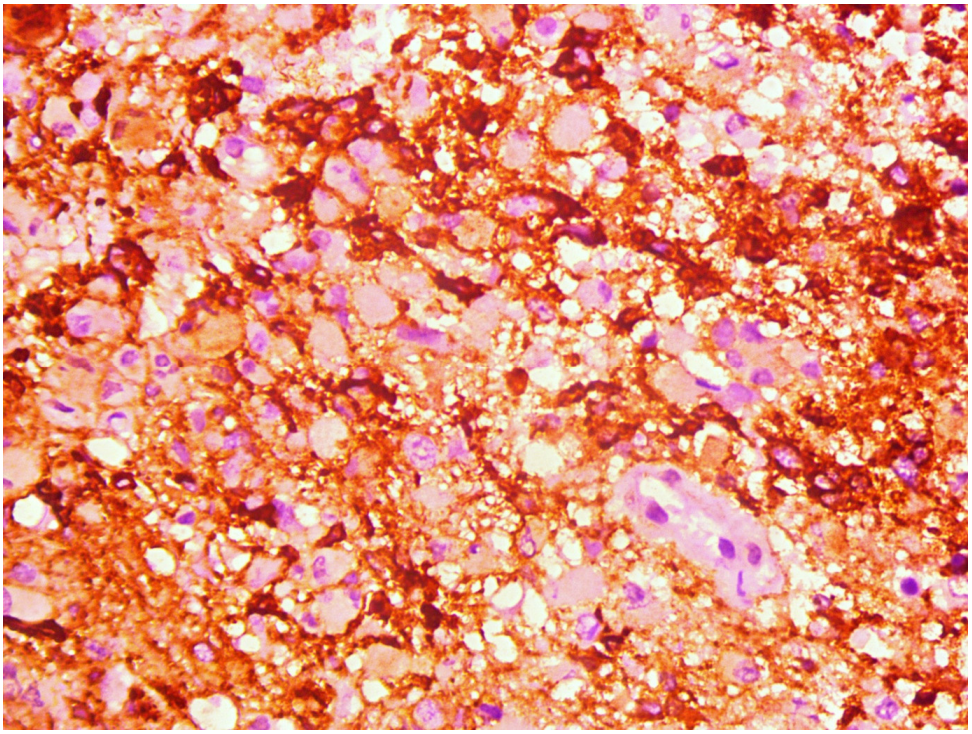


Figure 27 -S2053/19 glioblastoma EGFR WEAK POSITIVE (2+)

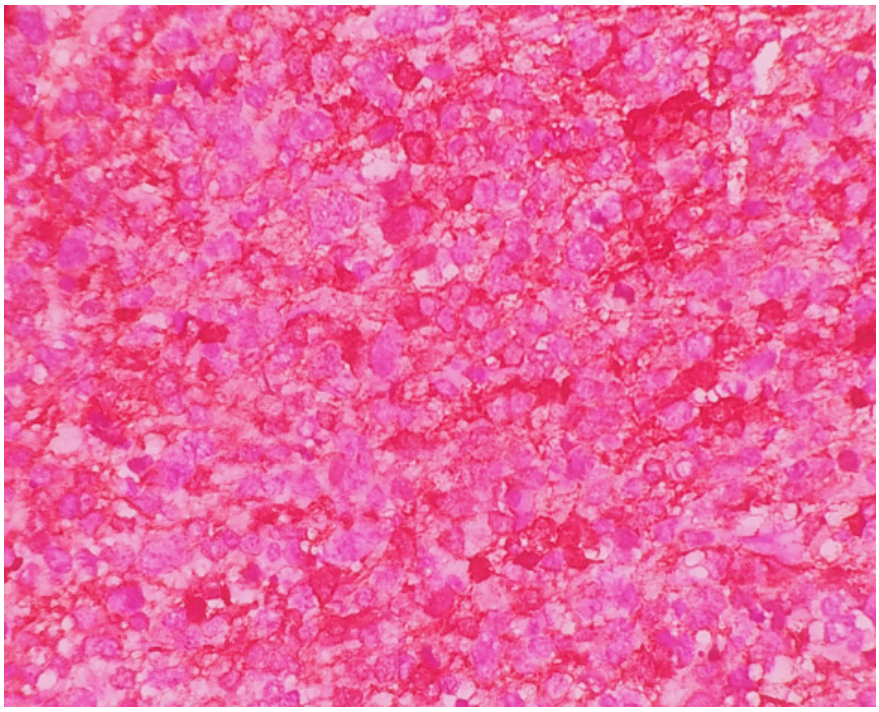


Figure 28 - S87/18 glioblastoma EGFR WEAK POSITIVE (2+)

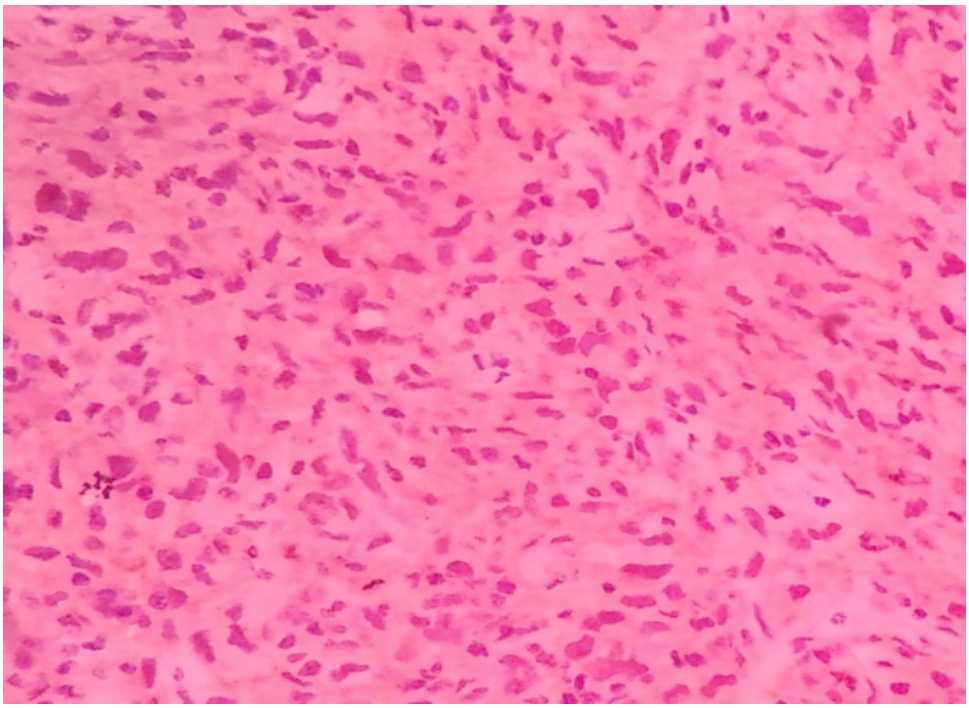


Figure 29 -S87/18 glioblastoma EGFR WEAK POSITIVE (2+)

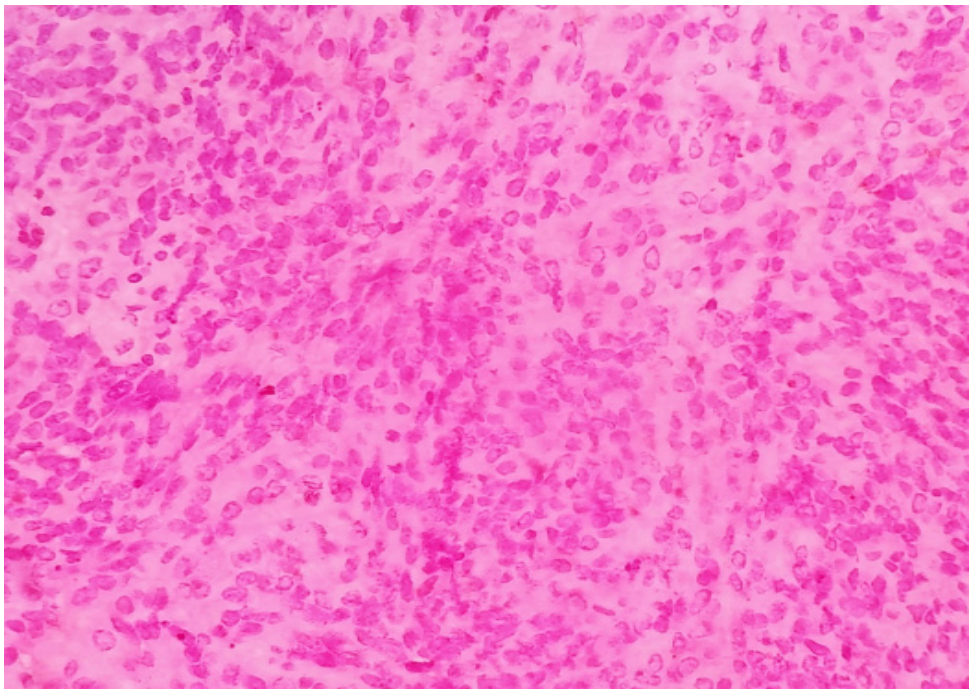


Figure 30 - S45/19 glioblastoma EGFR NEGATIVE

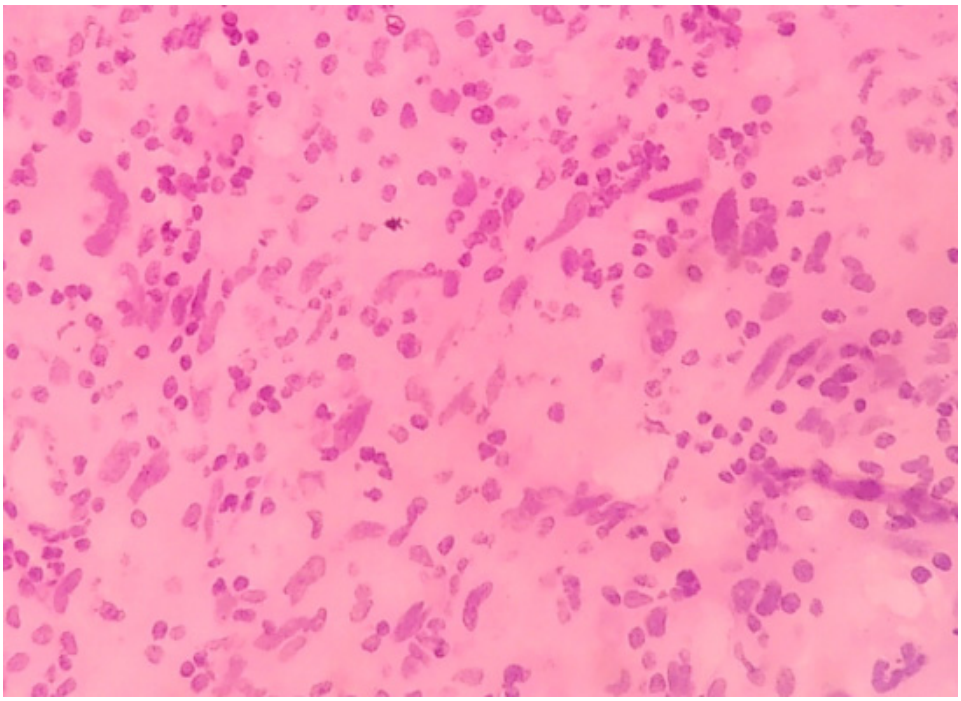


Figure 31 -S355/17 – glioblastoma IDH AND EGFR NEGATIVE

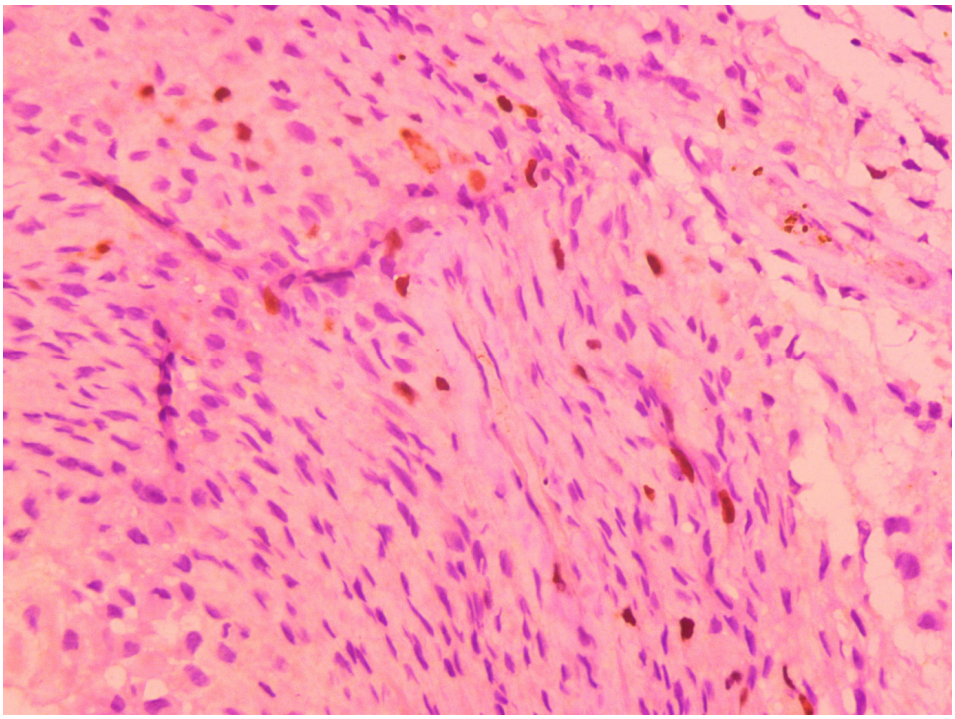


Figure 32 -S1477/19– Anaplastic astrocytoma Ki 67 – 9%

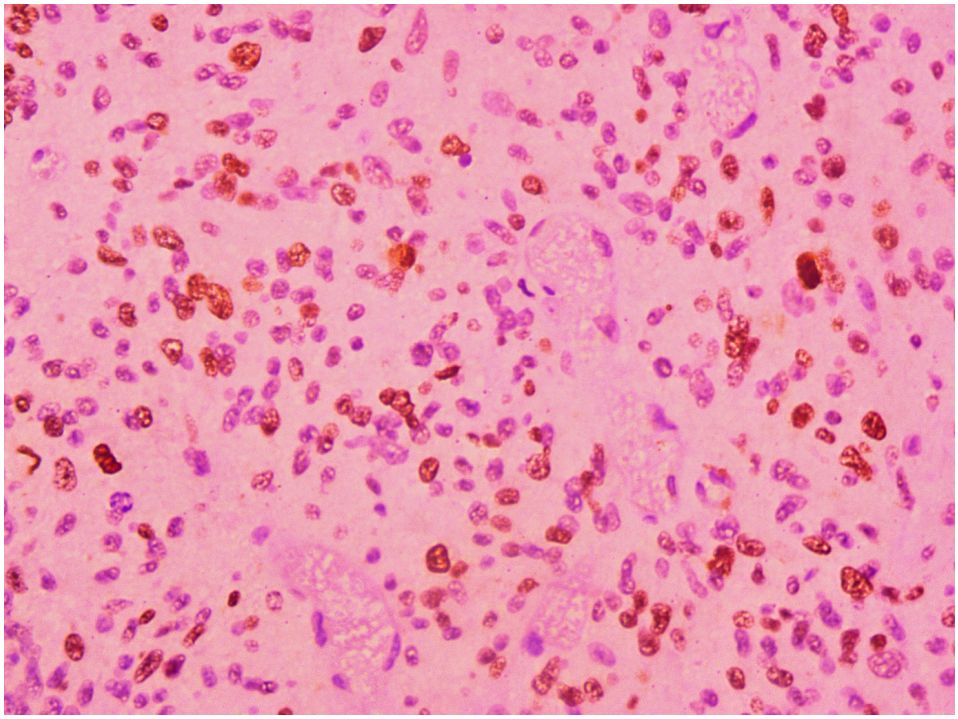


Figure 33 -S423/19- glioblastoma Ki 67 – 12%

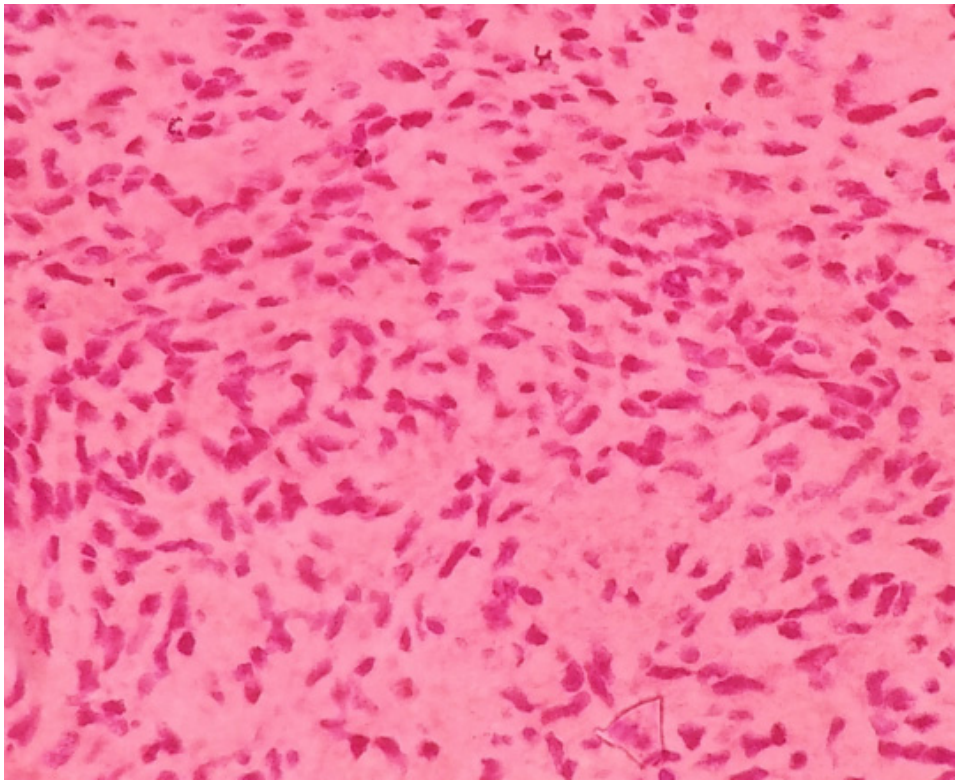


Figure 34 - S 1527/19 – glioblastoma- IDH AND EGFR NEGATIVE

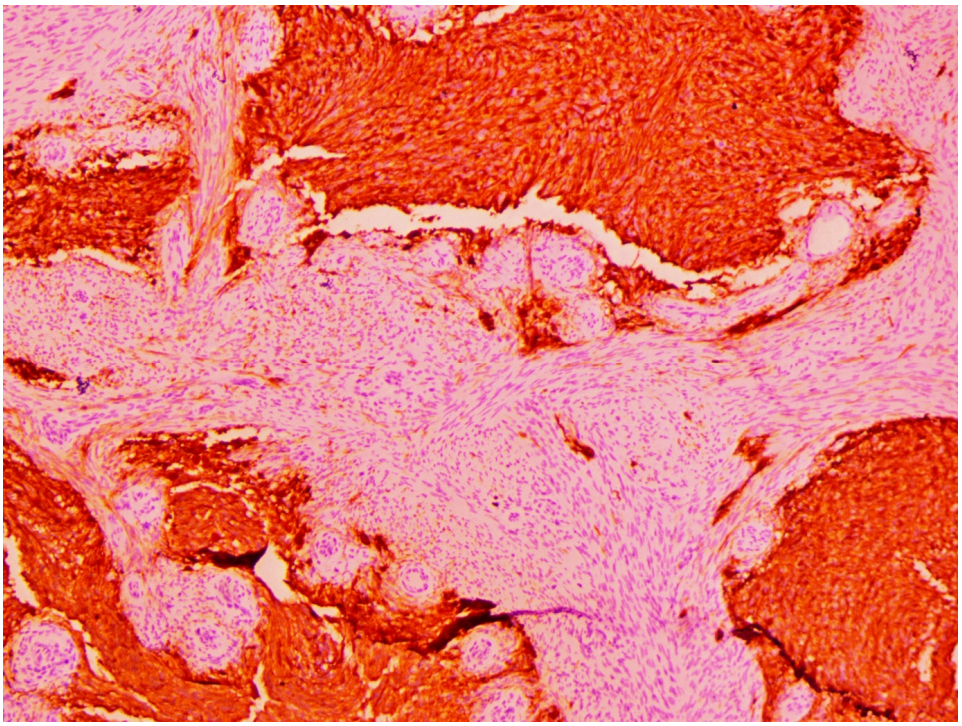


Figure 35 - S1198/18 – Gliosarcoma – GFAP positive in glial differentiation

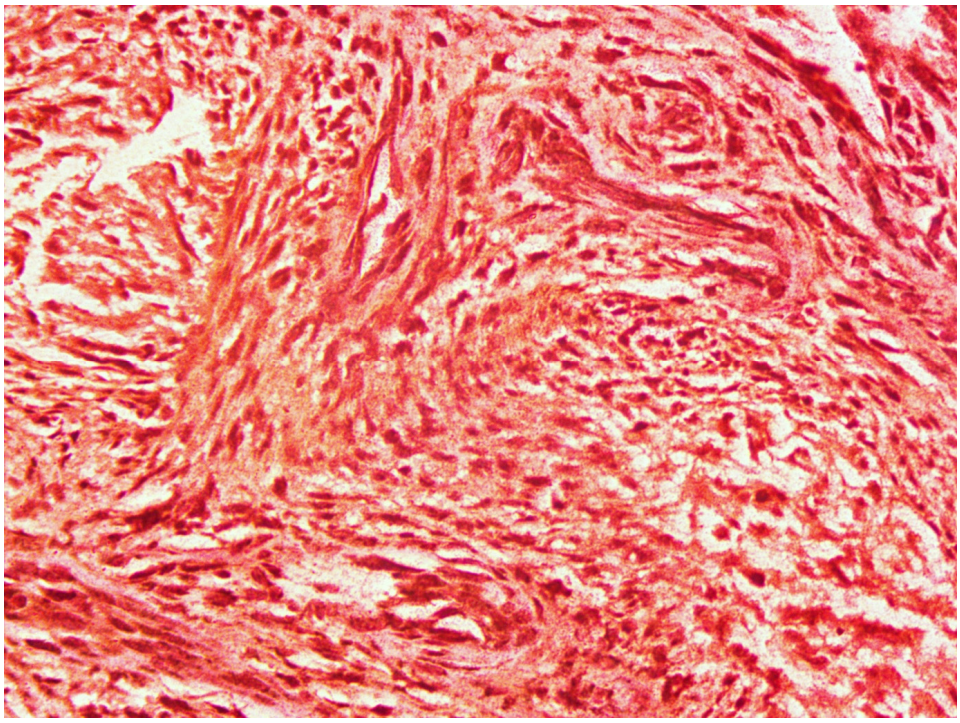


Figure 36 -S1198/18 – Gliosarcoma – reticulin positive in sarcomatoid areas

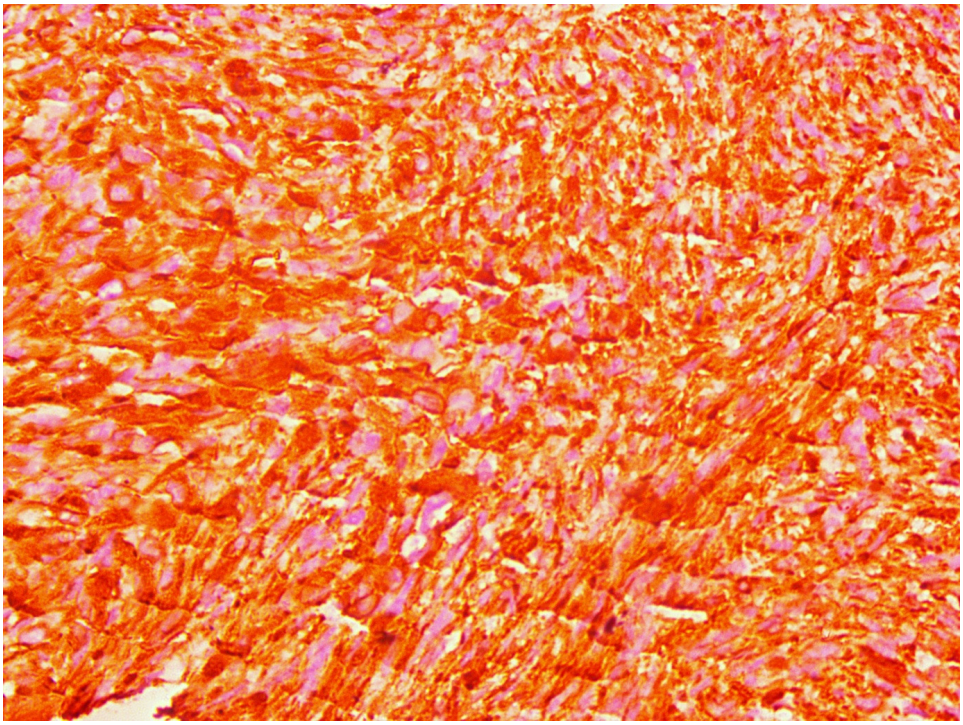


Figure 37 -S1198/18 – Gliosarcoma – Vimentin positive

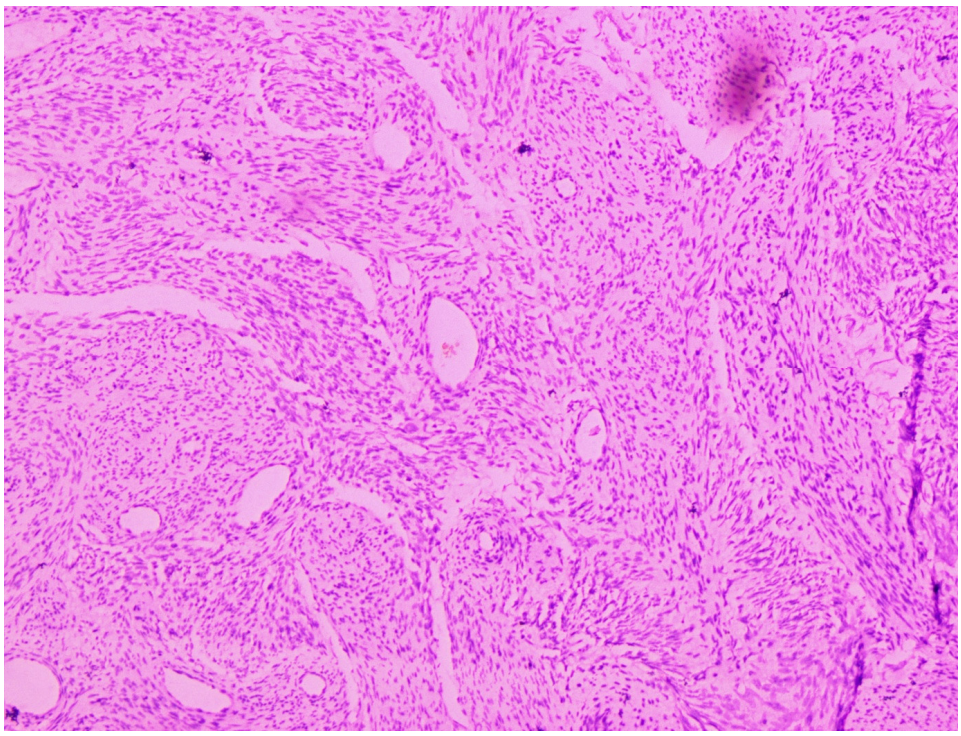


Figure 38 -S1198/18 – Gliosarcoma – IDH negative

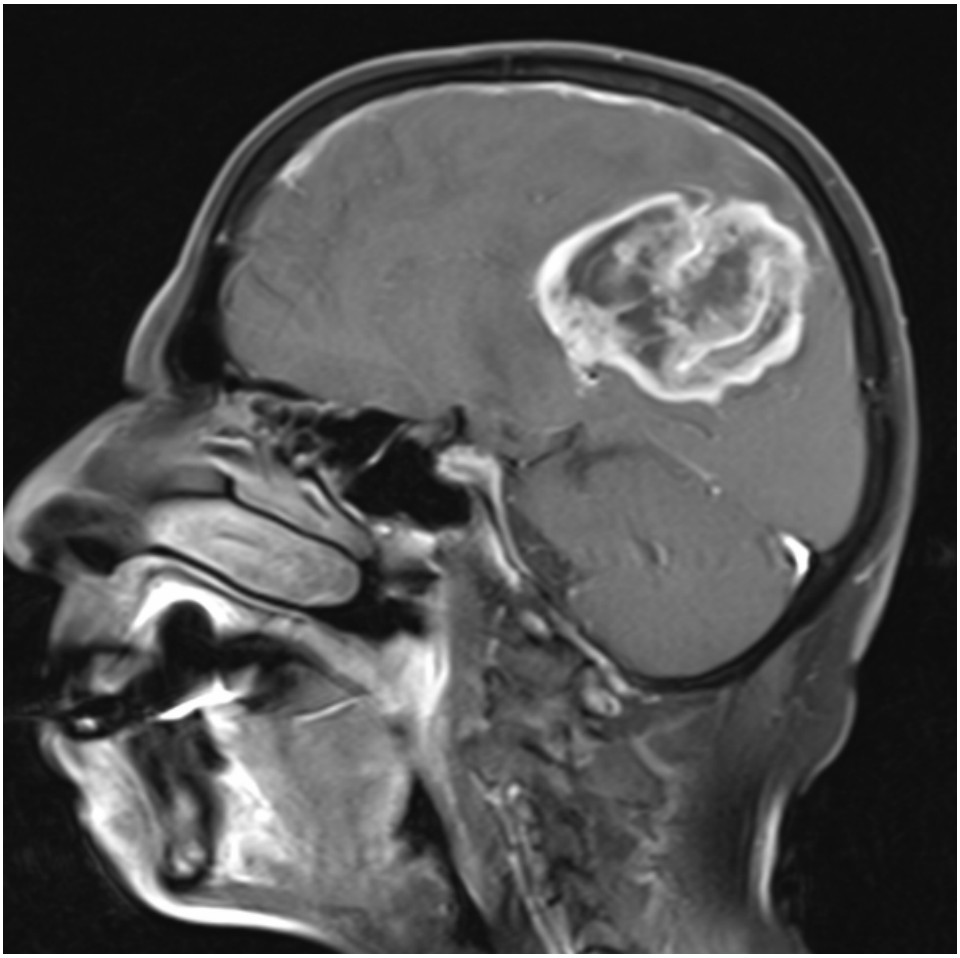


Figure 39 -MRI brain – S239/19, Pareital glioblastoma (IDH1 & EGFR negative)– ring enhancement present.

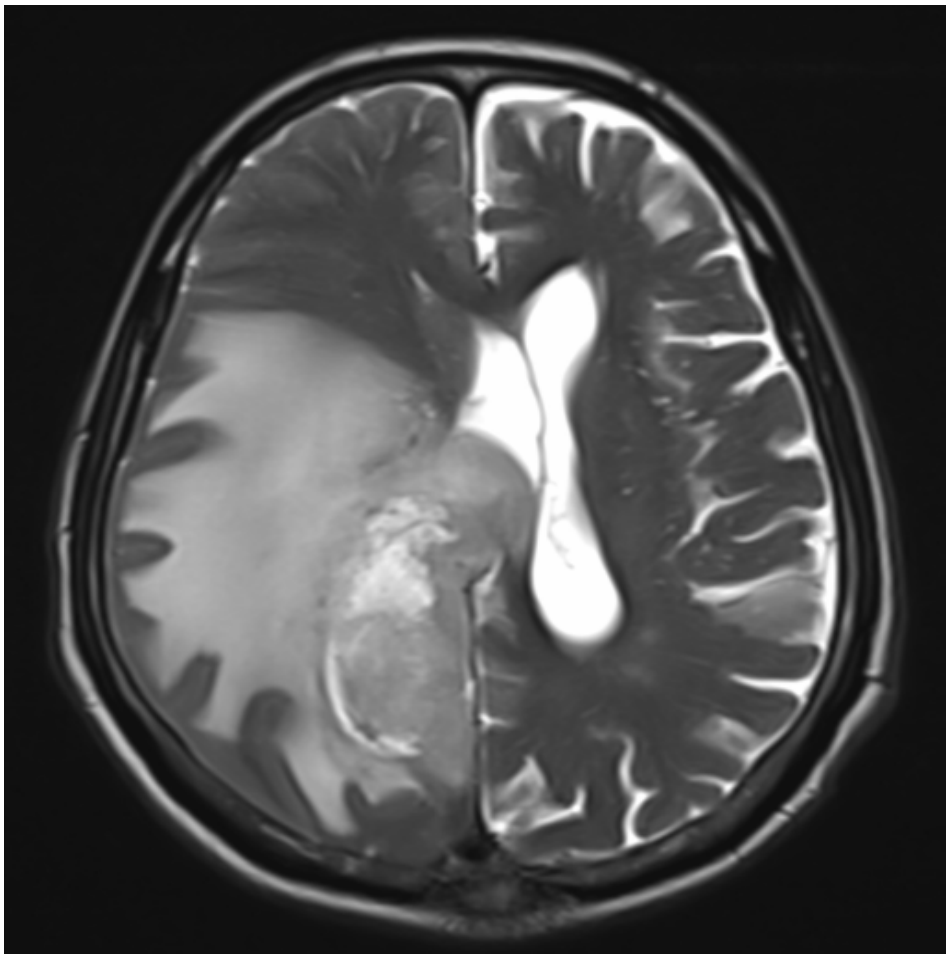


Figure 40 -MRI brain – S966/18, Pareito occipital glioblastoma (IDH1 & EGFR negative) – ring enhancement present

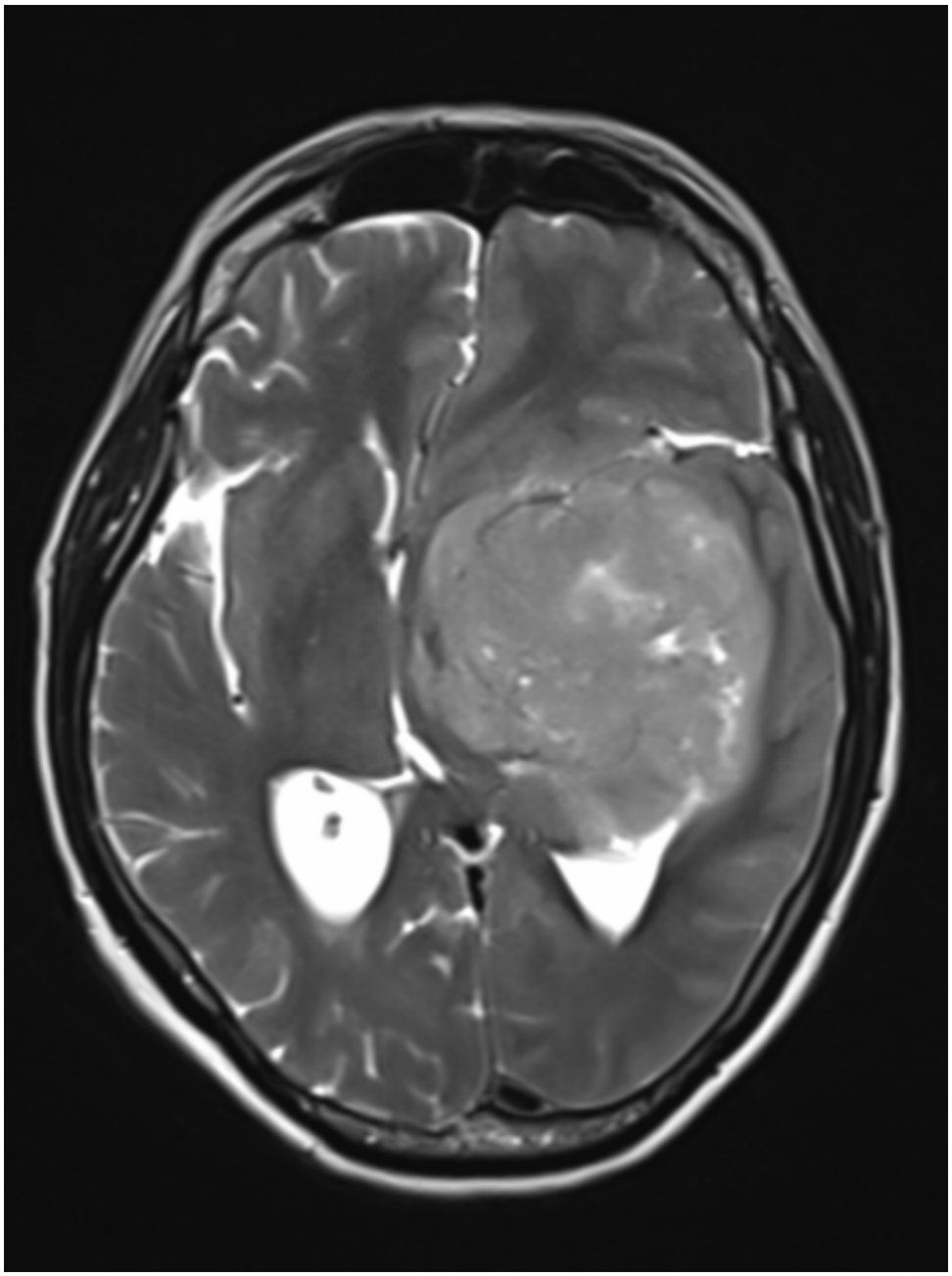


Figure 41 -MRI brain – S1527/19, Pareital glioblastoma (IDH1 & EGFR negative) – ring enhancement absent

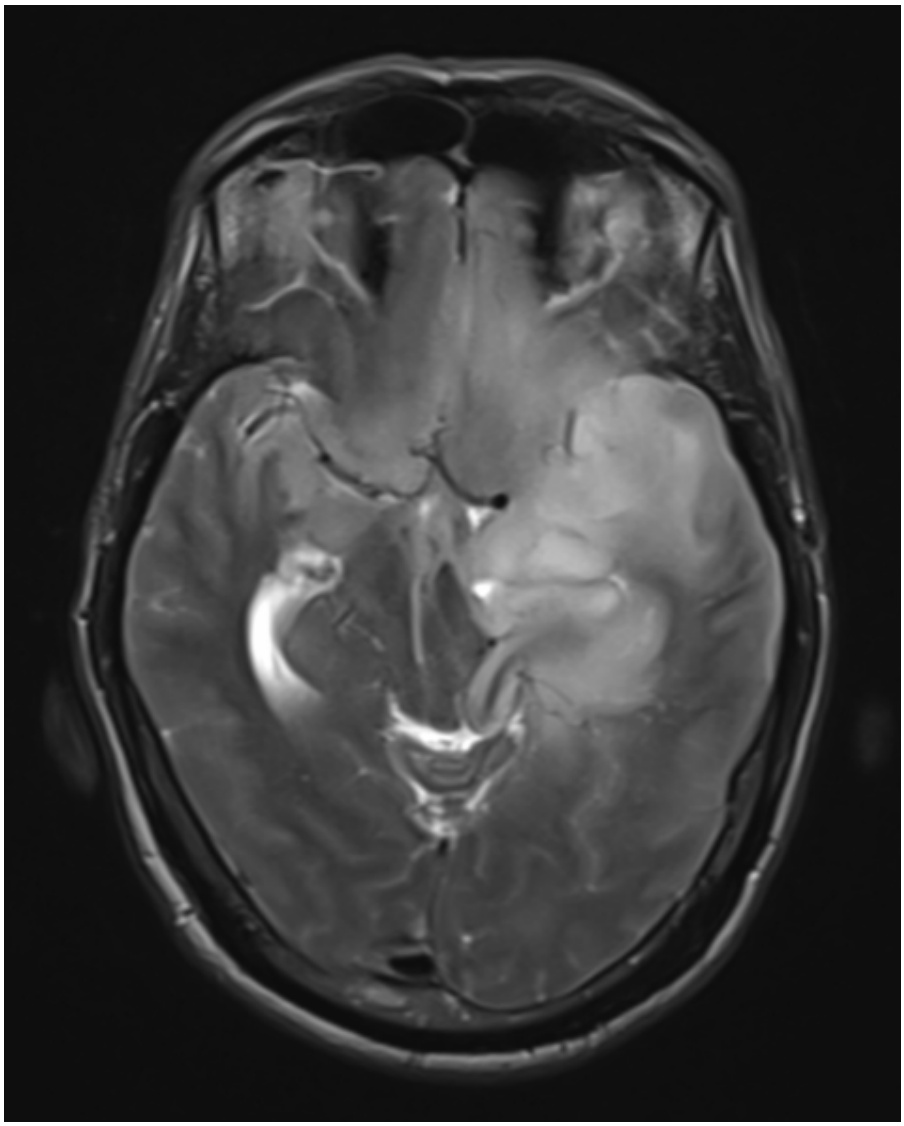


Figure 42 -MRI brain – 3704/18, Temporal glioblastoma (IDH1 positive) – ring enhancement absent

DISCUSSION

DISCUSSION

Brain tumors are among the rarest malignancies of the human body accounting for about less than 2% of all the tumors[74]. Their privileged status due to location, complex functionality and their rarity in causing extra-neural metastasis (<2%) make these tumors unique[184]. The treatment strategy of all malignant brain tumors, especially the gliomas has not undergone significant changes since many decades. The recent molecular advances in diagnosing the brain tumors have paved the way for a better prognosis. At this juncture, the ability to predict the prognosis by the new molecular marker – IDH1/2 has not only improved diagnostic accuracy but also opened doors for discovery of new drugs –IDH inhibitors and vaccines that have entered drug trials.

The presence of IDH 1/2 mutation is an independent prognostic, diagnostic and predictive marker for the gliomas. There is an increasing need to find a cure for gliomas as the population affected mainly ranges from young to middle aged adults. This invariably has a major bearing on the death adjusted life years (DALY) compared to other tumors. Also, the median age of presentation of the Indian population affected is almost a decade younger than the western population. This might be due to the presence of a higher proportion of younger population and a lower life expectancy[185].

According to the Global Burden of Diseases, Injuries, and Risk Factors Study 2016, the incidence rates of CNS cancers that were age standardized increased globally by 17.3%[186]. The countries that topped the list with increased incidence rates were China, the USA and India. The reported incidence has a significant variation in

geographical distribution which might be due to discrepant reporting practices and diagnostic techniques. Hence in view of rising incidence of gliomas in India, in order to standardize the overall protocol of treatment, proper screening and confirmatory tools are needed to improve the diagnosis of gliomas.

The present study analyses the histopathology of 50 cases of gliomas and the expression of IDH1 mutation in glioblastomas. An attempt has been made to study the incidence of occurrence of gliomas with respect to age, gender, location and lateralisation. Also, evaluation of IDH1 and EGFR expression in glioblastomas, categorization of them into primary and secondary types has also been done. To the best of our knowledge, this is the first time this study population has been subjected to the IDH1 expression analysis of glioblastomas in our Department of Pathology.

Studies have also been done to prove the comparable efficacy of immunohistochemistry and genetic sequencing namely Takano S et al (2016)[187] and Agarwal S (2013)[188].The above studies reported the successful use of antibody R132 (IDH1) in immunohistochemistry for glioblastomas and its role as a valuable substitute for genetic sequencing. Hence our study too reiterates the same fact regarding usage of IHC in glioma diagnosis.

Purkait S *et al* (2016)[189]study states that the gliomas with IDH mutation had a longer progression free survival. Also, the study has divided the IDH wild type glioblastomas into three groups by a combinatorial assessment of MGMT promoter mutation and TERT mutation. Group 1 cases had MGMT methylation only and accounted for the best outcome. Group 3 cases had TERT mutation only and showed the worst outcome. The prognosis was intermediate in group 2, where neither MGMT/

nor TERT was present or both were present. The presence of TERT correlated with older age and presence of EGFR mutation in 74% of these cases. This study further states that EGFR mutation is the commonest genetic alteration that occurs in 39.5 % of the glioblastomas. Hence in our study we decided to analyze the expression of these markers.

In the present study, 97 brain space occupying lesions have been diagnosed and categorised accordingly. Of these, the most common were neoplastic brain lesions accounting for 91.7% and non-neoplastic lesions comprising 8.3%.

The comparison is shown in the below table 24. This was comparable to the reports of Patil M B et al., (2018) [190]study.

Table 24: Distribution of neoplastic and non-neoplastic lesions of brain SOLs.

Study	Total cases	Neoplastic lesions (%)	Non-neoplastic lesions (%)
Patil M B et al (2018)[190]	**124**	**83.9**	**16.1**
Present study (2019)	**96**	**91.7**	**8.3**

Of the neoplastic lesions, primary CNS tumors were 97% and secondary brain tumors accounted for a mere 3%. This was comparable with Jalali et al (2008)[191] and Aryal G et al (2000).[192]

Table 25: Distribution of primary and secondary CNS tumors

Study	Total neoplastic brain lesions	Primary CNS tumors n (%)	Secondary CNS tumors n (%)
Jalali et al (2008)[191]	**656**	**580 (88.5)**	**78 (11.5)**
Aryal G et al (2000)[192]	**57**	**49 (86)**	**8 (14)**
Present study (2019)	**88**	**85(96.5)**	**3 (3.5)**

Of the non-neoplastic lesions, cysts formed the majority accounting for 5.2%. Among the primary brain tumors, the most common type of brain tumor was glioma (56.8%) followed by meningioma (27.3%). This is well supported by many studies namely Masoodi et al (2012)[193], Ahmed et al (2000)[194], Mondal. S et al (2016)[195] and Aryal G et al (2000)[192]

Table 26: Distribution of Primary CNS tumors

Study	Primary CNS tumors	Gliomas n (%)	Meningiomas n (%)
Mondal S et al (2016)[195]	**130**	**54(41.5)**	**20 (15.3)**
Aryal G et al (2000)[192]	**57**	**22 (38.6)**	**8 (14)**
Masoodi et al (2012)[193]	**106**	**53 (50)**	**53 (50)**
Ahmed et al (2001)[194]	**1110**	**538 (54.01)**	**234 (23.49)**
Present study (2019)	**85**	**50(56.8)**	**24 (27.3)**

The most frequent histological type of brain tumors among the primary brain tumors were the meningiomas accounting for 28.2% with glioblastomas coming a close second comprising 24.7%. A study- Lee et al (2005)[196] also had similar reports.

Table 27: Frequencies of histological types of Primary CNS tumors

Study	Primary CNS tumors	Meningiomas n (%)	Glioblastomas n(%)
Lee et al (2005)[196]	**3305**	**1026 (31.2)**	**316 (19.3)**
Present study (2019)	**85**	**28.2 (24)**	**25.7 (21)**

The least common histological type of brain tumors of our study were the embryonal tumors with a frequency of occurrence of 1.2% which was comparable

with reports of Lee et al (2005)[196], which reported only 2.2% frequency of occurrence of embryonal tumors.

Table 28: Frequencies of histological types of Gliomas

Study	Gliomas	Glioblastoma n (%)	Anaplastic astrocytoma n (%)	Diffuse astrocytoma n (%)	Pilocytic astrocytoma n (%)	Ependymoma n (%)
Jung KW *et al* (2016)[197]	**1160**	**474 (40.4)**	**118 (10.1)**	**83 (7.1)**	**67 (5.7)**	**97 (8.3)**
Present study (2019)	**50**	**21(42)**	**9 (18)**	**11 (22)**	**3(6)**	**6(12)**

Among the gliomas, the grade IV glioblastoma was the commonest histological type accounting for 42% and the least common was pilocytic astrocytoma comprising 6% of all gliomas. The frequency of occurrence was comparable to the study by Jung KW *et al* (2016)[197], where the commonest glioma was glioblastoma accounting for 40.4%.

Table 29: Distribution of Gliomas according to the WHO grade

Study	Gliomas	Grade I n (%)	Grade II n (%)	Grade III n (%)	Grade IV n (%)
Jaiswal *et al* (2016)[198]	**1468**	**89(6.1)**	**22(1.5)**	**112(7.6)**	**558(38)**
Ghangoria S *et al* (2014)[199]	**16**	**1(6.2)**	**0**	**11(68.7)**	**4(25)**
Present study (2019)	**44**	**3(6.8)**	**11(25)**	**9(20)**	**21(47.7)**

An analysis was done to assess the distribution of gliomas as per the WHO grade. This was compared to the study Jaiswal et al (2016)[198] and Ghangoria S et al (2014)[199]

While Grade IV accounted for the most common glioma, Grade II had a decreased frequency of occurrence in both the studies, compared to the present study.

Age wise distribution of gliomas:

Age wise distribution of gliomas showed increased occurrence of gliomas in the adult age group of 40-59 years accounting for 46%. This was comparable to the Jalali et al (2008)[191] where the most common age group of presentation of all gliomas was 41-60 years. The frequency of occurrence of gliomas in this age group was 37.3%

Table 30: Age wise distribution of Gliomas

Study	Total cases	Commonest age group 40-59 years n (%)	Least Common age group >60 years n (%)
Jalali *et al* (2008)[191]	**337**	**126 (37.3)**	**23 (6.8)**
Present study (2019)	**50**	**23(46)**	**5 (10)**

Similarly, the least common age group of occurrence of gliomas was above 60 years, accounting for 10% of gliomas which was comparable to the study Jalali et al (2008)[191] in which it was 6.8%.

Table 31: Age wise distribution of Glioblastomas

Study	Total cases	Commonest age group 50-70 years n (%)
Rasmussen, B. K *et al* (2017)[200]	**1364**	**767 (52)**
Li K *et al* (2018)[201]	**28835**	**(46.3)**
Present study (2019)	**21**	**11 (52.4)**

Glioblastomas had a striking predilection in the age group of 50 -59 years with frequency of occurrence of 38.1%. A total frequency of 52.4% was observed in the age group of 50 -70 years.

Rasmussen, B. K *et al* (2017)[200] and Li K et al (2018)[201] where a total of 28835 glioblastomas were studied from SEER 9 databases. Here the commonest age group was 40 -64 years with a frequency of occurrence of 46.3 %.

Table 32: Distribution of Pilocytic Astrocytoma among Pediatric CNS tumors

Study	Total Pediatric CNS tumors	Pilocytic astrocytoma n (%)
Khan MA et al (2012)[202]	**102**	**22(21.6)**
Present study (2019)	**6**	**2 (33.3)**

In our study, the pilocytic astrocytomas comprised of 33.3% of the total pediatric CNS tumors diagnosed. This was nearly comparable with Khan *et al.* (2012)[202] study composed of 102 pediatric CNS tumors.

Gender distribution of gliomas:

Of the fifty gliomas received, 31 (62%) were present in male. So, a definite male preponderance was identified. This was comparable with various other studies, few of which are compared below.

In our study, the anaplastic astrocytomas a sex ratio (M: F) of 1:1.2. This slight female preponderance in anaplastic astrocytomas can be attributed to the limited number of cases received.

The M: F ratio in glioblastomas is 2.5:1 which has a striking male predilection. This is supported by the study Jaiswal et al (2016)[198] which also have a similar M: F ratio of 2.4:1.

Another comparison of gender wise distribution of gliomas according to their histological types is shown in the table

Table 33: - Gender wise distribution of Anaplastic Astrocytomas and Glioblastomas

Study	Anaplastic astrocytomas	Male n (%)	Female n (%)	Glioblastomas	Male n (%)	Female n (%)
Jung KW *et al* (2016)[197]	**9**	**5(55.5)**	**4(44.5)**	**523**	**287(54.8)**	**236(45.2)**
Present study (2019)	**9**	**4(44.5)**	**5(55.5)**	**21**	**15(71.4)**	**6(28.6)**

The slight differences in incidence proportions can be attributed to the narrow range of cases.

Significance of gender in glioblastomas:

The significance of gender predilection in gliomas, especially glioblastomas can be attributed to the fact that men have a shorter cancer specific survival rate. According to Tian M et al (2018)[203], cancer specific survival rates (CSS) of 6586 patients were analyzed by the data obtained from SEER database over a period of eight years. Among the patients 61.5% were men and 38.5% were women. The five-year CSS rate revealed that men had lower rate of 6.8% as against 8.3% in women. Thus, gender has prognostic value in determining risk of glioblastomas, thereby paving way for researching the role of sex hormones in gliomagenesis.

Location wise distribution of gliomas:

In our study, out of the 50 gliomas, 44 occurred in the cerebrum, accounting for 88%.

This is in support of the observations made by Larjavaara S et al (2007)[26] study, where 331 gliomas were studied, out of which 86% occurred in the cerebrum and 14% in other sites.

Table 34: - Location wise distribution of Gliomas

Lobe	Larjavaara S et al (2007)[26] (%)	Present study (2019) (%)
Frontal	**40**	**30**
Temporal	**29**	**8**
Parietal	**14**	**14**
Occipital	**3**	**8**

In both the studies, frontal lobe seems to be the most frequent site. As far as gliomas of grades II, III are concerned, they too had specificity for frontal and parietal lobes. The slight discrepancy regarding the temporal lobe incidence in our study might be due to the location of gliomas in overlapping lobes. While in our study all the

pilocytic astrocytomas occurred in the cerebrum- supratentorial compartment, Collins VP *et al* (2015)[204] reports that 36% of pilocytic astrocytomas occurred in the supratentorium.

Significance of location in glioblastomas:

Simpson J R *et al* (1993)[205] studied 645 glioblastomas with respect to certain known prognostic factors like age, size and extent of surgery. According to this study, patients of frontal lobe glioblastomas survived longer than temporal and parietal lobe tumors (11.4 months, 9.1 months, and 9.6 months, respectively).

Also, unusual sites of occurrence of gliomas in our study were corpus callosum and cerebellum accounting for 4% and 6% respectively. According to Larjavaara S et al (2007)[26], bilateral tumors were 13 cases comprising 4.9% and cerebellar gliomas accounted for 1.5%.

The glioblastomas are solitary tumors usually and its spread across the corpus callosum leads to the classic pattern of a "butterfly" in imaging studies. The survival rate of such bilaterally located glioblastomas is dismal, according to Tunthanathip T et al (2017)[206], at 8.49 months. Also the study by Zakrzewska et al (2007)[207], reports that molecular profiles of butterfly glioblastomas do not show any TP53, EGFR alterations. In our study, we received two such gliomas, one was an anaplastic astrocytoma and the other was glioblastoma. The latter was IDH wild type and EGFR positive. Hence in view of the newly added entity in WHO 2016 classification of CNS tumors, namely the midline glioma with K27M mutation, molecular investigation and genetic profile must be done in all midline cases to explore and distinguish these unique subtypes.

Laterality of gliomas:

There is a certain correlation between the sides of presentation of gliomas with studies stating that high grade gliomas prefer right side of the brain (Inskip P *et al* 2003)[208].

In the present study, overall frequency of distribution of gliomas was increased in the right side of the brain, accounting for 46% comparable to Coluccia D *et al* (2018)[209]. The high-grade gliomas had a predilection for right side with 54.5% and 61.1% of diffuse astrocytomas and glioblastomas occurring there.

Table 35: - Frequency of Glioblastomas according to laterality

Study	Glioblastomas	Right hemisphere (%)	Left hemisphere (%)
Coluccia D *et al* (2018)[209]	**235**	**64.8**	**37.7**
Present study (2019)	**21**	**61.1**	**38.9**

This study has analysed the neurological outcome, survival and extent of resection in relation to tumor lateralization. The Karnofsky performance score (KPS) is used in all neurology patients to assess their functional impairment and prognosis. The study reported that all patients with left hemisphere tumors had a diminished KPS, 6 months postoperatively, increase in dysphasia and lack of complete resection. Hence a diminished survival has been reported in left hemisphere gliomas (7.4 months) as against (10.1 months) in right sided ones. Though this can be partially attributed to a more conservative resection, the difference is statistically significant.

Analysis of IDH mutant and IDH wildtype glioblastomas:

We studied the analysis of IDH1 expression in the 21 cases of glioblastomas received, in an attempt to know the frequency of distribution of the mutant and wild type tumors. In the present study IDH wild type accounted for 71.4%. The results were statistically significant with a p value of 0.0327 ($p<0.05$).

Table 36: Frequency of distribution of IDH mutant and IDH wild type Glioblastomas

Study	Total glioblastomas	IDH mutant n (%)	IDH wild type n (%)
Senhaji N et al (2016)[210]	**62**	**8(12.6)**	**54(87.1)**
UNO M et al (2011)[211]	**161**	**19 (11.8)**	**142 (88.2)**
Present study (2019)	**21**	**6 (28.6)**	**15(71.4)**

We had near comparable results with Senhaji N et al (2016)[210]. Another study by UNO M et al (2011)[211] states that out of 161 glioblastoma cases, only 19 (11.8%) were IDH1 positive and the rest 142 (88.2%) were IDH1 negative. Hence like in our study, wild type glioblastomas are more prevalent than IDH mutants. This is in correlation with the WHO 2016 classification of CNS tumors which states that IDH wild type glioblastomas are the commonest malignant gliomas accounting for 90% while the rest 10 % are IDH mutant tumors. Various literature studied have reported that IDH1 mutation occurs predominantly in secondary glioblastomas, whereas IDH wild type cases are primary glioblastomas. Out of 155 primary glioblastomas studied by UNO M et al (2011)[211], 15 were only IDH1 positive accounting for 9.7%.

According to the latest WHO, a diagnosis of IDH wild type is incompatible in the presence of an IDH1/2 mutation.

Significance of age in IDH mutant glioblastomas:

There is striking difference in age distribution of primary and secondary glioblastomas. The patients with IDH mutation are younger (mean age, 32–41 years) compared to IDH wild type glioblastomas (mean age, 56–59 years), in a study by Ohgaki H et al(2013)[212] which is in concordance with the WHO revised 4th edition, that states that IDH mutant patients are a decade younger than their wild type counterparts.

Of the six IDH mutant glioblastomas we studied, majority (83.3%) were in the age group of 40-60 years. According to Erik L et al (2019)[213] study, age is an independent prognostic factor with glioblastoma patients over the age of 65 years having a dismal prognosis. This is found to be due to immunosuppression as age advances. The difference in tumor microenvironment is said to be the reason behind the varying degrees of prognosis between the IDH mutant and wild type glioblastomas. Like various studies, in our study too we received 26.7% of IDH wild type glioblastomas above the age of 60 years whereas the percent of IDH mutant cases received in the same age group was nil.

Significance of localization in IDH mutant and wild type glioblastomas:

The correlation of location as a prognostic factor is derived from the fact that IDH mutant glioblastomas are preferentially located in the frontal lobes. Of the glioblastomas that we studied, 50% of the IDH mutant glioblastomas occurred in the right side of the brain and 50% was located in the frontal lobe. The results were

statistically significant with a p value of 0.028 ($p<0.05$).Similar observations were made by Jiang *et al* (2017)[214]

Table 37: Localisation of IDH mutant Glioblastomas

Study	IDH mutant glioblastomas	Frontal lobe (%)
Jiang H et al (2017)[214]	**267**	**46**
Present study (2019)	**6**	**50**

The presence of molecular subtypes of glioma stem cells has been recently identified. They are of three types - proneural, mesenchymal and classical. According to Behnan J *et al* (2019)[215] glioblastomas with the mesenchymal signature have a shorter survival rate.

The importance of localization lies in the fact that the preference of frontal lobe delineates the IDH mutant glioblastoma from the wild type as the former has proneural type signature as against the mesenchymal signature of the wild type glioblastoma. The importance of this molecular signature in the glioma stem cells is that all the intra tumoral regions sampled from the same anatomic location of gliomas were homogenous, regardless of the patient. Also, infiltrating edges of the tumor had a proneural type signature whereas the necrotic regions had a mesenchymal signature. The transition from proneural to mesenchymal has been the suggested mechanism for tumor resistance to radiation and chemotherapy in recurrent cases. Hence this molecular sub typing appears to be a promising strategy to provide specific

predictions of glioblastoma evolution, and rational treatment strategies. Therefore, our results can elucidate and provide newer insights into tumorigenesis, presurgical clinical decision and personalized treatment.

In the IDH wild type glioblastomas, our study showed increased predilection for frontal lobe (33.3%) compared to the Jiang et al (2016)[214] that stated the temporal lobe (42%) to be the commonest site. This discrepancy might be corrected if we further expand our dataset.

Significance of gender in IDH mutant and wild type glioblastomas:

In our study, both the IDH mutant and wild type glioblastomas had a male predilection with a M: F ratio of 4.9:1 in the IDH mutant tumor while the IDH wild type had a M: F ratio of 2:1. The percentage of males was 83.3% in IDH mutant glioblastomas. The results were statistically significant with a p value of 0.0464($p<0.05$).As per Jiang et al (2017)[214], M: F of IDH mutant and wild type glioblastomas were 1:1 and 1.9:1 respectively. Also, Ohgaki H et al (2013)[212] observed that IDH mutant glioblastomas had a female preponderance with a M: F 1:1.8 compared to wild type tumor with a M: F ratio of 1.33:1.The reason substantiated was that as IDH mutant glioblastomas are secondary type glioblastomas that progress from low grade gliomas like diffuse astrocytomas, the sex ratio could reflect that of the low-grade gliomas. The high male preponderance of IDH mutant glioblastomas in our study could be attributed to the minimal number of cases studied.\

Significance of EGFR in IDH wild type glioblastomas:

The presence of EGFR mutation correlates with the detection of primary glioblastoma as various studies indicate it to be over expressed in them. It occurs in 30-45% of primary glioblastomas according to Ohgaki H et al [212]. This is similar to our study where out of 15 IDH wild type glioblastomas, 6 were EGFR positive accounting for 40 %. The importance of EGFR analysis is that it has paved the way for many novel treatment modalities like EGFR-targeted monoclonal antibodies, tyrosine kinase inhibitors (TKIs), as per Xu H et al (2017)[216]. Also a study by Lee M (2018) [217]correlated the EGFR over expression, EGFR vIII mutation and gene amplification, using IHC, FISH and PCR. The results yielded showed that the sensitivity and specificity of EGFR IHC, in predicting EGFR gene amplification were 100% and 46.5% respectively. The tumors with EGFR amplification showed intense EFGR IHC staining (3+). In our study, out of the 6 EGFR positive cases, intense staining (3+) was noted in 4 cases. Therefore in our study, we were able to identify glioblastomas with EGFR amplification, the information of which can impact the further treatment. Tripathy K et al (2007)[218] studied the prognostic significance of EGFR in glioblastomas and found that among the 52 cases of glioblastomas analysed, the responders to treatment were 86.4% in EGFR negative cases and 46.7% in EGFR positive cases. Hence the analyses of EGFR positive cases help identify non responders to treatment. Data from our study can be used for the same.

The significance of IDH1 analysis and its prospects:

The imaging modality of choice of gliomas is the contrast enhanced MRI. Wang K *et al* (2016)[219] observed certain distinctive radiological features with prognostic value like – tumor contrast enhancement, multifocality, location, edema

and cystic degeneration. Out of the 280 glioblastomas, 73.3% of IDH1 mutant glioblastomas had contrast enhancement as against 94.9% of IDH wild type tumors. Also 71.1 %(32/45) had peritumoral edema among the IDH mutant glioblastomas while 77 %(181/235) of the IDH wild type tumors had similar imaging findings. In our study, most of the glioblastomas (16/21) presented with ring enhancement. In the IDH mutant glioblastomas (4/6) 66.7 % had contrast enhancement whereas 80% (12/15) of the IDH wild type tumors had similar findings. The study by Wang K et al.[219], observed that among the IDH mutant glioblastomas, the ones with non-enhancement had a longer duration of median progression free survival and overall survival. This had no prognostic value in IDH wild type tumors. Thus, radiological biomarkers can be combined with IDH1 status for accurate prediction of prognosis.

In this context, a promising new paradigm that needs to be discussed is 'Radiogenomics'. This rapidly growing field correlates molecular signature in tumors with conventional and advanced imaging techniques. By obtaining imaging phenotypes of glioblastomas from routine MRIs, correlation with their molecular signature is established thereby providing information regarding diagnosis, prognosis and treatment. This has the potential of becoming a surrogate non-invasive marker.

According to Choi C *et al.* (2012)[220], detection of the oncometabolite 2 – hydroxyl glutarate (2 HG) that accumulates in IDH mutant gliomas, can provide a non-invasive method of monitoring IDH mutant tumor cells in treatment and progression. Pope *et al* (2012)[221] demonstrated in 27 patients that 2 HG levels were significantly raised in IDH mutant cases with MRS (magnetic resonance spectroscopy). This provides preoperative knowledge of the IDH status to the

neurosurgeons, impacting the desired extent of surgical resection. Thus, the database from our study can be used in the future for monitoring progression/response and in further studies regarding the same.

The method of sampling the tumor tissue for diagnosis can be a stereotactic biopsy or an open surgical resection. In cases of stereotactic biopsy, in a study by Jackson RJ et al (2001)[222], it has been stated that inaccurate diagnosis of about 10% occurs, due to histological heterogeneity. IDH1 allows a possibility of differential diagnosis between neoplastic and non-neoplastic lesions of brain. Also among the neoplastic lesions, glial and non-glial tumors of CNS can be identified by IDH1/2 positivity[223, 224]

IDH helps to differentiate infiltrating gliomas from Grade I gliomas. It also discriminates gemistocytes from minigemistocytes in oligodendrogliomas. The former has a less stained centre while the latter stains intensely[224]. According to Horbinski *et al* (2009)[225], in suboptimal biopsies, IDH1 can be used in peripheral biopsy of the outer edge of tumor tissue to improve diagnostic accuracy. This demonstrates that IDH1 mutations help even in equivocal biopsies.

In cases of open surgical resections, Kim *et al* (2014)[226] found that volume of the resected tumor influenced the diagnosis of glioblastoma. Resections less than 20 ml caused a lower rate of glioblastoma diagnosis. However, this was not affected by IDH1 mutation status, proving that molecular genotyping usage improves the diagnostic accuracy. Beiko *et al* (2009)[227] observed that IDH1 status was associated with complete resection in the mutant group with 93% as against 67% in the wild type

group. This suggests that IDH mutant glioblastomas are amenable to resection. Further Thon N et al (2019)[228] reported that the application of molecular signature like IDH1 in surgical strategies is of prime importance because if the molecular profile indicates a chemo/radiation resistant glioma, extent of resection can be increased. Conversely in tumors with molecular profiles responsive to treatment, delayed resection can be attempted, especially in cases where tumor is situated in the eloquent regions of the brain. So, our study too can be a valuable tool in assisting the operating neurosurgeon in taking a decision regarding the extent of resection.

Apart from this, IDH finds a use in intraoperative enhancement of tumor demarcation and visualization also. Hadjipanayis CG et al (2015)[229]and Zhao S et al (2013)[230] both discussed the utility of 5-amino levulinic acid (5-ALA) in fluorescence guided neurosurgery. This has been approved for treatment in Europe and Japan. As the glioblastoma cells lack ferrochelatase (the enzyme that chelates protoporphyrin IX {PP IX} with iron), PP IX accumulates in the tumor cells thereby enabling distinction from normal adjacent brain. Schucht P et al (2014)[231] study validates that the above technique is more sensitive than Gadalonium enhancement. Hence an extensive resection beyond the radiologically evident margins can be performed. Kim JE et al (2015)[232] in his study observed that IDH1 mutant glioblastomas showed enhanced 5-ALA fluorescence due to low levels of NADPH in them. Thus, such specific metabolic characteristic features of IDH mutant glioblastomas can help the neurosurgeon in precise resection.

Another modality helpful in intraoperative sampling that depends on the IDH mutant status is DESI-MS –desorption electrospray ionisaton mass spectrometry. This

rapid imaging technique can be used to diagnose tumors based on their lipid profile. St John ER et al (2016)[233] and Jarmusch A K et al (2016)[234] both observed that DESI-MS detects 2HG levels in IDH1 mutant glioblastomas thereby discriminating normal brain from glioma. Hence intraoperative evaluation of tumors for rapid diagnosis can be done in IDH mutant gliomas.

Regarding the histopathological features of glioblastomas, certain unique characteristics have been identified that can differentiate IDH mutant and wild type glioblastomas based on morphology. The wild type glioblastoma exhibits certain variants like – gliosarcoma, giant cell glioblastoma and epithelioid glioblastoma. Though rare, these unique histopathological features help in predicting the molecular signature of the tumor even before IHC/DNA sequencing. According to the study by Neumann JE et al (2016)[235] certain morphological features like microcysts occurred predominantly in IDH mutant glioblastomas (75%) as against IDH wild type (14.3%), whereas certain features occurred exclusively in IDH wild type tumors like – PNET like foci (21.4%), calcification (10.7%), giant cells (50%), epithelioid cells (21.4) and fascicular growth (10.7 %). In our study, we received two cases with unique histomorphological features –namely gliosarcomas. The tumor tissue showed a typical biphasic pattern of growth with both neoplastic astrocytes and fascicles of spindle shaped cells. The glial cells were GFAP positive and spindle cells were vimentin positive, GFAP negative. Reticulin was also done which turned out be positive in the mesenchymal component. IDH1 mutation was absent, proving it to be a wild type glioblastoma. The study by Nobusawa S *et al* (2009)[236] observed two distinct morphological features of IDH mutant glioblastomas- presence of oligodendroglial

components in 54.1% cases against 19.5% of IDH wild type cases and pseudo pallisading necrosis which was more common among IDH wild type glioblastomas (90%) than IDH mutant (50%). In our study, too, pseudo pallisading necrosis was more common among the IDH wild type tumors.

The current standard of care (SOC) for infiltrating gliomas is maximal safe resection, concurrent chemotherapy/radiation and adjuvant treatment with temzolide. Houillier C et al (2010)[237]study showed that IDH1 mutant gliomas had a better response to chemotherapy. Similarly, presence of 1p/19q co deletion and MGMT methylated tumors also showed increased sensitivity to therapy. The relationship between IDH mutant and MGMT methylated glioblastomas was subjected to further detailed analysis in the study by Yang P et al (2015)[238]. This study observed that the overall survival and progression free survival was the best in glioblastomas harbouring both IDH mutation and MGMT methylation, whereas if either one was present, there was an intermediate prognosis. The glioblastomas with lack of both alterations had the worst survival rates. This study also states that IDH wild type glioblastomas had improved survival with addition of temzolide to radiotherapy than the latter alone. On the other hand, IDH mutant patients fared well compared to their wild type counterparts receiving temzolide+radiotherapy (TMZ+RT). The comparison of individual groups of IDH mutant patients receiving TMZ+RT with those receiving RT alone showed no difference in survival rates, proving that presence of IDH mutation causes resistance to TMZ as time passes. This area needs more research due to the differential impact of these biomarkers on TMZ clinical response.

As the IDHI/2 mutations are inaugural events in tumorigenesis, they have become attractive therapeutic targets. As IDH1 is a neoantigen with uniform penetrance in all tumor cells, it represents a potential target for immunotherapy. Kaminska B et al (2019)[239] study has briefly described about the various IDH1/2 inhibitors. Currently targeted inhibitors of *IDH1* (AG120, IDH305) are being evaluated in phase I trials for the therapy of hematologic malignancies like refractory AML, MDS as well as solid tumors – gliomas, cholangiocarcinomas and chondrosarcomas. AG 221, AGI-6780, IDH 305 are some of the growing list of IDH inhibitors currently being tested. The latest addition to the list of novel anti –IDH treatment modalities is the IDH R 132H protein targeting peptide vaccine. Taking advantage of the fact that, a fraction of IDH mutant glioma patients displayed IFN γ T cell response against the IDH1 mutant protein; a peptide vaccine has been derived. According to the study by Platten M *et al* (2018)[240], NOA-16 -the vaccine demonstrated safety and immunogenicity when tested on newly diagnosed IDH mutant malignant astrocytoma cases.

Thus the knowledge of the molecular signature (IDH1) of the glioblastomas is of supreme importance. It helps the pathologist, radiologist and the treating neurosurgeon bring about a holistic approach in the diagnosis, therapy and prognostication of the patient.

SUMMARY

In the present prospective study of 96 cases of brain SOLs, the following results were obtained:

- Out of the 96 cases, 92% were neoplastic lesions and 97% were primary brain tumors.
- Gliomas comprised of 50 cases out of the 96 brain SOLs and were the commonest among the primary CNS tumors.
- Of the non glial tumors, meningiomas were the commonest constituting 28.2%.
- The commonest glioma was glioblastoma accounting for 42%.
- The most common age group affected by gliomas was 40-59 years with an overall frequency of 46% and the glioblastomas was 50 -59 years accounting for 38.1%
- Males had a definite sex predilection for both gliomas overall and glioblastomas specifically, accounting for 62% and 71.4% respectively.
- The commonest location of all gliomas and glioblastomas in specific was the frontal lobe.
- Unusual sites of distribution of gliomas were – corpus callosum and cerebellum.
- The analysis of IDH1mutation showed IDH wild type glioblastomas as the most predominant, comprising 71.4% of the glioblastomas. The results were statistically significant with a p value of 0.0327 ($p<0.05$).

- The most common sex affected in IDH1 mutant glioblastomas was male and the most common age group was 50-59 years. The results were statistically significant with a p value of 0.0464($p<0.05$).
- IDH wild type glioblastomas accounted for 26.7% of the seventh decade patients.
- Frontal lobe was the predilected site for IDH1 mutant glioblastomas. The results were statistically significant with a p value of 0.028.
- The analysis of EGFR mutation in all IDH wild type glioblastomas showed 40% positivity.

CONCLUSION

The new WHO 2016 classification of CNS tumors has broadened the horizons of the knowledge about primary brain tumors, especially gliomas. The incorporation of a molecular signature has paved the way for great prospects regarding the treatment of gliomas, particularly glioblastomas. Despite the multimodal strategies of synergism and personalized treatment, available therapies are of limited utility. Patients still have poor prognosis with dismal progression free survival and overall survival.

By analyzing the expression of IDH1 and EGFR in glioblastomas, distinction into primary and secondary types has been made. This helps to know the prognosis, molecular subtype, sensitivity to chemotherapy/radiation and plan the preclinical surgical decision and post surgical treatment. Further the database of our study, being the first of its kind in our department, can facilitate etiological studies, establish awareness of differences in approach to diagnosis of the disease and provide ground for novel techniques in treatment.

Evidence established by our study which is in corroboration with various others is that there is concordance between immunohistochemistry and DNA sequencing, in the diagnosis of gliomas. In view of cost effectiveness and feasibility,IHC can be used as a surrogate marker in place of DNA sequencing.

Hence to conclude, IDH1 mutations are present in the Indian patients with gliomas similar to the literature reported from the West. Its emergence as an independent prognostic marker has now given rise to great challenges like establishing the complex molecular biology of gliomas,in order to discover novel techniques.

Our study can be a stepping stone in analysis of frequency of IDH mutant glioblastomas, their preferential localization, lateralization and predilection for younger population. This can start the ball rolling in the following arenas, with respect to gliomas:

- Identifying techniques with maximum precision in diagnosis.
- Planning maximum safe resection while preserving the quality of life of the patient.
- Increasing the sensitivity to radiation and chemotherapy.
- Providing targeted and tailored therapies for gliomas.

ANNEXURE I

WHO CLASSIFICATION OF TUMOURS OF THE CENTRAL NERVOUS SYSTEM

Diffuse astrocytic and oligodendroglial tumours

Diffuse astrocytoma, IDH-mutant
Gemistocytic astrocytoma, IDH-mutant
Diffuse astrocytoma, IDH-wildtype
Diffuse astrocytoma, NOS
Anaplastic astrocytoma, IDH-mutant
Anaplastic astrocytoma, IDH-wildtype
Anaplastic astrocytoma, NOS
Glioblastoma, IDH-wildtype
Giant cell glioblastoma
Gliosarcoma
Epithelioid glioblastoma
Glioblastoma, IDH-mutant
Glioblastoma, NOS
Diffuse midline glioma, H3 K27M-mutant
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted
Oligodendroglioma,
Anaplastic oligodendroglioma, IDH-mutant and1p/19q-codeleted
Anaplastic oligodendroglioma, NOS
Oligoastrocytoma, NOS
Anaplastic oligoastrocytoma, NOS

Other astrocytic tumours

Pilocytic astrocytoma
Pilomyxoid astrocytoma
Subependymal giant cell astrocytoma
Pleomorphic xanthoastrocytoma
Anaplastic pleomorphic xanthoastrocytoma

Ependymal tumours

Subependymoma
Myxopapillary ependymoma
Ependymoma

Papillary ependymoma
Clear cell ependymoma
Tanycytic ependymoma
Ependymoma, *RELA* fusion-positive

Anaplastic ependymoma

Other gliomas
Chordoid glioma of the third ventricle
Angiocentric glioma
Astroblastoma

Choroid plexus tumours
Choroid plexus papilloma
Atypical choroid plexus papilloma
Choroid plexus carcinoma

Neuronal and mixed neuronal-glial tumours
Dysembryoplastic neuroepithelial tumour
Gangliocytoma
Ganglioglioma
Anaplastic ganglioglioma
Dysplastic cerebellar gangliocytoma(Lhermitte-Duclos disease)
Desmoplastic infantile astrocytoma and ganglioglioma
Papillary glioneuronal tumour
Rosette-forming glioneuronal tumour
Diffuse leptomeningeal glioneuronal tumour
Central neurocytoma
Extraventricular neurocytoma
Cerebellar liponeurocytoma
Paraganglioma

Tumours of the pineal region
Pineocytoma
Pineal parenchymal tumour of intermediate differentiation
Pineoblastoma
Papillary tumour of the pineal region

Embryonal tumours

Medulloblastoma
Medulloblastoma, NOS
Medulloblastomas, genetically defined
  Medulloblastoma, WNT-activated
  Medulloblastoma, SHH-activated and TP53-mutant
  Medulloblastoma, SHH-activated and TP53-wildtype
  Medulloblastoma, non-WNT/non-SHH
Medulloblastomas, histologically defined
  Medulloblastoma, classic
  Desmoplastic/nodular medulloblastoma
  Medulloblastoma with extensive nodularity
  Large cell / anaplastic medulloblastoma
Embryonal tumour with multilayered rosettes C19MC-altered
Embryonal tumour with multilayered rosettes, NOS
Other CNS embryonal tumours
   Medulloepithelioma
  CNS neuroblastoma
  CNS ganglioneuroblastoma
  CNS embryonal tumour, NOS
Atypical teratoid/rhabdoid tumour
CNS embryonal tumour with rhabdoid features

Tumours of the cranial and paraspinal nerves
Schwannoma
  Cellular schwannoma
  Plexiform schwannoma
Melanotic schwannoma
Neurofibroma
  Atypical neurofibroma
  Plexiform neurofibroma
Perineurioma
Hybrid nerve sheath tumours
Malignant peripheral nerve sheath tumour (MPNST)
  MPNST with divergent differentiation
  Epithelioid MPNST
  MPNST with perineurial differentiation

Meningiomas

Meningioma

Meningioma variants

- Meningothelial meningioma
- Fibrous meningioma
- Transitional meningioma
- Psammomatous meningioma
- Angiomatous meningioma
- Microcystic meningioma
- Secretory meningioma
- Lymphoplasmacyte-rich meningioma
- Metaplastic meningioma
- Chordoid meningioma
- Clear cell meningioma
- Atypical meningioma
- Papillary meningioma
- Rhabdoid meningioma

Anaplastic (malignant) meningioma

Mesenchymal, non-meningothelial tumours

Solitary fibrous tumour / haemangiopericytoma

Haemangioblastoma

Haemangioma

Epithelioid haemangioendothelioma

Angiosarcoma

Kaposi sarcoma

Ewing sarcoma / peripheral primitive neuroectodermal tumour

Lipoma

Angiolipoma

Hibernoma

Liposarcoma

Desmoid-type fibromatosis

Myofibroblastoma

Inflammatory myofibroblastic tumour

Benign fibrous histiocytoma

Fibrosarcoma
Undifferentiated pleomorphic sarcoma / malignant fibrous histiocytoma
Leiomyoma
Leiomyosarcoma
Rhabdomyoma
Rhabdomyosarcoma
Chondroma
Chondrosarcoma
Osteoma
Osteochondroma
Osteosarcoma

Melanocytic tumours
Meningeal melanocytosis
Meningeal melanomatosis
Meningeal melanocytoma
Meningeal melanoma

Lymphomas
Diffuse large B-cell lymphoma of the CNS
Corticoid-mitigated lymphoma
Sentinel lesions
Immunodeficiency-associated CNS lymphomas
AIDS-related diffuse large B-cell lymphoma
EBV+ diffuse large B-cell lymphoma, NOS
Lymphomatoid granulomatosis
Intravascular large B-cell lymphoma
Miscellaneous rare lymphomas in the CNS
Low-grade B-cell lymphomas
T-cell and NK/T-cell lymphomas
Anaplastic large cell lymphoma (ALK+/ALK-)
MALT lymphoma of the dura

Histiocytic tumours
Langerhans cell histiocytosis
Erdheim-Chester disease
Rosai-Dorfman disease

Juvenile xanthogranuloma
Histiocytic sarcoma

Germ cell tumours

Germinoma
Embryonal carcinoma
Yolk sac tumour
Choriocarcinoma
Teratoma
Mature teratoma
Immature teratoma
Teratoma with malignant transformation
Mixed germ cell tumour

Familial tumour syndromes

Neurofibromatosis type 1
Neurofibromatosis type 2
Schwannomatosis
Von Hippel-Lindau disease
Tuberous sclerosis
Li-Fraumeni syndrome
Cowden syndrome
Turcot syndrome
Mismatch repair cancer syndrome
Familial adenomatous polyposis
Naevoid basal cell carcinoma syndrome
Rhabdoid tumour predisposition syndrome

Tumours of the sellar region

Craniopharyngioma
Adamantinomatous craniopharyngioma
Papillary craniopharyngioma
Granular cell tumour of the sellar region
Pituicytoma
Spindle cell oncocytoma

Metastatic tumours

ANNEXURE II: WHO GRADES OF CNS TUMOURS

Astrocytic tumours	I	II	III	IV
Subependymal giant cell astrocytoma	•			
Pilocytic astrocytoma	•			
Pilomyxoid astrocytoma		•		
Diffuse astrocytoma		•		
Pleomorphic xanthoastrocytoma		•		
Anaplastic astrocytoma			•	
Glioblastoma				•
Giant cell glioblastoma				•
Gliosarcoma				•
Oligodendroglial tumours				
Oligodendroglioma		•		
Anaplastic oligodendroglioma			•	
Oligoastrocytic tumours				
Oligoastrocytoma		•		
Anaplastic oligoastrocytoma			•	
Ependymal tumours				
Subependymoma	•			
Myxopapillaryependymoma	•			
Ependymoma		•		
Anaplastic ependymoma			•	
Choroid plexus tumours				
Choroid plexus papilloma	•			
Atypical choroid plexus papilloma		•		
Choroid plexus carcinoma			•	
Other neuroepithelial tumours				
Angiocentricglioma	•			
Chordoidglioma of the third ventricle		•		

ANNEXURE III

PROFORMA

1. Name :

2. Age :

3. Sex :

4. Ip number :

5. Address :

6. H/o presenting illness :

7. Past history :

8. Family history :

9. Clinical diagnosis :

10. Radiological diagnosis

    -Site of tumor :

    -Size of tumor :

11. Grossing notes :

12. Histopathological diagnosis :

13. Immunohistochemistry :

ANNEXURE: IV

HAEMATOXYLIN AND EOSIN STAINING METHOD FOR HISTOPATHOLOGY

1. Sections will be dewaxed with xylene for 20 minutes.

2. Sections will be hydrated through descending concentrations (absolute alcohol, 90%, 70%, 50%) of ethanol to water solutions.

3. Sections will be rinsed in distilled water.

4. Sections will be placed in Ehrlich haematoxylin stain for 20-30 minutes.

5. Sections will be rinsed with water.

6. Differentiation will be done by immersing the sections in 1% acid alcohol for 10 seconds.

7. Sections will be rinsed with water.

8. Blueing will be done by keeping the sections in scott's tap water for 2-10 minutes.

9. Counterstaining will be done with 1% aqueous eosin for 1-3 minutes.

10. Sections will be rinsed with water.

11. Sections will be dehydrated through increasing concentration of ethanol solutions (50%, 70%, 95%, absolute alcohol) and cleared with xylene.

12. Sections will be mounted with DPX.

ANNEXURE V

KEY TO MASTER CHART

HPE NO: Histopathological number

Gender:

M – Male

F – Female

Laterality:

R- Right

L-Left

Radiology:

RE- Ring enhancement

NA – Not applicable

ANNEXURE VI A

MASTER CHART

Sl. No	HPE NO	Age	Sex	Laterality	Lobe	Subtype	WHO Grade
1	S2295/17	35	F	R	Frontal	Anaplastic Astrocytoma	Grade III
2	S201/17	30	M	R	Pareital	Anaplastic Astrocytoma	Grade III
3	S355/17	16	M		Cerebellum	Glioblastoma	Grade IV
4	S376/17	55	M	R	Frontal	Glioblastoma	Grade IV
5	S456/17	32	F	L	Temporal	Diffuse Astrocytoma	Grade II
6	S1212/17	9	F	L	Pareital	Diffuse Astrocytoma	Grade II
7	S1296/17	25	M	R	Frontal	Diffuse Astrocytoma	Grade II
8	S1761/17	17	M	R	Occipital	Diffuse Astrocytoma	Grade II
9	S1905/17	17	F		Filum Terminale	Ependymoma	Grade II
10	2355/18	29	F	L	Pareital	Diffuse Astrocytoma	Grade II
11	3704/18	54	M	R	Temporal	Glioblastoma	Grade IV
12	234/18	29	M	R	Frontal	Diffuse Astrocytoma	Grade II
13	1194/18	30	M	R	Frontal	Glioblastoma	Grade IV
14	1331/18	7	F	L	Occipital	Pilocytic Astrocytoma	Grade I
15	S87/18	60	F	L	Fronto Pareital	Glioblastoma	Grade IV
16	S114/18	17	M		Filum Terminale	Ependymoma	Grade II
17	S387/18	32	M	L	Fronto Pareital	Glioblastoma	Grade IV
18	S414/18	50	M	L	Fronto Pareital	Anaplastic Astrocytoma	Grade III
19	S648/18	55	F	L	Pareital	Diffuse Astrocytoma	Grade II
20	S1135/18	14	M	R	Occipital	Pilocytic Astrocytoma	Grade I
21	S1198/18	51	F	R	Frontal	Glioblastoma	Grade IV
22	S691/18	56	M	L	Frontal	Glioblastoma	Grade IV
23	S768/18	57	F	L	Fronto Pareital	Anaplastic Astrocytoma	Grade III
24	S1385/18	32	M	R	Pareital	Anaplastic Astrocytoma	Grade III
25	S1744/18	21	M	L	Frontal	Pilocytic Astrocytoma	Grade I
26	S1892/18	73	F		Cerebellum	Glioblastoma	Grade IV
27	S2053/18	35	F	R	Frontal	Glioblastoma	Grade IV
28	S2226/18	52	F	L	Frontal	Glioblastoma	Grade IV
29	S2329/18	50	M	R	Frontal	Diffuse Astrocytoma	Grade II
30	S45/19	44	M		Corpus Callosum	Glioblastoma	Grade IV
31	S151/19	5	M	L	Lateral Ventricle	Ependymoma	Grade II
32	S239/19	60	M	L	Pareito occipital	Glioblastoma	Grade IV
33	S1291/18	43	M	R	Pareito occipital	Glioblastoma	Grade IV
34	S966/18	59	M	R	Pareito occipital	Glioblastoma	Grade IV
35	3704/18	54	M	R	Tempero Parietal	Glioblastoma	Grade IV

36	S423/19	60	M	R	Fronto Pareital	Glioblastoma	Grade IV
37	S1595/18	40	M	R	Fronto Pareital	Diffuse Astrocytoma	Grade II
38	S912/18	30	M	L	Temporal	Diffuse Astrocytoma	Grade II
39	S797/18	50	M	L	Fronto Pareital	Anaplastic Astrocytoma	Grade III
40	S968/18	46	F		Corpus Callosum	Anaplastic Astrocytoma	Grade III
41	S872/19	60	F	R	Frontal	Anaplastic Astrocytoma	Grade III
42	S57/19	42	M		Cerebellum	Ependymoma	Grade II
43	S203/19	41	F	R	Temporal	Diffuse Astrocytoma	Grade II
44	S973/19	45	F	L	Frontal	Glioblastoma	Grade IV
45	S1527/19	52	M	R	Parietal	Glioblastoma	Grade IV
46	S1548/19	42	M	L	Fronto Pareital	Glioblastoma	Grade IV
47	S1547/19	40	M	R	Occipital	Ependymoma	Grade II
48	S1477/19	22	F	L	Parietal	Anaplastic Astrocytoma	Grade III
49	975/19	19	F	L	Frontal	Ependymoma	Grade I
50	239/19	45	M	R	Frontal	Glioblastoma	Grade IV

ANNEXURE VI B GLIOBLASTOMAS – IDH MUTANT AND WILD TYPE

Sl. No	HPE	Age	Sex	Side	Lobe	IDH Status	EGFR Status	Radiology
1	S355/17	16	M		Cerebellum	Negative	negative	RE
2	S376/17	55	M	R	Frontal	Negative	Positive	RE
3	3704/18	54	M	R	Temporal	Positive	NA	
4	1194/18	30	M	R	Frontal	Negative	negative	RE
5	S87/18	60	F	L	Fronto Pareital	Negative	Positive	RE
6	S387/18	32	M	L	Fronto Pareital	Positive	NA	RE
7	S1198/18	51	F	R	Frontal	Negative	Positive	RE
8	S691/18	56	M	L	Frontal	Positive	NA	RE
9	S1892/18	73	F		Cerebellum	Negative	negative	RE
10	S2053/18	35	F	R	Frontal	Negative	Positive	
11	S2226/18	52	F	L	Frontal	Positive	NA	
12	S45/19	44	M		Corpus Callosum	Negative	Positive	RE
13	S239/19	60	M	L	Pareito occipital	Negative	negative	RE
14	S1291/18	43	M	R	Pareito occipital	Positive	NA	RE
15	S966/18	59	M	R	Pareito occipital	Negative	negative	RE
16	3704/18	54	M	R	Tempero Parietal	Negative	negative	RE
17	S423/19	60	M	R	Fronto Pareital	Negative	Positive	RE
18	S973/19	45	F	L	Frontal	Negative	negative	
19	S1527/19	52	M	R	Parietal	Negative	negative	
20	S1548/19	42	M	L	Fronto Pareital	Negative	negative	RE
21	239/19	45	M	R	Frontal	Positive	NA	RE

ANNEXURE VII

LIST OF ABBREVIATIONS

H & E – Hematoxylin and Eosin

CNS – Central Nervous System

CP angle – Cerebellopontine angle

SOL – Space occupying lesion

CSF – Cerebrospinal fluid

GFAP – Glial fibrillary acidic protein

IDH – Isocitrate dehydrogenase

EMA – Epithelial membrane antigen

EBV – Epstein – Barr virus

CMV- Cytomegalo virus

HSV- Herpes simplex virus

RNA – Ribonucleic acid

DNA- Deoxyribonucleic acid

STAT- Signal transducer and activator of transcription

IL - Interleukin

ROS- Reactive oxygen species

PXA - Pleomorphic xanthoastrocytoma

SEGA- Sub ependymal giant cell astrocytoma

PCR – Polymerase chain reaction

CT – Computered tomography

MRI – Magnetic resonance imaging

IARC- International Agency for Research on Cancer

SEER- Surveillance, Epidemiology, and End Results

CD – Cluster differentiation

PDGFR- Platelet derived growth factor

FGF – Fibroblast growth factor

VEGF- Vascular endothelial growth factor

EGFR- Epidermal growth factor

MDM- Mouse double minute homolog

IGFBP- Insulin-like growth factor-binding protein

MMP - Matrix metalloproteinases

Rb- Retinoblastoma

PTEN - Phosphatase and tensin homolog

CDKN2A - Cyclin-dependent kinase inhibitor 2A

ATRX- Alpha thalassemia/mental retardation syndrome X-linked

MYB - Myeloblastosis

NCAM- Neural cell adhesion molecule

TERT- Telomerase reverse transcriptase

OLIG - Oligodendrocyte transcription factor

SOX_-Sry-related HMG box

MAP- Mitogen-activated protein

CpG- Cytosine guanine sites

G-CIMP- CpG island methylator phenotype

NF- Neurofibromatosis

NOS- Not otherwise specified

RELA- v-rel avian reticuloendotheliosis viral oncogene

TTF- Thyroid transcription factor

TCA- The citric acid cycle

AML- Acute myeloblastic leukemia

MDS- Myelodysplastic syndrome

MPN – Myeloproliferative neoplasm

WHO – World Health Organization

ANNEXURE VIII

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ANNEXURE X

INSTITUTION ETHICAL COMMITTEE APPROVAL

MADURAI MEDICAL COLLEGE
MADURAI, TAMILNADU, INDIA -625 020
(Affiliated to The Tamilnadu Dr.MGR Medical University, Chennai, Tamil Nadu)

Prof Dr V Nagaraajan MD MNAMS DM (Neuro) DSc.,(Neurosciences) DSc (Hons)
Professor
Emeritus in Neurosciences,
Tamil Nadu Govt Dr MGR Medical University
Chairman, IEC

Dr.K.Raadhika, MD.,
Member Secretary,
Asso.Professor of Pharmacology,
Madurai Medical College,
Madurai.

Members

1. Dr.C.Anitha Mohan, MD,
Asso.Professor of Physiology &
Vice Principal,
Madurai Medical College

2. Dr.P.Raja, MCh., Urology,
Medical Superintendent Govt.
Rajaji Hospital, Madurai

3.Dr.R.Balajinathan MD., (General Medicine) Professor & HOD of Medicine, Madurai Medical & Govt. Rajaji Hospital, College, Madurai.

4.Dr.P.Amutha, MS., (General Surgery) Professor & H.O.D Madurai Medical College & Govt. Rajaji Hospital, Madurai.

5.Dr.N.Sharmila thilagavathi, MD., Professor of Pathology, Madurai Medical College, Madurai

6.Mrs.Mercy Immaculate Rubalatha, M.A., B.Ed., Social worker, Gandhi Nagar, Madurai

7.Thiru.Pala.Ramasamy, B.A.,B.L., Advocate, Palam Station Road, Sellur.

8.Thiru.P.K.M.Chelliah, B.A., Businessman,21, Jawahar Street, Gandhi Nagar, Madurai.

ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.J.Niveditha
Designation	:	PG in MD., Pathology
Course of Study	:	2017- 2020
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Histopathological study of gliomas and evaluation of IDH-1 expression in Glioblastoma by immunohistochemistry
Ethical Committee as on	:	08.04.2019

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary Chairman Dean / Convenor

Prof Dr V Nagaraajan
M.D., MNAMS, D.M., Dsc.,(Neuro), Dsc (Hon)
CHAIRMAN
Madurai Medical College
Madurai

DEAN
MADURAI MEDICAL COLLEGE,
MADURAI 625 020.

மதுரை மருத்துவக்கல்லூரி
26 APR 2019

ANNEXURE XI

ANTI – PLAGIARISM CERTIFICATE

This is to certify that this dissertation work titled **"HISTOPATHOLOGICAL STUDY OF GLIOMAS & EVALUVATION OF IDH-1 EXPRESSION IN GLIOBLASTOMA BY IMMUNOHISTOCHEMISTRY"** of the candidate **Dr.J.NIVEDITHA** with registration Number **201713101** for the award of **M.D** in the branch of **PATHOLOGY**. 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 pages and result shows **01%** percentage of plagiarism in the dissertation.

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