ROLE OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF GLIOBLASTOMA AND ANAPLASTIC ASTROCYTOMA AT KENYATTA NATIONAL HOSPITAL

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Submission date: 28-Sep-2017 05:43PM (UTC+0300)
Submission ID: 853949962
File name: MA_AND_ANAPLASTIC_ASTROCYTOMA_AT_KENYATTA_NATIONAL_HOSPITAL.docx (7.11M)
Word count: 7511
Character count: 42854

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H58/68976/2013

ABSTRACT

Background

Cancer is a growing disease burden in our country. Contributing to it are Central Nervous tumours and most commonly glial tumours which are intracranial. Knowing the type of tumour the patient suffers from dictates treatment options and the patient's prognosis. The most common malignant glial tumour is glioblastoma which maybe primary or IDH1 mutation glioblastoma (secondary). The IDH1 mutation tumours arise from a previous lesion i.e. from diffuse astrocytoma World Health Organization (WHO) grade II or from anaplastic astrocytoma WHO grade III. The primary GBM arise de novo though as a result of multiple gene alterations. Primary GBM is associated with other gene mutations e.g. Epidermal growth factor receptor (EGFR) amplication, Phosphatase and tensin homolog PTEN/MMAC1 mutation and loss of chromosome 10, CDKN2A (p16) deletion and less frequently MDM2 amplication. Secondary GBM is associated with TP53 mutations though it is more of TP53 protein accumulation rather than 19q loss.

Objective: The purpose of the study was to describe the role of immunohistochemistry in the diagnosis of glioblastoma and anaplastic astrocytoma at Kenyatta National Hospital.

Methodology: The study was a laboratory based retrospective descriptive study conducted at KNH/UON Histopathology Laboratory. The study included all available previously diagnosed glioblastoma and anaplastic astrocytoma cases which were forty six. Patient's data and histopathological reports were retrieved from the archives, and re-analyzed to identify those with a glioblastoma WHO grade IV and anaplastic astrocytoma WHO grade III diagnosis. Histopathology evaluation was done using paraffin embedded blocks, routine H/E, Ki-67 a cell proliferative marker, immunohistochemical markers isocitrate dehydrogenase (IDH1) and Alpha Thalassemia/Mental Retardation Syndrome X-linked (ATRX) were also done. The data was entered into spreadsheets in the computer and processed using statistical package for social sciences. Descriptive analysis and also bivariate analyses was done to correlate ATRX expression with IDH-1 in cases diagnosed as glioblastoma and anaplastic astrocytoma. The results were presented in form of charts, tables and figures.

Results: The population characteristics are as follows: the age range of the study population was 7 to 70 years with a median (interquartile range IQR) of 44 (22) years. Males were 24 cases (52.2%) and female were 22 cases (47.8%). There were 3 pediatric cases and 92.7%of the subjects were > than 12 years.

Many cases did not have their tumour sites indicated (35%). Of those indicated many had multiple sites, the common ones being the frontal, parietal and temporal cerebral cortex. On review of the previous versus current diagnosis: Previous WHO grade III and IV (GBM 89.1%, anaplastic astrocytoma (AA) 6.5% Atypical Teratoid/Rhabdoid Tumour (AT/RT) 2.2% Oligoastrocytoma 2.2%) current diagnosis after consensus of the principle investigator and 2 qualified pathologists GBM 95.6%, AA 2.2% and 1 case of no tumour. There were 44 cases of GBM majority were the Classical subtype (77.3%) and 1 case of Oligodencytric (2.3%).

Immunohistochemistry: 45 cases underwent IHC. Majority of the cases (28/44) in Ki-67 had a mitotic score mean 27.1% (SD 15.1%) of the GBM cases. The AA case mitotic rate was 7%. IDH1 mutations were present in 11/44 of the GBM and in the AA case. ATRX loss was in 17/44 GBM cases and the AA case. The anaplastic astrocytoma is the only case that displayed both IDH1 mutation and ATRX loss. The occurrence of IDH1 mutations was found to be significantly associated with the occurrence of ATRX loss (Chi sq 4.682, p-value 0.030). There was no significant association between ATRX loss and patient age (Chi sq 0.0002, p-value 0.987) and tumour proliferation rate (Chi sq 0.396, p-value 0.941)

Conclusion: GBM is a common supratentorial tumour rarely seen in the cerebellum (7%). The most common subtype is the classical type with monotonous nuclear pleomorphism and atypia, brisk mitotic activity, necrosis and endothelial proliferation. Anaplastic Astrocytoma is a rare tumour as only 5 cases were diagnosed in 5years and had both IDH1 mutation and ATRX loss. There are 2 GBM subpopulations i) GBM with IDH1 mutation but no ATRX loss ii) GBM with no IDH1 mutation and no ATRX loss. The GBM with IDH1 mutation are Glioblastoma, IDH-mutant which is now classified in the WHO 2016 guide line.

We **recommend** further studies on patient survival time with a diagnosis of WHO grade III anaplastic astrocytoma and IV glioblastoma to correlate with immunohistochemistry findings with prognosis as evidenced by patient survival.

CHAPTER ONE

INTRODUCTION

Gliomas are the most common primary tumors of the central nervous system arising from the glial tissue, which are the support tissue for nerves. They are classified as grade I to grade IV on the basis of histological criteria established by the World Health Organization (WHO) (1) Glioblastoma may grow de novo (primary glioblastoma, P-GBLs) or through advancement from low grade diffuse astrocytoma WHO grade II or from anaplastic astrocytoma WHO grade III.

Over the years the nomenclature of glioblastoma has evolved with the introduction of molecular findings in the 2016 (Table 1) WHO classification of central nervous system (CNS) tumours update versus the 2007 (Table 2) that relied on clinico-pathology, tumour variants and histo-morphology. The inclusion of molecular properties of the tumours will lead to a greater diagnostic accuracy as well as improved patient management and more accurate determinations of prognosis and treatment response.(2)

The primary and secondary GBM are morphologically indistinguishable. The use of immunohistochemistry is able to distinguish them. They constitute unique diseases that affect patients of different age groups. They develop through diverse genetic pathways (3)(4) that show different RNA and protein expression profiles and may differ in their response to treatment. Among the glial tumours glioblastoma, has shown the most genetic variation which are as a result of multiple mutations (5).

Glioblastoma can manifest at any age but preferentially affects adults with a peak age of 49-56 years. The glioblastoma tumours are more commonly located in the supratentorial region (frontal, temporal, parietal, and occipital lobes) and are rarely seen in the cerebellum. And the classical subtype is the most common in our population.

Table 1: The 2016 World Health Organization Classification of Tumors of the Central Nervous System.

WHO classification of tumours of the central nervous system

Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, IDH-mutant	9400/3
Gemistocytic astrocytoma, IDH-mutant	9411/3
Diffuse astrocytoma, IDH-wildtype	*9400/3*
Diffuse astrocytoma, NOS	9400/3
Anaplastic astrocytoma, IDH-mutant	9401/3
Anaplastic astrocytoma, IDH-wildtype	*9401/3*
Anaplastic astrocytoma, NOS	9401/3
Glioblastoma, IDH-wildtype	9440/3
Giant cell glioblastoma	9441/3
Gliosarcoma	9442/3
Epithelioid glioblastoma	*9440/3*
Glioblastoma, IDH-mutant	9445/3*
Glioblastoma, NOS	9440/3
Diffuse midline glioma, H3 K27M–mutant	9385/3*
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	9450/3
Oligodendroglioma, NOS	9450/3
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted	9451/3
Anaplastic oligodendroglioma, NOS	*9451/3*
Oligoastrocytoma, NOS	*9382/3*
Anaplastic oligoastrocytoma, NOS	*9382/3*
Other astrocytic tumours	
Pilocytic astrocytoma	9421/1
Pilomyxoid astrocytoma	9425/3
Subependymal giant cell astrocytoma	9384/1
Pleomorphic xanthoastrocytoma	9424/3
Anaplastic pleomorphic xanthoastrocytoma	9424/3
Ependymal tumours	
Subependymoma	9383/1
Myxopapillary ependymoma	9394/1
Ependymoma	9391/3
Papillary ependymoma	9393/3
Clear cell ependymoma	9391/3
Tanycytic ependymoma	9391/3
Ependymoma, *RELA* fusion-positive	9396/3*
Anaplastic ependymoma	9392/3
Other gliomas	
Chordoid glioma of the third ventricle	9444/1
Angiocentric glioma	9431/1
Astroblastoma	9430/3
Choroid plexus tumours	
Choroid plexus papilloma	9390/0
Atypical choroid plexus papilloma	9390/1
Choroid plexus carcinoma	9390/3
Neuronal and mixed neuronal-glial tumours	
Dysembryoplastic neuroepithelial tumour	9413/0
Gangliocytoma	9492/0
Ganglioglioma	9505/1
Anaplastic ganglioglioma	9505/3
Dysplastic cerebellar gangliocytoma (Lhermitte–Duclos disease)	9493/0
Desmoplastic infantile astrocytoma and ganglioglioma	9412/1
Papillary glioneuronal tumour	9509/1
Rosette-forming glioneuronal tumour	9509/1
Diffuse leptomeningeal glioneuronal tumour	
Central neurocytoma	9506/1
Extraventricular neurocytoma	9506/1
Cerebellar liponeurocytoma	9506/1
Paraganglioma	8693/1
Tumours of the pineal region	
Pineocytoma	9361/1
Pineal parenchymal tumour of intermediate differentiation	9362/3
Pineoblastoma	9362/3
Papillary tumour of the pineal region	9395/3
Embryonal tumours	
Medulloblastomas, genetically defined	
Medulloblastoma, WNT-activated	9475/3*
Medulloblastoma, SHH-activated and *TP53*-mutant	9476/3*
Medulloblastoma, SHH-activated and *TP53*-wildtype	9471/3
Medulloblastoma, non-WNT/non-SHH	9477/3*
Medulloblastoma, group 3	
Medulloblastoma, group 4	
Medulloblastomas, histologically defined	
Medulloblastoma, classic	9470/3
Medulloblastoma, desmoplastic/nodular	9471/3
Medulloblastoma with extensive nodularity	9471/3
Medulloblastoma, large cell / anaplastic	9474/3
Medulloblastoma, NOS	9470/3
Embryonal tumour with multilayered rosettes, C19MC-altered	9478/3*
Embryonal tumour with multilayered rosettes, NOS	*9478/3*
Medulloepithelioma	9501/3
CNS neuroblastoma	9500/3
CNS ganglioneuroblastoma	9490/3
CNS embryonal tumour, NOS	9473/3
Atypical teratoid/rhabdoid tumour	9508/3
CNS embryonal tumour with rhabdoid features	*9508/3*
Tumours of the cranial and paraspinal nerves	
Schwannoma	9560/0
Cellular schwannoma	9560/0
Plexiform schwannoma	9560/0

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Table 2: The 2007 WHO Classification of Tumours of the Central Nervous System.

TUMOURS OF NEUROEPITHELIAL TISSUE

Astrocytic tumours	
Pilocytic astrocytoma	9421/1[1]
Pilomyxoid astrocytoma	*9425/3**
Subependymal giant cell astrocytoma	9384/1
Pleomorphic xanthoastrocytoma	9424/3
Diffuse astrocytoma	9400/3
Fibrillary astrocytoma	9420/3
Gemistocytic astrocytoma	9411/3
Protoplasmic astrocytoma	9410/3
Anaplastic astrocytoma	9401/3
Glioblastoma	9440/3
Giant cell glioblastoma	9441/3
Gliosarcoma	9442/3
Gliomatosis cerebri	9381/3
Oligodendroglial tumours	
Oligodendroglioma	9450/3
Anaplastic oligodendroglioma	9451/3
Oligoastrocytic tumours	
Oligoastrocytoma	9382/3
Anaplastic oligoastrocytoma	9382/3
Ependymal tumours	
Subependymoma	9383/1
Myxopapillary ependymoma	9394/1
Ependymoma	9391/3
Cellular	9391/3
Papillary	9393/3
Clear cell	9391/3
Tanycytic	9391/3
Anaplastic ependymoma	9392/3
Choroid plexus tumours	
Choroid plexus papilloma	9390/0
Atypical choroid plexus papilloma	*9390/1**
Choroid plexus carcinoma	9390/3
Other neuroepithelial tumours	
Astroblastoma	9430/3
Chordoid glioma of the third ventricle	9444/1
Angiocentric glioma	*9431/1**
Neuronal and mixed neuronal-glial tumours	
Dysplastic gangliocytoma of cerebellum (Lhermitte-Duclos)	9493/0
Desmoplastic infantile astrocytoma/ ganglioglioma	9412/1
Dysembryoplastic neuroepithelial tumour	9413/0
Gangliocytoma	9492/0
Ganglioglioma	9505/1
Anaplastic ganglioglioma	9505/3
Central neurocytoma	9506/1
Extraventricular neurocytoma	*9506/1**
Cerebellar liponeurocytoma	*9506/1**
Papillary glioneuronal tumour	*9509/1**
Rosette-forming glioneuronal tumour of the fourth ventricle	*9509/1**
Paraganglioma	8680/1
Tumours of the pineal region	
Pineocytoma	9361/1
Pineal parenchymal tumour of intermediate differentiation	9362/3
Pineoblastoma	9362/3
Papillary tumour of the pineal region	*9395/3**
Embryonal tumours	
Medulloblastoma	9470/3
Desmoplastic/nodular medulloblastoma	9471/3
Medulloblastoma with extensive nodularity	*9471/3**
Anaplastic medulloblastoma	*9474/3**
Large cell medulloblastoma	9474/3
CNS primitive neuroectodermal tumour	9473/3
CNS Neuroblastoma	9500/3
CNS Ganglioneuroblastoma	9490/3
Medulloepithelioma	9501/3
Ependymoblastoma	9392/3
Atypical teratoid / rhabdoid tumour	9508/3

TUMOURS OF CRANIAL AND PARASPINAL NERVES

Schwannoma (neurilemoma, neurinoma)	9560/0
Cellular	9560/0
Plexiform	9560/0
Melanotic	9560/0
Neurofibroma	9540/0
Plexiform	9550/0

[1] Morphology code of the International Classification of Diseases for Oncology (ICD-O) (614A) and the Systematized Nomenclature of Medicine (http://snomed.org). Behaviour is coded /0 for benign tumours, /3 for malignant tumours and /1 for borderline or uncertain behaviour.

* The italicised numbers are provisional codes proposed for the 4th edition of ICD-O. While they are expected to be incorporated into the next ICD-O edition, they currently remain subject to change.

Definition

Glioblastoma is a malignant astrocytic tumour. It is composed of poorly differentiated neoplastic astrocytic cells, with cellular pleomorphism, nuclear atypia, a high mitotic rate sometimes accompanied by vascular thrombosis, microvascular proliferation with areas of necrosis (1).

Anaplastic astrocytoma is a diffusely infiltrating astrocytoma with focal anaplasia with a marked proliferating potential and has no necrosis. They arise from low grade astrocytomas or even de novo without a malignant precursor. They may evolve to the malignant glioblastoma (2).

Molecular pathogenesis of the WHO grade IV GBM has been linked with isocitrate dehydrogenase IDH1 and IDH2 gene mutations in secondary tumours >50% (6) and less frequently in the primary GBM.

This study aimed to sub-classify the glioblastoma into GBM-IDH and primary GBM, using the biomarkers of antibodies against mutant IDH (IDH R132H), and ATRX, The sub classification of these tumours will include;

- Glioblastoma with no IDH mutation and ATRX retention
- Rare glioblastoma with no IDH mutation and ATRX loss.

This study would help further aid decision making in patient management and predict the prognosis and with frequent utility by the pathologist and laboratory personnel making it an affordable test for diagnostic and predictive value.

LITERATURE REVIEW

Primary and secondary GBM were terms first used by Scherer a German scientist who in 1940 was in exile and felt that the two needed to be distinguished. The secondary GBM developed from the astrocytomas and was responsible for the long clinical duration. (7) For purpose of discussion in this study the non-primary GBM tumours will be referred to as IDH-GBM.

Scherer was ahead of his time even as they wrote the WHO classification of nervous system tumours in 1993 as the two had not been identified as two distinct pathologies (8) They were not even recognized as astrocytic tumours, instead listed as poorly differentiated embryonal tumours. With the introduction of immunohistochemistry we are now able to distinguish the two with their various subtypes and identify its astrocytic origin.

The primary GBM accounts for majority of the cases in the elderly (mean age 55) with a short clinical duration of less than three months. They develop de novo with no clinical, radiological or histopathological evidence of a preexisting less malignant precursor. They result as a combination of multiple genetic mutations.

IDH-GBM develop from younger patients mostly under 45 years and as a malignant progression from diffuse astrocytoma WHO grade 2 and anaplastic astrocytoma grade 3. The progression differs from less than 1 year to more than 10years (9)(10)

TP53 mutations are uncommon in primary glioblastoma but occur more often in IDH glioblastoma; EGF receptor (EGFR) overexpression prevailed in primary glioblastoma but was rare in secondary glioblastoma. Only 1 of 49 glioblastoma showed *TP53* mutation and EGFR overexpression, indicating that these alterations are mutually exclusive events defining 2 different genetic pathways in the evolution of glioblastoma (11)

Primary GBM is associated with EGFR amplication, PTEN (MMAC1) mutation and loss of chromosome 10, CDKN2A (p16) deletion and less frequently MDM2 amplication. IDH-GBM is associated with TP53 mutations though more of TP53 protein accumulation and 19q loss. In GBMs, ATRX loss was seen to be associated with IDH1/2 mutation.

The GBM with IDH1 mutations are secondary now classified in the WHO Classification of Tumours of the Central Nervous System 2016 guide line as Glioblastoma, IDH-mutant. There is

glioblastoma IDH-wild type which have been shown to have a poor prognosis as compared to the longer survival time of the Glioblastoma, IDH-mutant.

DISEASE CLASSIFICATION

Brief Epidemiology

GBM are the most frequent brain tumour about 12-15% of all intracranial malignant tumours(2)and are the most frequent astrocytic tumours 69% in the European population. In Switzerland there are 3.55 new cases in 1000,000 per year and in the US 2.96 new cases in 100,000 per year. (Central Brain Tumor Registry of the United States: Central Brain Tumor Registry of the United States (CBTRUS). Though this doesn't account for the GBMs that develop from diffuse/ low grade astrocytomas (DA) grade II or the anaplastic astrocytoma (AA) WHO grade III.

Age and Sex distribution

GBM may occur at any age but occurs mostly in adults between age 45 and 70 years. A study done in Zurich of 1003 cases showed over 70% of patients within this age group. In the same study series it demonstrated a male preponderance of 3:2 to the female population.

The diagnosis of IDH-glioblastoma requires prior clinical or histological history of a less malignant astrocytoma i.e. WHO grade II OR III tumours. At a population level, at the University of Alabama they found that only 5% of all cases were secondary glioblastoma as compared to primary GBM with histopathological evidence of a precursor diffuse or anaplastic astrocytoma.(1)

Localization

Glioblastoma is found mainly in the cerebral hemisphere that is supratentorial with a high incidence in the temporal lobe and least commonly in the occipital lobes but can also be rarely located in the spine and cerebellum. GBM has been found in the brainstem and mainly affects children if in the brainstem

IDH-glioblastoma tend to have frontal lobe involvement, in especially the region surrounding the rostral extension of the lateral ventricles. Stockhammer and colleagues showed that IDH1/2 WHO

grade II astrocytomas tend to develop in a frontal location, while the anaplastic astrocytoma have a preference for the cerebral hemispheres (12).

Histopathology

An anaplastic pleomorphic tumour is composed of poorly differentiated astrocytes with, marked nuclear atypia and high mitotic activity with necrosis. There may be prominent endothelial proliferation. All display high cellularity with high degree of nuclear pleomorphism, and may be monomorphic i.e. CLASSICAL type, may have multinucleated giant cells i.e. GIANT CELL (GC) type, have spindling or ovoid cells i.e. GLIOSARCOMA or have a swollen clear cytoplasm OLIGODENCYTRIC.

The GC subtype has numerous multinucleated giant cells, small syncytial cells with a reticulin network. They may have prominent nucleoli and cytoplasmic inclusions.

Gliosarcoma is characterized by a biphasic pattern displaying both glial and mesenchymal differentiation which is sarcomatous with the typical herringbone pattern and long bundles of spindle cells.

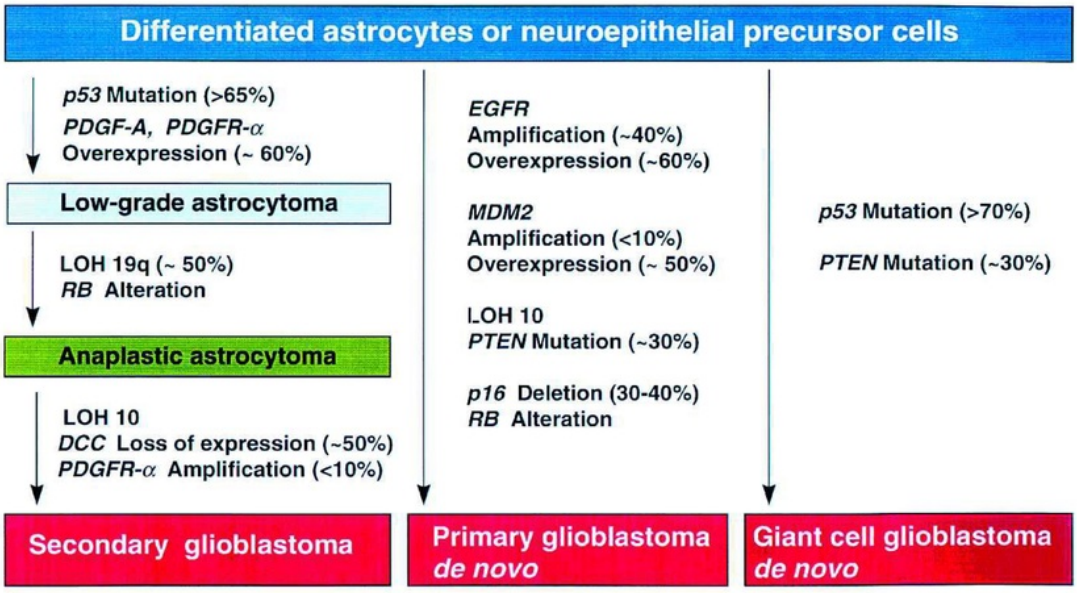
Spread and Metastasis

Usually spread is through infiltration. Extension may occur to the corpus callosum into the contralateral cerebral hemisphere forming a bilateral symmetrical tumor i.e. the 'Butterfly Glioma'. There is a rapid spread to the anterior commissure internal capsule and fornix enlarging them and distorting their normal anatomy.(1)

It rarely metastasizes via CSF into the subarachnoid space (13) Although it may extend within and along the perivascular spaces but not into the vessel lumen (14). Hematogenous spread to extraneural tissue is very rare especially in patients who have not undergone any surgical intervention. There may be intraperitoneal metastases through the venticuloperitoneal shunts (15).

The GBM tumours may occur as independent multifocal tumours and not as a result of metastasis as described by Batzford et al 2.4% (16) and Barnad et al 7.6% (17).

Figure 1 Genetic pathways operative in the evolution of glioblastoma.



Modified from Kleihues et al.1997

HISTOPATHOLOGY

According to the 2007 WHO classification, histologic criteria for the diagnosis of glioblastoma include nuclear atypia, cellular pleomorphism, mitotic activity, vascular thrombosis, microvascular proliferation, and necrosis(1). Glioblastoma may show significant intertumoral and intratumoral heterogeneity, both histologically and genetically(18)(19)(20)and this may also apply to glioma-initiating cells(20). This heterogeneity reflects genomic instability and, occasionally, focal new clones arising as a result of additional genetic alterations can be seen histologically(21)(22). Areas with oligodendroglioma-like components are significantly more frequent in secondary than primary glioblastoma (42% vs. 18%; $P = 0.0138$; (18) and, accordingly, more frequent in $IDH1^{mut}$ glioblastoma than in $IDH1^{wt}$ glioblastoma (54% vs. 20%; $P < 0.0001$;(4); **Table 1**). An increase in the fraction of tumor cells with oligodendroglial morphology in IDH glioblastomas was also reported in a large study of 618 cases(23). This is not surprising as secondary glioblastoma assumedly share IDH1 mutation precursor cells with oligodendrogliomas.

Anaplastic astrocytoma histopathologic findings are similar to those of a diffuse infiltrating astrocytoma with increased cellularity, distinct nuclear atypia and marked mitotic activity. When you see microvascular glomerular or festoons and of necrotic foci then it has progressed to glioblastoma(12).

IMMUNOHISTOCHEMISTRY FINDINGS

Ki-67 Antigen

Routine assessment of the cell proliferation rate may be determined by using Hematoxylin and Eosin where by the pathologist counts the number of mitosis seen in a high power field. Using cell proliferative markers we are able to better assess it. The cell proliferative rates help tell us the tumour biology. Most glial cells are you usually in a non-proliferative state. Tumour cells are always multiplying and this can be used to prognosticate and also in treatment choices so we are able to use targeted therapy.

Ki-67/MIB-1 a monoclonal antibody is commonly used and has proven prognostic and diagnostic power in astrocytic tumours. The Ki-67 antigen is a large nuclear protein (345, 395 kDa) preferentially expressed during all active phases of the cell cycle (G_1, S, G_2 and M-phases), but absent in resting cells (G_0-phase). The name Ki-67 is derived from its town of origin Kiel in Germany and 67 was the plate number of the original clone of the 96 well. The antibody is useful in classification of a variety of tumors.(24)

Anaplastic astrocytomas typically displays mitotic activity with Ki-67/MIB usually in the range of 5-10% while that of glioblastoma proliferative activity is usually prominent with numerous typical and atypical mitoses. The growth fraction shows great variation with a mean of 15-20%(25).

Isocitrate Dehydrogenase (IDH)

Isocitrate dehydrogenase (IDH)1 and IDH2, are metabolic enzymes involved in the citric acid cycle (Kreb's Cycle) and produce cytosolic NADPH which is utilized in the regeneration of reduced glutathione, a major cellular antioxidant (26). The IDH -1 mutation is found in the first or

second base pair of Arginine 132 (6) and is observed in 89-91% of astrocytic and oligodendrocytic gliomas(27).

The substitution mutation of an arginine 132 residue with histidine leads to reorganization of the active site R132H; causing loss of enzymatic function for oxidative decarboxylation of isocitrate; and causes a gain of function for the NADPH-dependent reduction of α-ketoglutarate leading to the production of 2-hydroxyglutarate (2HG) (28)(29). Mutations in the enzyme cytosolic isocitrate dehydrogenase 1 (IDH1) are found in approximately 80% of grade II-III gliomas and secondary glioblastoma in humans.

In 2008 Parsons et al was able to identify the IDH-1 gene mutation(6) especially in the secondary GBM of the younger GBM patients. >80% and <5% in PGBM. It is now agreed that *IDH1* mutation is a definitive diagnostic molecular marker of secondary glioblastoma and more reliable and objective than clinical and/or pathologic criteria.

Using a commercially produced antibody, 90% of *IDH* mutations can be detected. IDH is a strong perinuclear cytoplasmic stain which may sometimes display weak nuclear staining.

ATRX

Inactivating alterations in Alpha Thalassemia/Mental Retardation Syndrome X-linked (*ATRX*) were recently identified in 7% of adult GBMs and in 14-31% of pediatric GBMs (30). ATRX is critical for normal telomere homeostasis by regulating incorporation of histone variant H3.3 into telomeric chromatin(31)(32), and *ATRX* alterations are associated with an alternative lengthening of telomeres (ALT) phenotype among GBMs and this allows the cells to divide indefinitely, unlike normal cells which may only undergo a programmed number of cell divisions. The cell death induced by ATR inhibitors is highly selective for cancer cells that rely on ALT, suggesting that such inhibitors may be useful for treatment of ALT-positive cancers

ATRX stain is a strong nuclear stain. Non-neoplastic brain regions like the neuronrs show strong immunoreactivity to ATRX, whereas glial cells and blood vessels show weaker reactivity. These regions are used as internal controls. Nuclear ATRX loss is scored as specific if tumor cell nuclei

were completely unstained while nuclei of non-neoplastic cells such as endothelia, microglia, lymphocytes and reactive astrocytes were strongly positive.

CHAPTER TWO

JUSTIFICATION

Despite the extensive published studies of GBM internationally, there is a paucity of data available to describe the different types of GBM in Kenya. As has been proven in other parts of the world, molecular data on GBM has a major impact on prognosis and therapy of the patients.

The study sought to reclassify previously inaccurately diagnosed tumours, or tumours that were correctly diagnosed according to the 2007 WHO classification which is now superseded by the 2016 classification. This would give a very good idea about the utility of the biomarkers and necessity to carry out IDH ans ATRX sequencing in the clinical practice in young people with astrocytoma. The data from this study can be used to define the cost/effectiveness algorithm for our environment.

RESEARCH QUESTION

What is the role of immunohistochemistry in the diagnosis of glioblastoma and anaplastic astrocytoma at Kenyatta National Hospital?

BROAD OBJECTIVE

To describe the morphological patterns and immuno-histochemistry of glioblastoma and anaplastic astrocytoma at Kenyatta National Hospital.

SPECIFIC OBJECTIVE

1. To review all cases diagnosed as glioblastoma and anaplastic astrocytoma on light microscopy.
2. To determine the Immunohistochemistry staining pattern of glioblastoma and anaplastic astrocytoma using IDH-1 and ATRX.
3. To determine the cell proliferation rate of glioblastoma and anaplastic astrocytoma using Ki-67.

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CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

Laboratory based retrospective descriptive study.

3.2 Study Site

The study was conducted in KNH/UON Histo-Pathology Laboratory.

3.3 Study Population

Fifty six histopathological Formalin Fixed Paraffin Embedded (FFPE) tissue specimen with a diagnosis of glioblastoma WHO stage 4 and anapalastic astrocytoma WHO stage 3 over a retrospective period of four and a half years from January 2012 to June 2016.

3.4 Study Eligibility Criteria

3.4.1 Inclusion Criteria

Cases that were diagnosed as WHO grade III and IV astrogliomas on histopathology diagnosis at Kenyatta National Hospital from 2012 to June 2016, whose reports and tissue blocks were available.

3.4.2 Exclusion criteria

1. Laboratory reports of which the tissue blocks could not be retrieved or were damaged or lacked sufficient tissues for evaluation.
2. Poorly processed tissue.

3.5 Identification of Cases

The files containing histology reports at the Kenyatta National Hospital were perused to identify all cases that meet the inclusion criteria. The name, sex, ward, patients hospital number, hospital

name and laboratory number were noted in the proforma form from the histology report as the cases were identified. This information was used to retrieve the archived specimen.

3.6 Sampling Method

Consecutive sampling method was used to select the samples for the study. Sampling frame was created from the list of all the GBM collected between 1st January 2012 to 30th June 2016.

3.7 Sample Size Determination

Immuno-histochemistry is useful in the detection of mutation present in tissues diagnosed with Glioblastoma. In this study the proportion IDH mutations and ATRX loss was reported to describe the Immuno-histochemistry findings. Given the study design (descriptive cross-sectional) and outcome of interest, the sample size determination was based on precision using the formula by Daniel (1999)

$$n \geq \frac{NZ^2{}_{\alpha/2}P(1-P)}{d^2(N-1)+Z^2{}_{\alpha/2}P(1-P)}$$

Where;

n Minimum sample size

N Estimated population size within the study period (N=55)

P Estimated prevalence of cytoplasm mutation among glioblastoma cases (P was set up at 0.5 because there was no available information on the mutation prevalence from previous studies)

$Z_{\alpha/2}$ Critical value for standard normal distribution at α-level of significance (α=0.05, $Z_{\alpha/2}$=1.96)

D Margin of error (d=0.03)

Using the above formula, the calculated minimum sample size was 44 tissues. In this study, 46 tissue specimens were available from archives.

3.8 STUDY PROCEDURE

3.8.1 Data Collection Instruments

3.8.1.1 PROCESSING OF THE TISSUE AND REPORTING OF THE RESULTS

1. All reports filed in the department and diagnosed as glioblastoma and anapalastic astrocytoma were retrieved.
2. Corresponding paraffin blocks, were sectioned and stained using the routine Hematoxylin/ Eosin and Ki-67 method. (Appendix I)
3. Slides were microscopically examined with the help of my supervisors and a diagnosis using WHO classification of grading (identifying mitotic activity, microvascular proliferation and necrosis) was made.
4. Immunostaining for IDH1, ATRX and Ki-67 was performed. .

3.8.1.2 MATERIALS

Equipment

A semi- automated Rotary microtome and an Olympus microscope provided by the University of Nairobi department of Human Pathology.

IDH-1, ATRX and Ki-67 TMA block and immunostaining were perfomed manually at the University of Nairobi Histopathology laboratory using the standard operating procedure (Appendix II).

Reagents and other consumables

Gloves, cassettes, microtome blades, staining racks, slides, slide holder, labels, and reagents were purchased by the principal investigator.

3.9 METHODS

3.9.1 Paraffin Embedded Block Retrieval

Paraffin wax embedded blocks were retrieved from histopathology archives at the study site using the laboratory numbers on the retrieved pathology reports.

3.9.2 Histopathological Preparation

a) Heamatoxylin and Eosin

A 4µm section was cut from each of the blocks and stained with i)Hematoxylin & Eosin and ii) Ki-67 as per protocol. This procedure was performed at the University of Nairobi histopathology with assistance of laboratory technologists (Appendix II).

b) Tissue Miro-Array Preparation

For the tissue microarray (TMA) slides, they were prepared using a TMA machine (Beecher Instruments, Inc., Sun Prairie, WI, USA), two cylindrical representative tissue core biopsies of 1000 µm diameter were punched from each paraffin donor block and transferred to the pre-punched holes on recipient paraffin blocks at defined array coordinates.

Short sealing of the recipient blocks at 37 °C was done.

The TMA blocks were cut with a microtome (Microm International, Walldorf, Germany) into 4 µm thick sections, and mounted on SuperFrost Plus slides.

Afterwards, the slides were stained with hematoxylin and eosin and reviewed for adequate tumor representation in the individual cores.

3.9.3 Immunohistochemistry

IDH-1, ATRX and Ki-67 TMA blocking and immunostaining were perfomed manually at the University of Nairobi Histopathology laboratory using the standard operating procedure (Appendix III).

Settings for the ATRX OptiView method was: Cell Conditioner 1 pre-treatment for 40mins, primary antibody incubation for 20 min at 42 °C, antibody dilution 1:400, and counterstaining done with hematoxylin.

Twenty-four sample punches from four representative tissue microarrays were selected and ATRX re-staining performed in whole tissue sections of the same tumor for validation of the results.

Samples were considered positive when more than 10% of tumor cells showed nuclear immune reactivity for ATRX and were considered negative, when the tumor cells showed no nuclear positivity in the presence of endothelial cells, cortical neurons and infiltrating inflammatory cells were generally positive and served as internal positive controls.

Immunoreaction for IDH-1 was scored positive when tumor cells showed a strong cytoplasmic staining for mIDH1R132H. A weak diffuse staining and staining of macrophages was not scored positive.

The slides were reported by the principal investigator then reviewed with the two supervisors who are qualified pathologists.

3.11 Sample Analysis

- All tumors were initially classified and graded according to the current WHO 2007 guidelines (33)
- Mitotic activity was calculated in two different ways: (1) as the number of mitotic figures in 10 consecutive fields (mitotic activity index; MAI) represented as a mean or (2) as the percentage of mitotic cells out of a total of 1000 neoplastic cells (mitotic index; MI) which was represented as a range.

3.12 Data Management and Quality

Data collected was recorded on a hard copy register then cleaned, verified and thereafter entered into a Microsoft Excel worksheet. The cleaned worksheet was imported to the statistical analysis software and analysed. Confidentiality was maintained, as only the principal investigator had access to the data. Information on soft copy was protected from unauthorized persons by password enabling. All records were identified by study identification number (GBA).

3.13 Data Analysis and Presentation

The descriptive analysis of cases diagnosed as glioblastoma and anaplastic astrocytoma on light microscopy comparing mitotic activity using Ki-67 and also differentiation of primary and IDH-GBM using Immunohistochemistry staining pattern of IDH-1 on GBM was done.

Analysis of continuous data variables such as age was presented using means and respective standard deviations (SD) or medians and inter-quartile range (IQR) as deemed appropriate.

Counts and corresponding percentages (%) were used for categorical variables such as gender, site of lesion, immunohistochemistry categories etc.

In bivariate analysis, k-sample test for equality of medians was used to compare the distribution of mitotic index by age group and chi-square test of association to evaluate the association between mitotic index and Ki-67 staining pattern.

Test statistic and corresponding p-values were reported with a 0.05 α-level of significance. For correlation analyses unpaired, two-tailed Student's t-test and the Fisher's exact test were performed to identify possible significant associations or differences between two pairs.

Pictorial presentation of some results will be done using pie, bar and box charts as deemed appropriate.

Stata version 12 (Stata Corp, College Station Texas) was used for all statistical analyses.

3.14 Quality Assurance

3.14.1 Pre-Analytical Stage

- ✓ All reagents were prepared according to the manufacturer's instructions.
- ✓ Standard operating procedures were adhered to during all the procedures.
- ✓ The reagents were checked for expiry date, turbidity, odour and precipitates.
- ✓ Observation of recommended storage requirements for all reagents were observed.
- ✓ The slides were well labelled before mounting the sections and then arranged in order to avoid mix up of slides,

3.14.2 Analytical

- ✓ All TMAs were screened for internal positive controls such as endothelial cells and/or trapped cortical neurons.
- ✓ Positive controls were used while doing immunohistochemistry staining and interpretation.

3.15 Ethical consideration

Authority was sought from the KNH/UON ethical and research committee and study commenced after gaining formal approval from the committee.

Confidentiality of the participating subjects was maintained.

Any changes in diagnosis was to be communicated to the neurosurgeon (study supervisor) who would have initiated appropriate follow-up as deemed suitable to allow correct patient care.

CHAPTER FOUR

RESULTS

During the study period January 2012 to June 2016 there were fifty six (56) cases reported as glioblastoma and five (5) cases of anaplastic astrocytoma. One oligoastrocytoma was included in the study as it was felt it was under called and one atypical teratoid/rhabdoid tumour which on review was actually a glioblastoma case.Of the fifty six of the GBM cases forty six cases proceeded into the study as they met the inclusion criteria, the remaining ten had blocks missing. The anaplastic astrocytoma, 3 of the 5 cases were retrieved from the archives and the remaining two blocks were missing.

4.1 REVIEW OF CASES

4.1.1 Demographic Characteristics of Patients

A total of 46 tissue specimens with a diagnosis of WHO glial grade III and IV tumours were reviewed. The tissues had been obtained from both male patients (24/44; 52.2%) who were more compared to female (22/44; 47.8%). They were aged between 7 and 70 years.

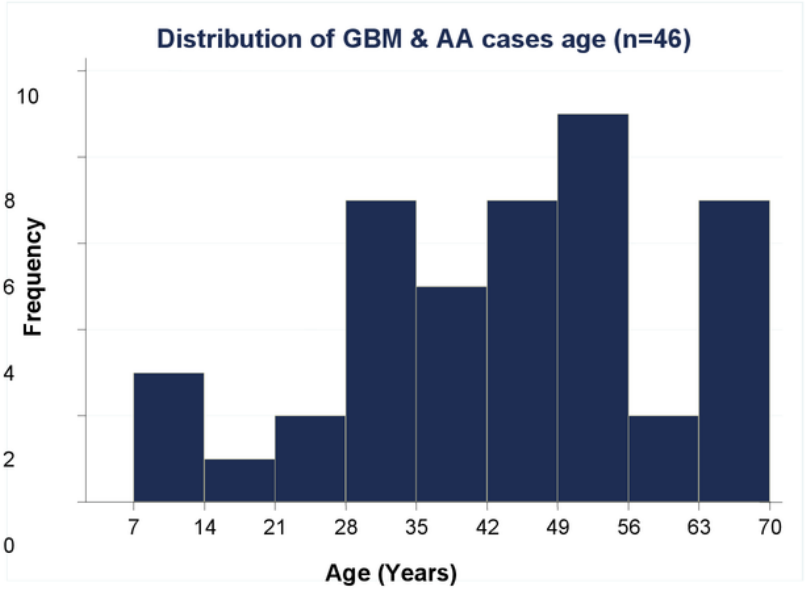


Table 1: Distribution of patients' age (years)

The age distribution showed a peak between 49-56 years. Median age was 44 years with an inter-quartile range of 22 years. Median age was used as the graph did not display normal distribution. There was a right sided skew since most of our cases were of patients above 40years.Adults (>20years) were the majority (92.7%). Paediatrics (≤20years) were 3: two aged 7 years and 10 years.

4.1.2 Review of Tumor Diagnosis

Table 2: Tumor sites

Variable	Category	Frequency
Site of tumor (n=46)	Frontal lobe	1
	Temporal lobe	7
	Parietal	4
	Cerebellum	2
	Temporal & parietal	4
	Frontal & parietal	4
	Frontal & temporal	3
	Occipital & Cerebellum	1
	Parietal & Occipital lobe	1
	Frontal, temporal & parietal	1
	Cerebro-pontine angle	1
	Lateral ventricles	1
	Not indicated	16

Fourteen (n=45) had tumors in more than one site. The most common tumour locations were in the frontal, temporal and parietal lobes. More than 50% of the cases had site location missing from their investigation request forms.

4.1.3 Tissue Specimen Findings

Table 3: Summary of the Tissue Specimen Review Findings

Variable	Category	WHO Grade classification 2007	Frequency	Proportion
Previous histomorphological Diagnosis (n=46)	Glioblastoma	IV	41	89.1
	Anaplastic Astrocytoma	III	3	6.5
	Atypical teratoid/Rhabdoid teratoid	IV	1	2.2
	Oligoastrocytoma	III	1	2.2
Current histomorphological Diagnosis (n=46)	Glioblastoma	IV	44	95.6
	Anaplastic Astrocytoma	III	1	2.2
	No tumor	-	1	2.2

According to the initial histomorphological diagnosis there were 41 glioblastoma (WHO grade IV), 3 anaplastic astrocytoma WHO grade II), 1 atypical teratoid (WHO IV) and 1 oligrastocytoma (WHO grade II) cases. The review after consensus of the investigator and the two supervisors was 44 glioblastoma (WHO grade IV), 1 anaplastic astrocytoma cases (WHO grade III) and 1 case was tumor negative having reviewed all of its blocks.

Glioblastoma Subtypes

Table 4: Distribution of Glioblastoma Subtypes

Variable	Subtype	Frequency	Proportion
Glioblastoma subtype (n=44)	Classical	34	77.3
	Giant cell	6	13.6
	Gliosarcoma	3	6.8
	Oligodencytric	1	2.3

Majority of the glioblastoma were the classical subtype 34/44, one case was of oligodencytric subtype.

4.2 Immuno-Histochemistry Staining Pattern

Forty five of the forty six cases (both GBM and AA) included in the study underwent IDH1 and ATRX immunohistochemistry since one case on review was found to be tumour free.

4.2.1 IDH1 and ATRX

Table 5: IDH1 and ATRX Staining Pattern

Immunohistochemistry tests	Category	Frequency	Proportion
ATRX (n=45)	Positive	23	51.1
	Negative	18	40.0
	Inconclusive	4	8.9
IDH1 (n=45)	Positive	12	26.7
	Negative	32	71.1
	Inconclusive	1	2.2

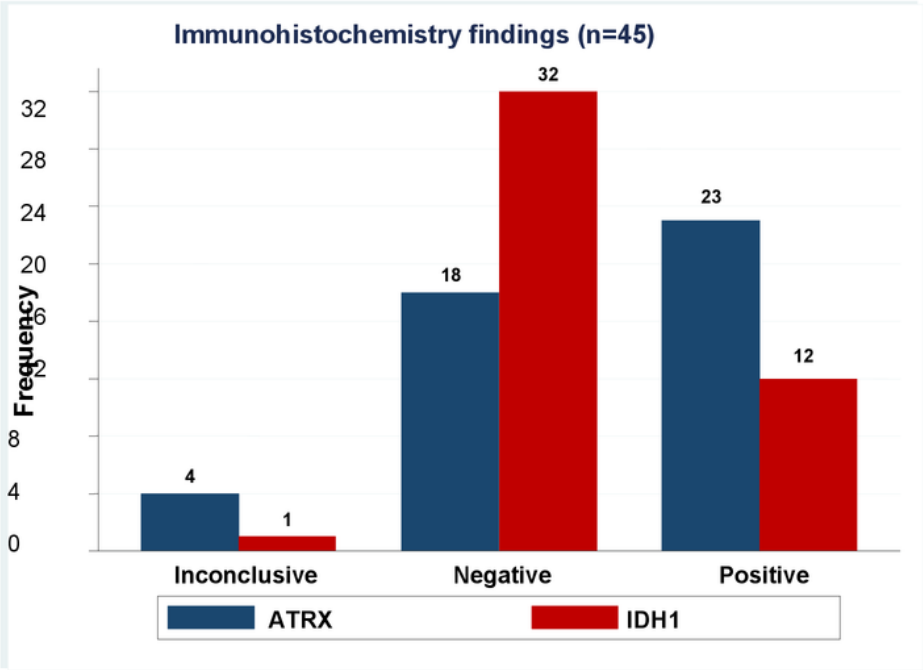


Figure 2: ATRX and IDH1 Immuno-Histochemistry Results

The majority of positive cases demonstrated a strong perinuclear cytoplasmic staining with additional weaker nuclear staining, the negative and positive cases were very clear. Of the GBM cases 71.1% were IDH1 negative and twelve (n=45) cases were positive for IDH1 mutation 26.7% which included one AA case and 11 GBM cases.

Out of the 45 cases, 17(37.8%) GBM and 1 (2.2%) Anapalstic astrocytoma had ATRX loss as all tumor cell nuclei were completely unstained while nuclear positivity was seen in vessels, microglia, reactive astrocytes and entrapped neurons. 23 of the GBM had a positive nuclear stain for ATRX and four glioblastoma cases were inconclusive as no immunoreaction was observed in the entire tissue, these cases were not scored and consequently not considered for statistical evaluation. (Table 5 and Figure 4)

Eight did not have either of the mutations (ATRX positive test and IDH1 negative test). The only anaplastic astrocytoma case was positive for IDH1 evaluation. Overall one case had both IDH1 mutations and ATRX loss and that was an anaplastic astrocytoma case.

Table 6: Summary Statistics for Patient's Age of by Immunohistochemistry Findings

Immunochemistry test	Category	Median age	IQR	Minimum	Maximum
ATRX	Negative	44	33-52	7	64
	Positive	43	34-60	10	70
IDH1	Negative	43	33-52	7	70
	Positive	52	30-63	15	69

The age distribution for patients was similar between the ATRX negative and ATRX positive (Median test chi-sq=0.108; p-value=0.742) and between IDH1 positive and IDH1 negative (Median test chi-sq=0.407; p-value=0.524).

4.3 CELL PROLIFERATION RESULTS

4.3.1 Tissue Staining Patterns

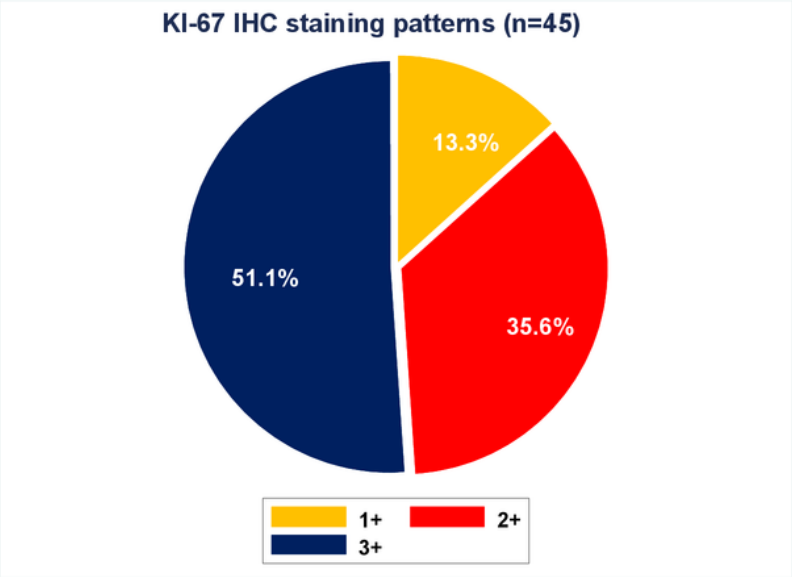


Figure 3: Distribution of Tissue Specimens by Ki-67 Staining Patterns

Most (51.1%) of the tissues specimens stained strongly (3+), 35.6% moderately (2+) and the rest 13.3% (1+) were weakly staining.

4.3.2 Mitotic Activity

A total of 45 cases underwent Ki-67 staining as one case was tumour free. All cases stained positive for KI-67 Antigen.

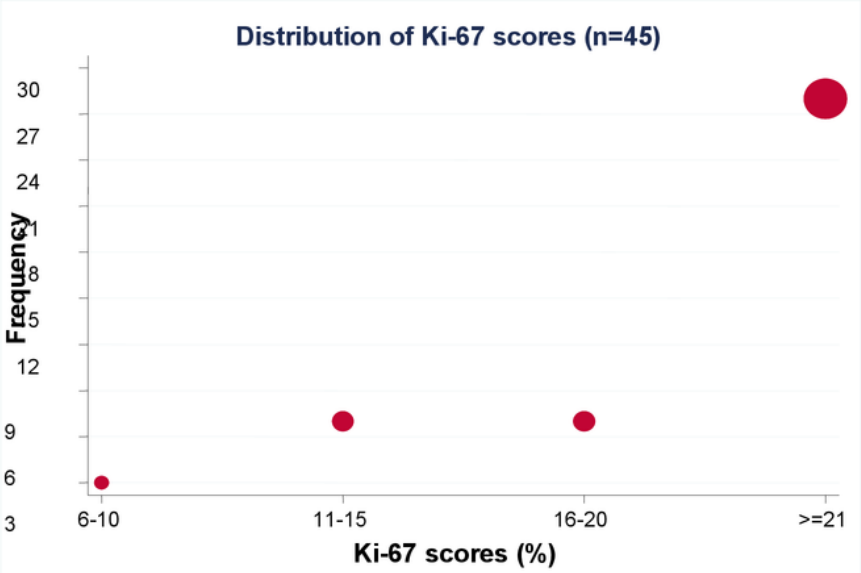


Figure 4: Distribution of Mitotic Activity Score

The size of the dots corresponds to the frequency (number of tumors having a specific Ki-67 score). Among the glioblastomas, majority (42/44) had a mitotic activity rate of 11% and above; 28 (63.6%) had a score of 21% and above the highest score was 75%. The anaplastic astrocytoma mitotic activity index was 7%.

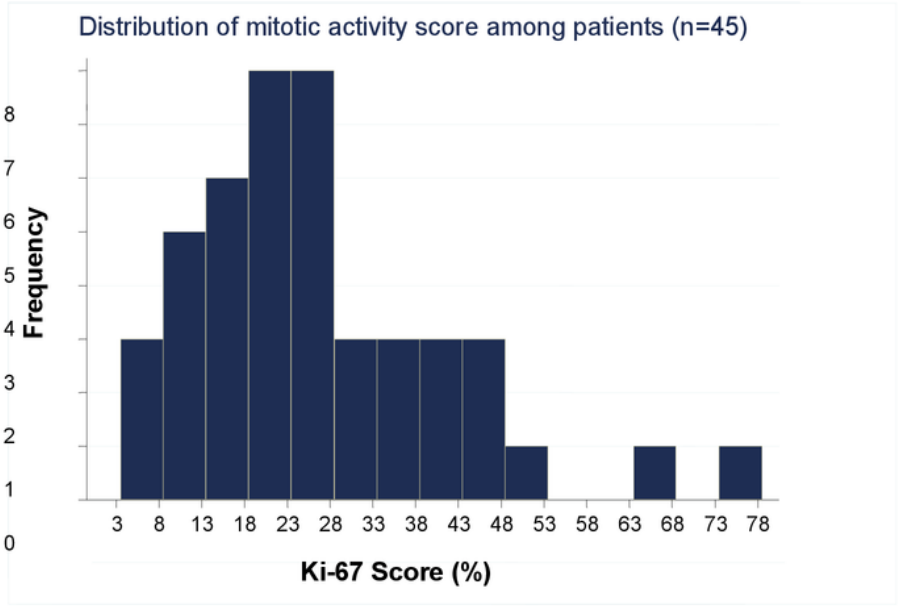


Figure 5: Graph showing the Distribution of Mitotic Activity Score among Patients.

The number of patients increased gradually with the increase in mitotic activity score until the peak (mode- 23%) after which a sudden decline in the number for scores above 28%. The median (IQR) mitotic score for glioblastoma was 24% (35%-17%). The mean (SD) mitotic score was 27.1% (15.1%)

Distribution of mitotic index by age group

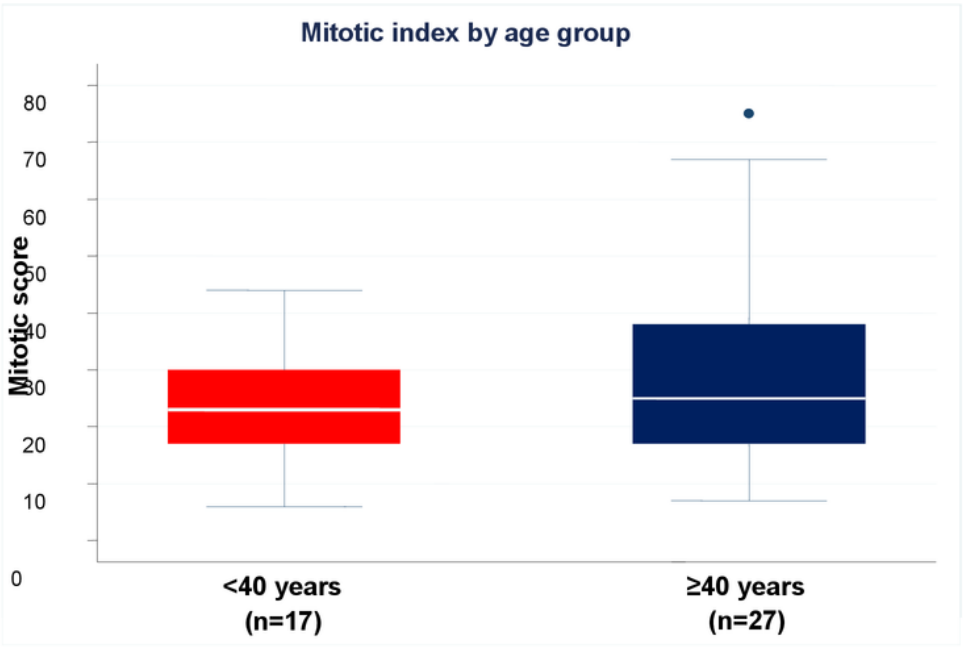


Figure 6: Mitotic Index by Age Group

Using the K-sample equality of medians test, there was no significant (Pearson chi-square=0.582; p-value=0.445) difference in the median mitotic score between glioblastoma patients aged below 40 years (median=20) and those aged 40 years and above (median=24).

Among patients aged below 40 years, mitotic index varied from 6% to 44% with 75% having a score of 30% and below. Among the 40 year old and above patients, mitotic score ranged between 7% and 75% with 75% having a mitotic score of 38% and below.

4.3.4 Correlation of the three Immunohistochemistry Antibodies

Pearson chi-square test was done to evaluate the association between ATRX loss, IDH1 mutations, age group and proliferation rate as shown in table below;

Table 1: Comparison of ATRX Loss versus IDH1, Age Group & Proliferation Rate

Variable	Category	ATRX Loss	ATRX Presence	Fisher's exact Statistic	P-value
IDH1	Negative	16	15	4.682	0.030
	Positive	1	8		
Age group	<40 years	7	9	0.0002	0.999
	≥ 40 years	11	14		
Proliferation rate	6-10	1	1	0.396	0.999
	11-15	3	3		
	16-20	2	4		
	≥ 21	12	15		

There was a statistically significant association between the IDH1 test findings and ATRX test findings (Fisher's statistic=4.682; p-value=0.030). Out of the 31 tissue specimens that did not have cytoplasm mutations (IDH1 negative result), 16 (51.6%) had ATRX loss whereas among the 9 tissues with cytoplasm mutations (IDH1 positive result), 1 (11.1%) had nucleus mutations which was the only case of anaplastic astrocytoma.

There was no significant association between ATRX test findings and patient's age group (Fisher's statistic=0.0002; p-value=0.999) or the tumor proliferation rate (Fisher's statistic=0.396; p-value=0.999)

Table 2: Comparison of IDH1 mutations versus age group & proliferation rate

Variable	Category	IDH1 Negative	IDH1 Positive	Fisher's Exact statistic	P-value
Age group	<40 years	13	4	0.196	0.739
	≥ 40 years	19	8		
Proliferation rate	6-10	3	0	*	*
	11-15	4	3		
	16-20	5	2		
	≥ 21	20	7		
Glioblastoma subtypes	Classical	25	8	*	*
	Giant cell	5	1		
	Gliosarcoma	1	2		
	Oligodencytric	0	1		

* *Test not done*

There was no significant association between cytoplasm mutation and tissue staining pattern (Fisher's statistic =1.373; p-value=0.633) or age group (Fisher's statistic =0.196; p-value=0.739). At each level of proliferation rate, there were more IDH1 negative compared to IDH1 positive.

4.4 HISTOPATHOLOGY MICROGRAPHS REPERSENTATION

Figure :1(a) and (b) shows brain tissue exhibiting increased cellularity with nuclear atypia and mitotic activity in the absence of vascular proliferation and necrosis.

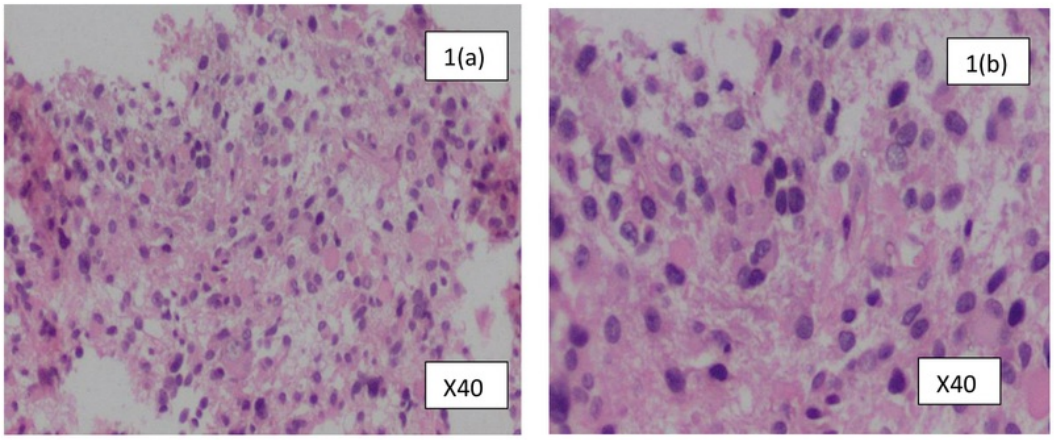
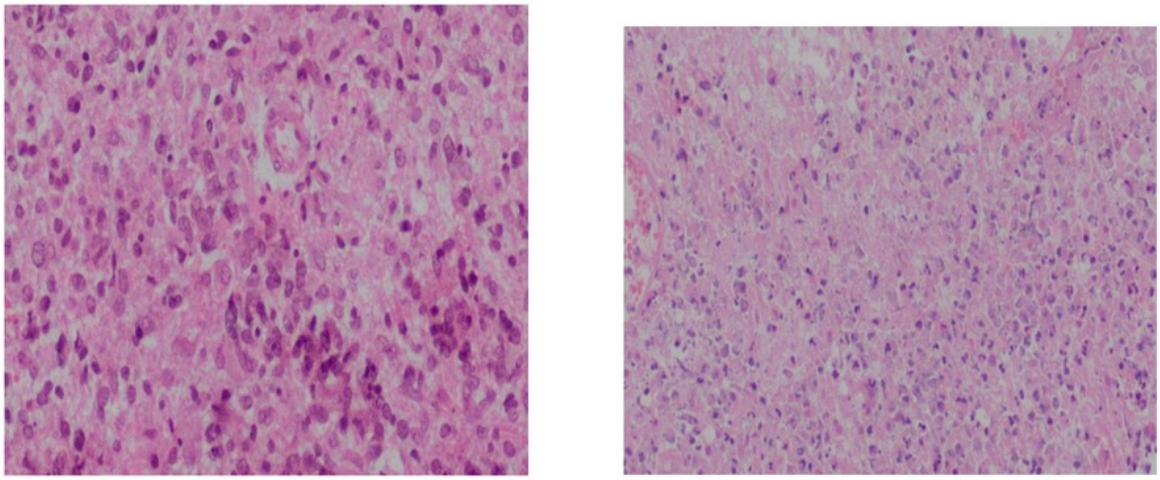


Figure 7: Anaplastic Astrocytoma

4.4.1 GLIOBLASTOMA DIAGNOSTIC CRITERIA

Figure: The photomicrographs show section of brain tissue demonstrating diagnostic criteria for glioblastoma stained with H/E at various magnification 2(a) cellular atypia and pleomorphism(black arrow) 2(b) necrosis with cellular debris 2(c) abnormal mitosis(blue arrow)



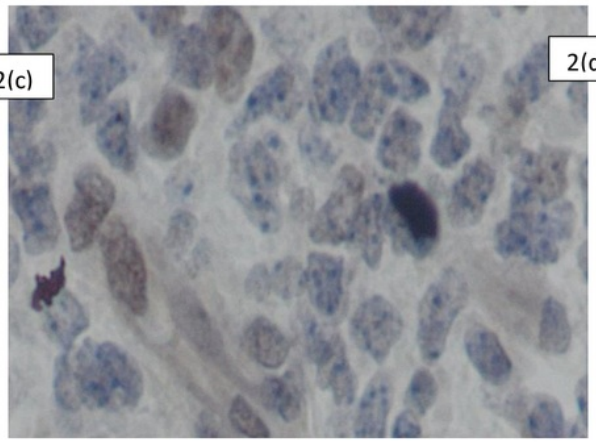
2(d) endothelial proliferation showing glomeruloid formation

2(a)

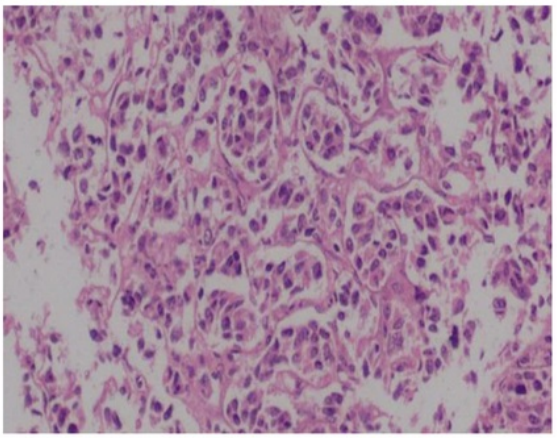
X2(b)

X40

2(c)



2(d



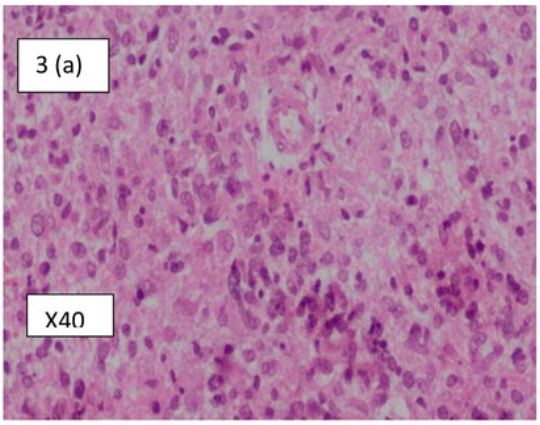
X100

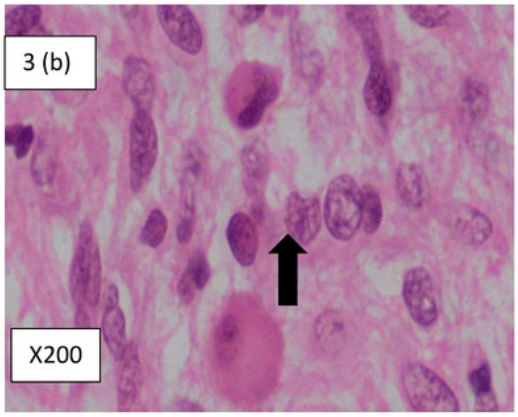
Figure 8: Morphology of Glioblastoma

4.4.2 GLIOBLASTOMA SUBTYPES

I) Classical

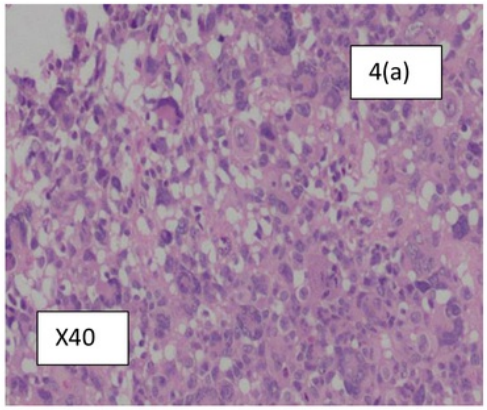
3(a) shows brain tissue stained with H/E exhibiting cellular pleomorphism and nuclear atypia. A line of parallel nuclei forming a pseudo palisade can be seen. 3(b) demonstrates the cellular atypia at x200 magnification (thick black arrow). Changes in the genetic control of cell growthand cell cycle give rise to the hypoxia, necrosis and the new vessel formation.

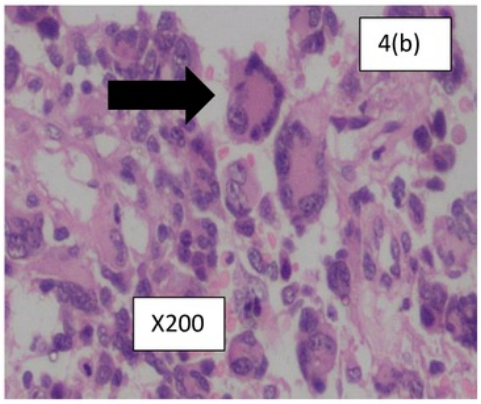




II) Giant cell

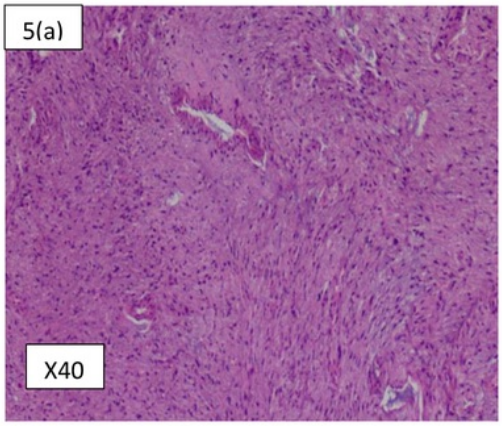
4(a) sections of the brain stained with H/E at x40 and 4(b) at x 200 infiltrated by an astrocytic tumour with areas of necrosis and multiple giant cells (black arrow)


4(a)
X40

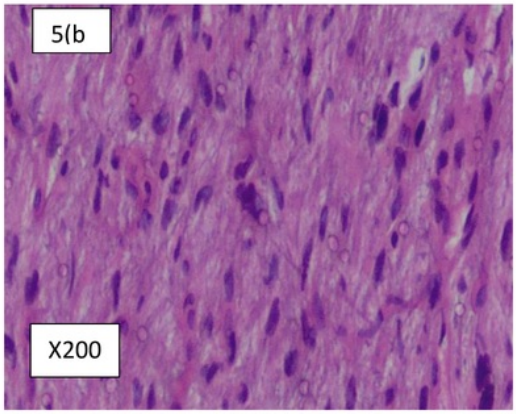

4(b)
X200

III) Gliosarcoma

Sections of the brain stained with H/E 5(a) x40 magnification with cellularity and arranged in fascicles 5(b) x200 the cells are spindle shapes with abnormal mitosis.



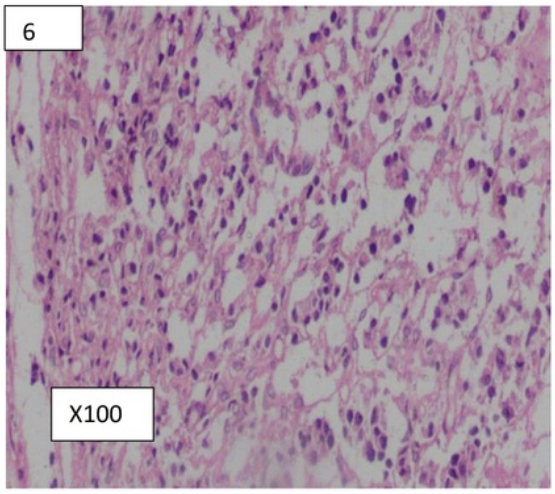
Low Magnification



High Magnification

IV) Oligodencytric

6 sections show nuclear pleomorphism and atypia with perinuclear clearing "fried egg" appearance.



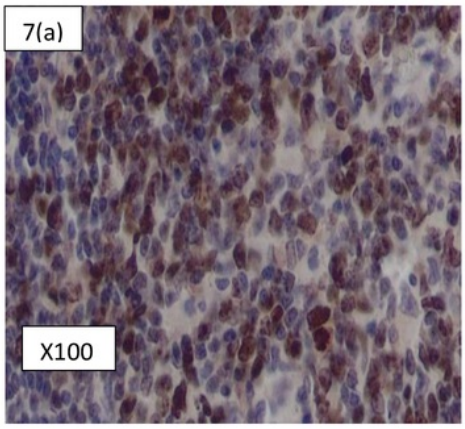
High Magnification

4.4.3 IMMUNOHISTOCHEMISTRY: KI-67, ATRX, IDH1

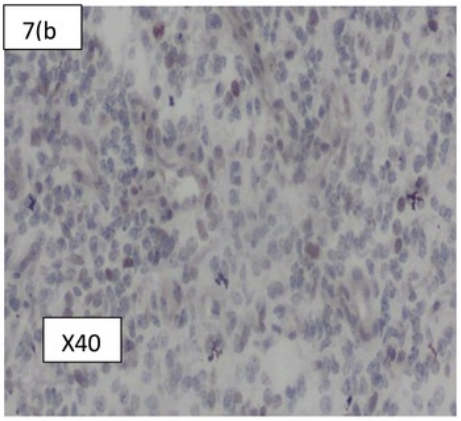
Figure 4: Immunohistochemistry

1. **KI-67 Antigen**

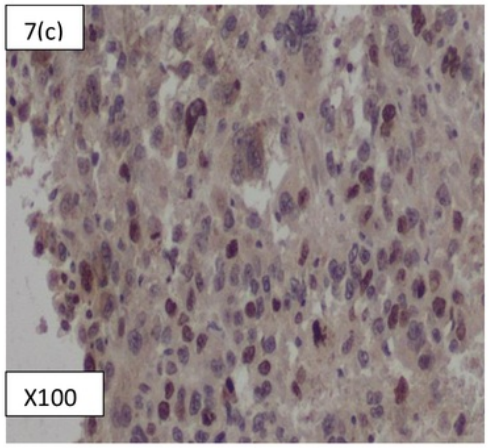
Sections show use of Ki-67 cell proliferation marker with different staining intensity and proliferation rate. It is a nuclear stain though the cytoplasm may weakly stain too



Positive Control



Weak Staining Intensity 1+



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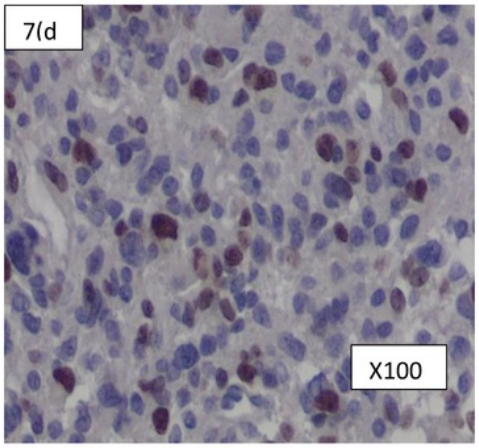
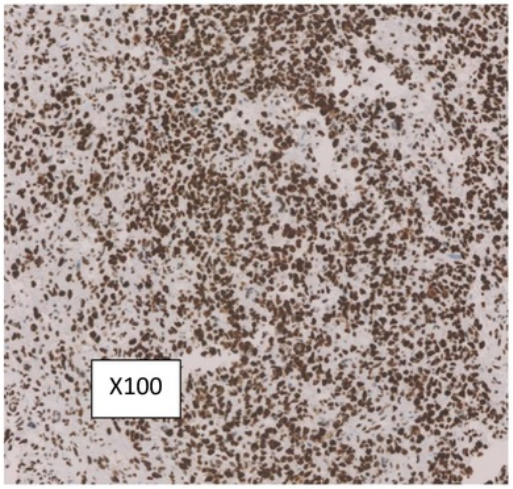
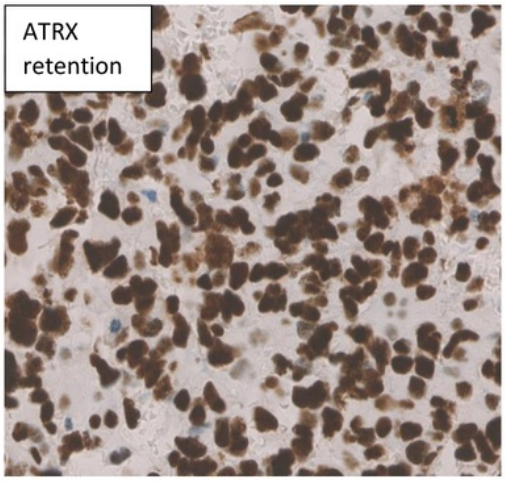
Moderate Staining Intensity 2+



Strong Staining Intensity 3+

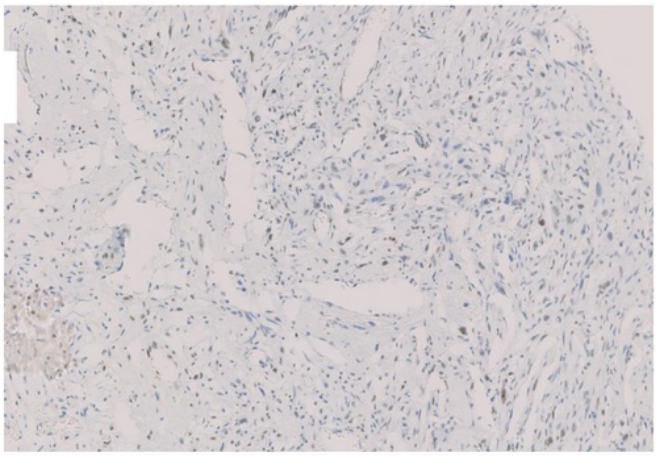
ATRX Nuclear Staining





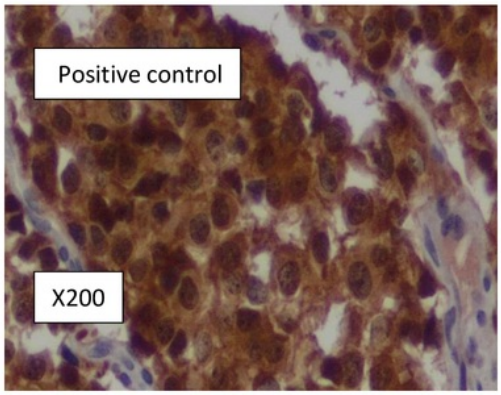
Samples were considered positive when more than 10% of tumor cells showed nuclear immune reactivity for ATRX and were considered negative, when the tumor cells showed no nuclear positivity in the presence of endothelial cells, cortical neurons and infiltrating inflammatory cells were generally positive and served as internal positive controls.

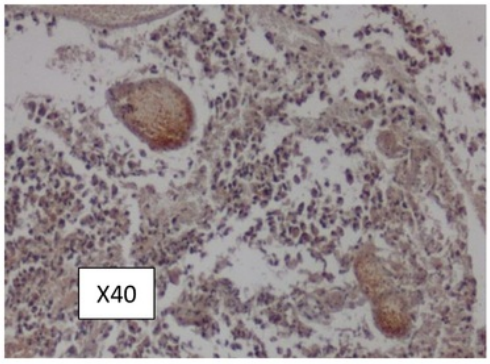
ATRX Loss

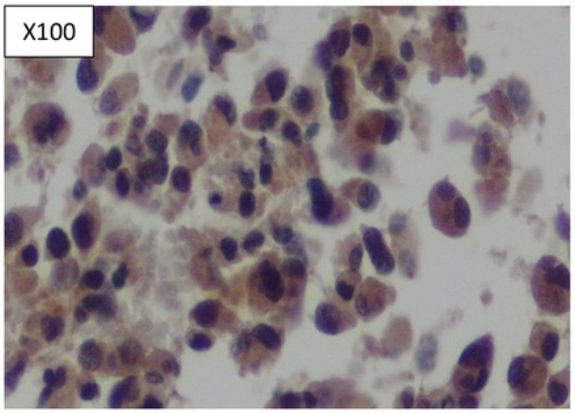


IDH 1 Cytoplasm Staining

Positive control with cytoplasmic staining, Immunoreaction for IDH-1 was scored positive when tumor cells showed a strong cytoplasmic staining for mIDH1R132H. it may also display weak nuclear staining







CHAPTER FIVE

DISCUSSION

A total of 46 tissue specimens with a diagnosis of WHO glial grade III and IV tumours were reviewed. The tissues had been obtained from patients aged between 7 and 70 years.

Age distribution in the study showed multiple peaks. Median age was 44 years with an inter-quartile range of 22 years. In a study carried out in Zurich by Ohgaki et al between 1980 -1994, the mean age of 680 glioblastoma patients (including secondary glioblastoma) was 61.3 years. These patients had been followed up for a 15 year period or to their death. The mean age of the Zurich study fairly corresponds to our study and the small difference could be attributed by the difference in sample size. Majority of the Zurich cases were elderly adults. More than 79% were greater than 50 years.

In this study tissues from male patients (24/45; 52.2%) were comparable to the females (22/45; 47.8%) this is not similar to what was observed by Ohgaki et al (1) where there was a male preponderance 3:2 male to female ratio.

The GBM tumours may occur as independent multifocal tumours and not as a result of metastasis as described by Batzford and Barnad et al (16)(17) as a result of metastasis. This corroborates with the study done in KNH where (14/30) of the patients had tumors in more than one cerebral site. Majority of the tumours were located in the frontal, temporal and parietal lobes. This was also observed in a Patient Care Evaluation Study conducted by the Commission on Cancer of the American College of Surgeons. Data from US hospital cancer registries were submitted directly to the National Cancer Database. Where they found GBM are more commonly located in the supratentorial region (frontal, temporal, parietal, and occipital lobes), are rarely seen in the cerebellum, and are very rare in the spinal cord (34).

IDH 1 mutations are seen within our study population at a rate of 26.7%. On morphology both the IDH1 positive and negative are indistinguishable. IHC helps to further classify it in that IDH1 mutations are seen in secondary GBM and rarely in primary GBM. WHO classifies secondary GBM as IDH-mutant glioblastoma (1).

Ohgaki et al found that secondary GBMs were predominantly found in younger patients (median age of ~45 years compared with median age of ~60 years for primary GBM) and tend to occur less frequently than primary GBMs, making up ~5% of total GBMs (4). Compared to our study where 8 (n=12) where >40 years this shows a difference which could be attributed to having few pediatric cases.

In a case series review done in Department of Neuropathology in Heidelberg Germany, 38/42 (90 %) of WHO grade II and 101/113 (89 %) of WHO grade III astrocytomas presented with loss of ATRX expression. In our case the anaplastic astrocytoma WHO III showed ATRX loss (100%), 17/45 (37.8%) of GBM showed ATRX loss. In the same German study a set of 23 (n=136, 17%) GBM cases demonstrated nuclear ATRX loss compared to this study which showed 37.8%. Among the 23 GBM, 11 carried an *IDH* mutation (35). Of the ATRX loss cases only one was IDH1 Positive and this happened to be the 7 year old case (the only pediatric case).

In Tokyo, Japan a study of 191 patients who were at least 20years, underwent surgery in the hospital between to 2012 and had a diagnosis of diffuse glioma, anaplastic astrocytoma or GBM. In the GBMs, ATRX loss was also associated with IDH1/2 mutation. The incidence of IDH1/2 mutation in tumours with ATRX loss, however, was lower in GBMs than in grades II/III gliomas (six of 15, 40.0 versus 24 of 26, 92.3%, $P < 0.001$) (36).

Jiao et al who carried out a review of cases from 3 different study sites on 363 brains demonstrated *ATRX* alterations in 93 of which grade III astrocytomas (73%, n=44), secondary GBMs (57%, n=14). In contrast, *ATRX* loss was rare in primary GBMs (4%, n=94)(37). *IDH* mutations were observed in 87 of 88 adult gliomas in the same study with an *ATRX* alteration (99%). The tumors with this genetic signature were called "I-A gliomas", denoting that these tumors had alterations in either *IDH1* or *IDH2* and in *ATRX*(37).

This molecular classification system demonstrates the prognosis of gliomas. ATRX and IDH mutant anaplastic astrocytomas have a favourable prognosis compared with anaplastic astrocytomas with IDH mutation only (38). *IDH* mutations are associated with improved survival in gliomas (39). The I-A gliomas were noted to have a longer survival (51 months, $P = 0.007$) and those that did not have any ATRX and IDH1 alterations median survival (28 months).(37)

In the glioblastomas, majority (42/44) had a mitotic activity rate of 11% and above; of which 28 (63.6%) had a score of 21% and above. The tissue diagnosed with anaplastic astrocytoma had a mitotic activity rate of 7%.

Routine assessment of the cell proliferation rate may be determined by using Hematoxylin and Eosin where by the pathologist counts the number of mitosis seen in a high power field. Using cell proliferative markers we are able to better assess it. The cell proliferative rates help tell us the tumour biology. Most glial cells are you usually in a non-proliferative state. Tumour cells are always multiplying and this can be used to prognosticate and also in treatment choices so we are able to use targeted therapy.

In Turkey, Kayaselcuk (40) et Al (2002) found the mean mitotic rate to be 33.57% in a study with 20 GBM cases and Range 6.40–64.40% (SD 28.51 ± 19.35) Median 23.95. Zuber (41)et Al (1988) in 51 frozen sections ,27 were GBM had a cell proliferation rate of 1.7-32.3% (mean 11.1% SD 8.2%) and in 8 Anaplastic astrocytoma cases 0.7-7.4% (mean 3.5 and SD 2.2%). Both studies were in concordance with our cohort.

STUDY LIMITATION

This study was expensive as the immuno-panels are not available locally and if sponsorship was available it would have taken a shorter time to conduct the study. In addition the archiving system was not up to date and we missed some blocks.

The study population was mostly elderly with only one paediatric case.

There was no local data available

CONCLUSION

This study has characterized anaplastic astrocytoma and GBM in our population and revealed that there are IDH1 mutation and ATRX alterations present. Majority of the GBM were Primary Glioblastoma, IDH-wild type which also displayed high proliferative cell rates.

This study established two populations of glioblastoma WHO grade IV. Those that are

- GBM with no IDH1 mutation and no ATRX loss
- GBM with IDH1 mutation but no ATRX loss.

Anaplastic astrocytoma is a rare tumour seen within our population with only 5 cases in a six year period. The anapalastic astrocytoma displayed IDH1 mutation and ATRX loss.

Immuno-histochemistry markers are used in classification of gliomas especially glioblastoma where molecular testing is not available. Tumours with both IDH1 mutations and ATRX loss have a better prognosis than tumours that do not have this mutations.

STUDY LIMITATION

The study aimed to look at cases as far back as five years ago, and the investigator was faced with missing blocks from the archives. This also paused a challenge in carrying out the immunohistochemistry where the fixative recommended is 10% neutral buffered formalin. ATRX immunohistochemistry was significantly affected by the quality of tissue for example tumor portions that were not sufficiently fixed did not provide satisfactory results.

RECOMMENDATION

Further studies on patient survival time with a diagnosis of WHO grade III anaplastic astrocytoma and IV glioblastoma to correlate with immunohistochemistry findings with prognosis as evidenced by patient survival.

ROLE OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF GLIOBLASTOMA AND ANAPLASTIC ASTROCYTOMA AT KENYATTA NATIONAL HOSPITAL

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