ASSESSMENT OF ISOCITRATE DEHYDROGENASE MARKERS, CLINICAL PRESENTATION AND IMAGING CHARACTERISTICS IN PATIENTS UNDER MANAGEMENT OF GLIAL NEOPLASMS AT KENYATTA NATIONAL HOSPITAL

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A DISSERTATION PROTOCOL SUBMITTED IN PART FULFILLMENT FOR THE AWARD OF MASTER DEGREE OF MEDICINE IN NEUROSURGERY, SCHOOL OF MEDICINE UNIVERSITY OF NAIROBI

STUDENT'S DECLARATION

I, Daniel Kanyata Nduati, do declare that this research project is my original work and has never been presented for the award of a degree in any other university.

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DEDICATION

To my loving and supportive family.

I would like to extend my warmest gratitude to my family for being me with every step of the way to making this project and this course possible.

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ABBREVIATIONS

ADC-	Apparent Diffusion coefficient
ATRX gene -	alpha thalassemia/mental retardation syndrome X-linked gene
CT SCAN-	Computer tomography scan
CECT-	Contrast enhanced Computer tomography scan
CNS-	Central nervous system
CTBRUS-	Central Brain Tumor Registry of The United States
DWI-	Diffusion Weighted Imaging
DNA-	Deoxyribonucleic acid
FLAIR-	Fluid-attenuated inversion recovery Magnetic resonance Imaging
GCS-	Glasgow Coma Scale
ICP-	Intracranial pressure
IDH-	Isocitrate dehydrogenase
KFS-	Karnofsky Performance Score
MRI-	Magnetic resonance Imaging
MRI Gadolinium-	Contrast enhanced Weighted Magnetic resonance Imaging
MDM2/HDM2-	Mouse double minute 2 homolog/Human double minute 2 homolog
NIH-	National Institute of Health
NAD^+ -	Nicotinamide adenine dinucleotide (oxidized form)
NADH-	Nicotinamide adenine dinucleotide (reduced form)
NECT-	Non-enhanced Computer tomography scans
T1WI- T1	Weighted Magnetic resonance Imaging
T2WI- T2	Weighted Magnetic resonance Imaging
WHO-	World Health Organization

ABSTRACT

Background

Tumors of the central nervous system are caused by mutations in genes that regulate cell growth. Improved molecular techniques have enabled scientist to investigate this mutation in detail and even correlate them with prognosis. Isocitrate dehydrogenase mutations are the most important prognosticating factors in patients with glial tumors. This has led to changes in the classification of glial tumors in the new 2016 World Health Organization classification of Tumors of the central Nervous system.

Study Broad Objective

To characterize the IDH-1 status and clinical characteristics of glial tumors managed surgically at the Kenyatta National Hospital

Study Design and Site

This was a cross sectional observational study involving all patients with glial tumors managed at the Kenyatta National Hospital. The study was conducted in the Kenyatta National Hospital, outpatient department, trauma and main theatres, neurosurgical ward(4c) and intensive care unit. The lab work was done in the university of Nairobi histopathology labs.

Participants/Materials and Methods

The cases were accrued over a period of 18 months and included six months of retrospective data review and 1year prospective accrual of patients. Patients who had surgery within the last 6 months, had their records traced in the surgical operations book and files in the records department. Patients were contacted using the contacts given in the files and seen in the clinic. New patients were recruited during routine neurosurgical clinics.

Participants included all patients with new onset glial tumors as determined by histology, those on follow-up for confirmed glial tumors managed within 6 months of the start of the study, and patients with recurrent tumors. All patients received routine standard of care and clinical data collected. Tumors were examined after extirpation by the pathology department and glial tumors identified. Immunohistochemistry was done for oligodendrogliomas, Astrocytomas and Glioblastomas. Grade I tumors and ependymomas were excluded from IDH-1 characterization as it is not prognostic in these tumors. Once sample size was achieved the tumor blocks were processed and tested for isocitrate dehydrogenase mutation. The IDH-1 staining was done

using Anti-IDH1 R132H antibody clone H09 (Heidelberg 2009) Patients were followed up for 3 months to detect any early postoperative morbidity or mortalities.

Results

A total of 33 glioma patients were operated and managed within an 18-month period. Twenty-three cases were prospective and 10 retrospectives. Twenty two cases were eligible for IDH-1 characterization (Astrocytomas, Oligodendrogliomas and Glioblastomas). Five (24%) of tumors had IDH-1 mutation compared to 16(76%) of tumors with the wild type genotype. The Male to female ratio 1:1.6 with mean age of patients 43.5 years(SD±20.62) with a range of 18-70 years. Most patients came from counties that bordered Nairobi county where the hospital is based. Fourteen patients (63%) of the patients presented with headache as the primary symptom followed by 4 patients(18%) who presented with seizures. Thirteen patients (59%) of the patients presented within 2 weeks and 3 months of onset of the symptoms. Of patients who thought a delay had occurred, physician delay at 22.73% was the commonest cause.

Fifteen patients (68%) of the patients had a karnofsky performance score of 70 and above. Twenty-one patients (95 %) had CT scan as an initial evaluation with sixteen patients (72%) having MRI for surgical planning. Nine patients (45%) had tumors in the frontal lobe followed by 5 patients (23%) with tumours in the parietal lobe. Sixteen patients (76%) of the tumours had well defined margins. Seventeen patients (85%) of the tumours had surrounding oedema.

In 3-month, post-operative follow-up, ten patients (45%) of the patients were alive compared to 8 (38.1%) of patients who had died. Three patients(14.2%) of patients were lost to follow-up. Tumour grade was a predictor of early postoperative mortality with a diagnosis of low-grade glioma having a 6 times protective effect on odds ratio. Whilst Absence of oedema and IDH-1 mutation were also significantly protective from early post-operative mortality.

1.0 CHAPTER ONE: INTRODUCTION

1.1 Background

Prior to 1956 numerous classifications for grading of central nervous system tumors existed. This created a situation where researchers could not collaborate as they had not agreed on the nomenclature of describing the tumors. This led to a resolution of the WHO executive board in 1956 with adoption at the world health assembly in 1957, mandating the WHO to establish a classification and grading system that was acceptable and to be used worldwide. The WHO blue book on tumors was the result of this efforts and it has undergone 4 editions with the latest being the 2016 version. The revisions have been done to reflect the increasing knowledge of clinical patterns and advances in molecular characterization of glial tumors, both of which have implication in management and prognosis. Gliomas are the commonest brain tumors in Kenya. Currently there is limited information on the molecular characteristics of these gliomas. This study will provide some of the preliminary data on the molecular characteristics of gliomas seen at Kenyatta National Hospital, the busiest neurosurgical unit in Kenya.

1.2 Literature Review

2.1 Brain tumor classification

Tumors of the central nervous system are caused by mutations in genes that regulate cell growth. Early investigative processes relied on light microscopy for diagnosis. This utilized patterns of presentation on light microscopy. Later incorporation of immunohistochemistry to detect the lineage of the tumor cells increased the diagnostic accuracy of light microscopy. Currently immunohistochemistry markers for genetic mutations have further enabled scientist to improve the prognostic utility of light microscopy. The 2016 WHO classification and grading of tumors update was done incorporating molecular information with major bearing on prognosis thus reflecting improvement of molecular techniques over the years.(1,2)

CNS tumors can be classified into two major categories: primary tumors and secondary tumors. Primary tumors arise from cells that are intrinsic to the CNS or its coverings. Secondary tumors arise from sites remote from the brain. Tumors are also characterized by the location, either supratentorial or infratentorial. Information on the location is supplemented by imaging characteristics on CT-Scan and MRI. Many studies correlating imaging studies to histological

diagnosis form the basis for pre-operative diagnosis of brain tumors. Intraoperative diagnosis of tumors by frozen section has most utility in distinguishing areas of normal brain from those with tumor. After surgery tumors are prepared for light microscopy and stained with hematoxylin-eosin which is frequently adequate for diagnosis. Supplementary information using immunohistochemistry and electron microscopic observations can be used in cases that are equivocal in light microscopy. (3,4),(5)

1.3 Current Brain Tumor Registries

Brain tumors are rare, accounting for about 3% of all newly occurring tumors. Efforts to document clinical pattern of the different types of brain tumors require collaboration. This has led to centralized registry being developed in various countries. With probably the best known being the American CTBRUS tumor registry that collects information on brain tumors and covers 98% of the American population. Other regional tumor registries include the Eurocare tumor registry which was a centralized tumor registry for 67 European cancer registries. Individual country registries also exist. The registries provide population-based data for the various histologic types to enable research into new modalities of treatment. The Eurocare registry, from 1995-2002, documented that the commonest brain tumor in that region was glioblastoma occurring in 33 persons per 100,000/year followed by astrocytoma at 12.2 persons per 100,000/year. This was at variance with American population data that shows that meningioma at 36.4% was the most common diagnosis followed by tumors of the pituitary at 15.5% and glioblastoma at 15.1%(6–11)

In Kenya, we only have facility-based data on the prevalence of the different types of tumors. The earliest studies done showed that, from 1984-1993, gliomas were the most common tumors managed at 45.8% followed by meningioma at 34.4%. A more recent study showed that from 2012-2014, meningioma was the most common tumor at 41.4% followed by gliomas at 26.3%.(12,13)

Other registries that exist are brain tumor image registries. Their existence has led to research into advanced image analysis for automated characterization of radiological characteristics of brain tumors to provide diagnosis and predict prognosis. The best known is the NIH cancer imaging archive.(14)

1.4 Immunohistochemistry as an Adjuvant to Diagnosis

The main cell types of the CNS are neurons and glial/supportive cells. Other types of cells include meningeal cells and ependymal cells. The different types of cells in the central nervous system display different cell surface proteins dependent on the proteins assembled by the cell. The basis of diagnosis in neuropathology is histological pattern of the sample. This is supplemented by immunohistochemistry to identify lineage proteins aiding in diagnosis. Tumor grading is based on characteristic like nuclear pleomorphism, number of mitosis, microvascular proliferation and necrosis. Grading using histological characteristics has been shown to have prognostic implications. Some adjuvants to grading of tumors are Ki67 which accurately shows the number of mitosis in a tumor sample.(15)

Table 1:Immunohistochemistry markers used in diagnosis

Immunohistochemistry Marker	Function	Present
Glial Fibrillary acid Protein (GFAP)	Intermediate filament. Cytoplasmic filament for glial cytoskeleton	normal, reactive and neoplastic astrocytes and ependymal cells and developing and neoplastic oligodendrocytes
Neurofilaments (NFP)	Intermediate filament. Cytoskeleton of neurons	Tumors of neuronal origin or exhibiting neuronal or neuroendocrine differentiation. medulloblastoma, olfactory neuroblastomas, paragangliomas and carcinoid tumors.
S-100 protein	Protein in cytoplasm and nuclei of cells from neural crest cells	Found in schwannomas, neurofibromas and malignant peripheral nerve sheath tumors
Neuron –specific enolase	cell specific isoenzyme of the glycolytic enzyme enolase	highly specific marker for neurons and peripheral neuroendocrine
Synaptophysin (SYN)	Synaptic vesicle glycoprotein	Present in neuroendocrine cells neurons and nerves
Epithelial membrane protein (EMA)	Component of the cell membrane	Normal and neoplastic cells of meningothelial and perineural origin
Keratin	Fibrous structural protein	in benign and malignant tumors of the choroid plexus, craniopharyngioma and occasionally in meningioma and

		glioblastomas and gliosarcomas with metaplastic changes

Advances in molecular sciences have enabled advances in immunohistochemistry to encompass more immunohistochemistry stains that the ones mentioned above. Studies involving long term follow-up of patients has revealed that certain molecular characteristics of tumors have prognostic implications to the natural history of the disease. In the new 2016 WHO update on brain tumors, glial tumors have probably undergone the most changes in classification as these molecular signatures have been shown to have superior prognostication in addition to histological methods.

1.5 Pure Glial Tumors and new prognostic molecular signatures

1.5.1 Types of Glial Tumors

The glial cells include astrocytes, oligodendrocytes, ependymal and microglial cells. Astrocytes and oligodendrocytes are the predominant supportive cell types. They form tumors astrocytomas and oligodendrogliomas. Astrocytic tumors in the 2007 blue book consisted of pilocytic astrocytoma, subependymal giant cell astrocytoma, pleomorphic xanthoastrocytoma, diffuse astrocytoma, anaplastic astrocytoma and glioblastoma multiforme.

Table 2: Glial Tumors of the CNS

Tumor	WHO Grade	
Astrocytic tumors	Grade 1	Pilocytic astrocytoma Subependymal giant cell astrocytoma
	Grade II	Diffusely infiltrating astrocytoma
	Grade III	Anaplastic astrocytoma
	Grade IV	Glioblastoma Multiforme
Oligodendroglial Tumors	Grade II	Oligodendroglioma
	Grade III	Anaplastic oligodendroglioma
Oligoastrocytic tumors	Grade II	Oligoastrocytoma
	Grade III	Anaplastic oligoastrocytoma
Ependymal Tumors	Grade I	Subependymoma Myxopapillary ependymoma
	Grade II	Ependymoma
	Grade III	Anaplastic ependymoma

1.5.2 Isocitrate dehydrogenase (IDH) alterations in Glial tumors

An important genetic alteration in all glial tumors that affects prognosis is the IDH status. As shown in Figure 1, IDH is a Krebs cycle enzyme that catalyzes the change of isocitrate to α-Ketoglutarate with the gain of an $NADH^+$ and release of CO_2.

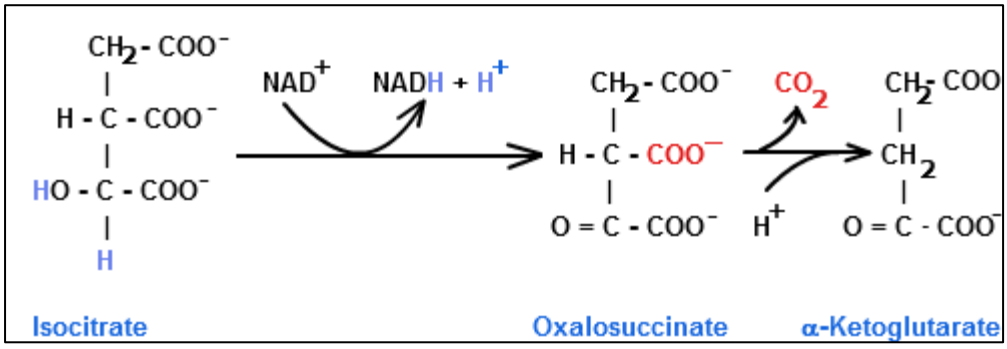


Figure 1: Isocitrate dehydrogenase reaction

IDH 1 mutations refer to the cystolic enzyme while IDH 2 mutations refer to the mitochondrial enzyme. IDH-1 and IDH-2 mutations have been found in a majority of grade II and grade III astrocytomas compared to glioblastoma specimens. The rate of IDH 1 mutation was found to be higher in grade II and III compared to IDH-2 mutations. In an examination of 1010 glial tumors, 716 IDH1 mutations and 31 IDH2 mutations. IDH-1 mutations therefore have more utility and have been found to have prognostic implications in glial tumors. Gliomas without mutant IDH 1 have a poorer prognosis than IDH-1 mutant tumors of the same grade. An analysis of the prevalence of IDH 1 and IDH 2 mutations is shown in the Table 1. below. Current therapeutic strategies targeting IDH mutant tumors are under clinical trials.(16,17)

Table 3:IDH-1 Mutations in grade II and III gliomas(16)

Type of Glioma	N=1010
Grade II astrocytomas	**72.7% (165/227)**
Grade III Astrocytomas	64% (146/228)
Grade II oligodendrogliomas	82% (105/128)
Grade III Oligodendrogliomas	69.5% (121/174)
Grade II Oligoastrocytomas	81.6% (62/76)
Grade III Oligoastrocytomas	66.1% (117/177)
Total	716 (70.9%)

1.5.3 Utility of IDH-1 Characterization in Gliomas

The current 2016 WHO update therefore classifies all gliomas as either IDH-1 mutant or IDH wild type. For Glioblastomas (GBM) this identifies whether the tumor is a primary GBM or it developed from a lower grade lesion, secondary GBM. IDH wildtype are extremely rare in lower grade glial lesions that it is almost exclusively considered a variant of glioblastoma. Of 120 IDH wild type tumors with histological III (astrocytomas), all but one based on molecular profiles could be considered glioblastoma. Of 40 IDH wild type tumors with histological grade II, 33 were glioblastomas in evolution. Since glioblastomas occasionally have epithelial or pseudoepithelial differentiation. Mimics of glioblastoma are metastatic carcinoma and other

primary tumors such as ependymoma, choroid plexus carcinoma, medulloepithelioma, craniopharyngioma, pituitary adenoma or papillary meningiomas. Immunohistochemistry with cytokeratin CAM 5.2 differentiates Glioblastomas from metastatic carcinoma. Cytokeratin CAM 5.2 is an antibody that recognizes cytokeratins 8 and to a lesser extent CK7, which are cytokeratins elaborated in cells of epithelial origin and not glial cells. Of the other glial cell tumors specifically astrocytomas, mesenchymal elements have been described. Glial fibrillary acid protein, an intermediate protein expressed in cells of the central nervous system can differentiate it from tumors of mesenchymal origin.(15,18,19)

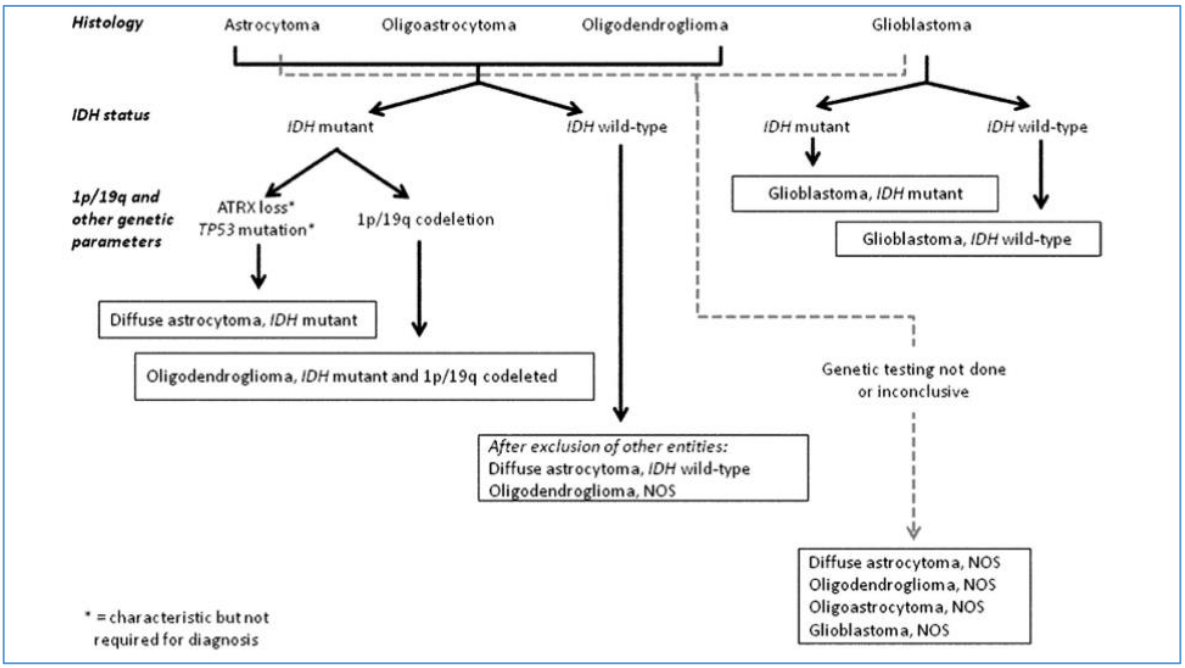
1.5.4 Additional Molecular markers not required for diagnosis

For Astrocytomas additional genetic parameters are used. ATRX loss, TP 53 mutations and 1p/19q codeletion or the other important prognostic genetic markers.(1,20) TP53 is the commonest mutated gene in human cancer. TP53 is a cell cycle check point protein that is induced by various cell cycle aberrations such as double strand breaks, inappropriate cell proliferation and other mechanisms. Once activated, TP53 induces multiple cellular responses such as cell cycle arrest, DNA repair, altered secretion of growth factors, apoptosis or inactivation of its own negative regulator, MDM2/HDM2. Uniquely normal TP53 protein has a very short life cycle in the cell compared to the mutant TP53 which accumulates and thus is detected using immunohistochemistry. TP53 mutations are rare in oligodendrogliomas. Most (80%) of the anaplastic astrocytomas and glioblastomas with mutated IDH1 or IDH2 genes also had a mutation of TP53 compared to 21% for grade II and 10% for grade III oligodendrogliomas. Therefore TP53 is used as a marker of astrocytic differentiation in IDH mutant glial tumors.(21–23) However, diagnosis of tumors of oligodendroglial lineage require demonstration of IDH gene mutation and combined whole arm losses of 1p and 19q (1p/19q codeletion). However, this is not a universal finding in oligodendrogliomas especially pediatric oligodendrogliomas. Of 40 pediatric patients assessed for 1p/19q codeletion, 10(25%) had the mutation. However between 37-45% of adult oligodendrogliomas with IDH mutation harbor this mutation.(24–26)

ATRX gene, is a useful marker of astrocytic lineage as it is mutually exclusive with 1p/19q co-deletion. Mutations are most commonly in grade II, grade II and secondary GBMs and uncommon in primary GBMs and oligodendrogliomas.(27)

Therefore, for glial tumors, IDH-1 or 2 mutations are the largest predictors of prognosis. Progression free survival and outcome survival. Most IDH wild type tumors develop into glioblastomas. Of the IDH mutant tumors, a subset has TP53 mutations and ATRX loss and these develop into diffuse astrocytomas, anaplastic astrocytomas and glioblastomas. Those with 1p19q deletion which is mutually exclusive to ATRX loss develop into oligodendrogliomas. (28)

Figure 2:Algorithm for molecular diagnosis of Gliomas



2,0 CHAPTER TWO: RATIONALE

Molecular characterization of certain glial tumors has shown to have a prognostic bearing on the outcome of the disease. Immunohistochemistry (IHC) for IDH-1 markers is central to the integrated diagnosis of gliomas. Currently at Kenyatta National hospital diagnosis follows the 2007 WHO manual on diagnosis of brain tumors. Characterization of molecular markers will enable classification of patients using the new 2016 WHO brain tumor classification.

2.1 Objectives

2.1.1 Broad Objective

To characterize the IDH-1 status and clinical characteristics of glial tumors managed surgically at the Kenyatta National Hospital

2.1.2 Specific Objectives

a) To determine patient characteristics and clinical presentation of patients with glial tumors at Kenyatta National Hospital

b) To describe the CT and MRI characteristics of patients with glial tumors at Kenyatta National hospital

c) To initiate classification of patients with glial tumor using the 2016 World Health Organization Classification of tumors of the Central Nervous system

d) To determine whether there is a 3-month prognostic value for sequencing IDH-1 in patients with Gliomas at KNH.

3.0 CHAPTER THREE: METHODOLOGY

3.1 Study Design

A cross-sectional observational study involving all patients with glial tumors with a retrospective and prospective arm.

3.2 Study Area

The study was conducted in Kenyatta National Hospital, Outpatient department, Trauma and Main theatres, neurosurgical ward (4c), the intensive care unit and university of Nairobi histopathology department.

3.3 Study Population

All patients with histologically confirmed glial tumors managed between January 2018 and June 2019

3.4 Recruitment Procedures

For the retrospective 6-month arm, patients had their records traced in the surgical operations book and files in the records department. Patients were contacted using the contacts given in the files and seen in the clinic. We picked patients sequentially as they present the clinic until 30 glioma patients were operated and histology confirmed to be gliomas with subsequent Immunohistochemistry done for IDH-1.

New patients were recruited during routine neurosurgical clinics. All patients received routine standard of care. A full hemogram and urea and electrolyte were done as well as a CT scan and MRI at admission. Vital signs were taken, and a full neurological examination done. Patients with medical comorbidities had medical review and management prior to surgery. Surgical extirpation was done for the tumor in the elective theatres and the specimen processed by the histopathology department. Glial tumors of uncertain histology were subjected to immunohistochemistry for lineage proteins i.e. GFAP, synaptophysin, S-100

Table 4:Patient Timelines

	Screening/ admission	48 hrs. Post-op	2 weeks (14+/- 7 days) Post-op	3-month post-surgery
Informed Consent/ procedure information	X			
Medical history/ demographics	X			
Comorbidities/concomitant medications	X	X	X	
Physical examination	X			
Hematology/biochemistry	X			
Clinical Examination	X			
Radiological examination	X			
Discharge information		X	X	
Histology and immunohistochemistry			X	
Prognostication and referral to oncologist			X	
Follow-up phone call/ clinic visit				X

3.5 Laboratory Procedures

A preliminary histological report was given to the patients to enable continuity of care. Tumor block were stored until sample size was achieved. Immunohistochemistry for IDH-1 was then done for oligodendrogliomas, Astrocytomas and Glioblastomas. Grade I tumors and ependymomas were excluded from IDH-1 characterization as it is not prognostic in these tumors. The slides were then prepped for IDH-1 sequencing following the protocol in appendix 7. Patients were followed up in clinic 24 with their results and further treatment or follow up recommended based on post-operative images and histology of the tumor.

3.6 Inclusion Criteria

a) All patients identified to have new onset glial tumors as determined by histology and in uncertain cases immunohistochemistry.

b) All patients with recurrent confirmed glial tumors whether they have had chemotherapy or radiotherapy

3.7 Exclusion Criteria

a) Patients with confirmed non-glial tumors.

3.8 Outcomes

3.8.1 Primary Outcome Measures

The molecular morphology of glial tumors operated at Kenyatta National Hospital.

3.8.2 Secondary Outcome Measures

a) Clinical presentation of patients with glial tumors at KNH as evaluated by screening tool in Appendix 2-Demographic, History and clinical examination tool.

Demographic data including sex, date of birth and county of origin was recorded. A medical history detailing the symptom at first presentation. The duration of symptoms and reasons for delay was ascertained. A neurological examination was done starting with a Glasgow coma scale, a mini-mental exam and Karnofsky performance score determined. All clinical protocols were guided by Hutchinson's clinical methods.(29)

b) The radiological characteristics of brain tumor including site, size in mm^3 using the ABC/2 method, and radiological characteristics was evaluated by screening tool in Appendix 2- Imaging Evaluation Screening Tool.(30)

c) An outcome survival and progression free survival was determined using a postoperative call and follow-up.

3.9 Sample Size

The prevalence of glial tumors was estimated by various authors as

Author	Review period	N.o.	
Mwangombe	1984-1993	214	**Gliomas – 97(45.8%)** Astrocytomas-81(37.8%) Ependymomas-10(4.6%) Oligodendrogliomas-6(2.8%)
Boore	2005	71	**Gliomas-19(26.6%)**
Mureithi	2012-2014	152	**Gliomas-39(25.6%)** Pilocytic astrocytomas-3 (2.19%) PXA-1 (0.73%) Diffuse fibrillary astrocytomas- 4(2.92%) Anaplastic astrocytomas-4(2.92%) Glioblastomas-22(16.06%) Oligodendrogliomas-2(1.46%) Ependymal tumors-3(2.19%)

The prevalence of glial tumor signature IDH-1 mutation is 70.9% in grade II and III astrocytomas, Oligoastrocytomas and oligodendrogliomas. The prevalence of IDH wild type tumor is 82.5% in glioblastomas and this data was used to calculate the sample size.

70.9% and 82.5% were our proportions of interest. The number of tumors operated per year was 73. With 25% of them being gliomas our population of interest was 18 tumors.

The sample size formula for a finite population (25 tumors per year) to estimate a proportion with specified precision was estimated using

$$\text{Unlimited population: } n = \frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2}$$

$$\text{Finite population: } n' = \frac{n}{1+\frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2 N}}$$

Where

Z- 1.96 (Z score from Gaussian curve covering 95% area under the curve and representing a confidence interval of 95%)

P= the expected true proportion(In this case 0.709 or 70.9% derived from Hartman et al.)(16)

e= Desired precision 0.05, Therefore

n = (Z^2 x P (1 - P))/e^2

n= 1.96^2 x 0.21 /0.0025

n=322

$$n' = \frac{n}{1+\frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2 N}}$$

n’ = 322/ 1 +(1.96^2 x 0.21 /0.0625)

n’=322/13.9

n’= 22

The desired sample size was 22.

Adding a dropout rate of 25%, our final sample size was 30 patients.

3.10 Data Collection Procedures

Three data collection points were envisioned

a) Screening, clinical examination and image evaluation

b) Histology and immunohistochemistry findings

c) 3-month follow-up

3.11 Variables

3.11.1 Quantitative data

Categorical data

1. Nominal data
    - sex- male/Female
    - County of origin
    - Literacy/level of education (5)
    - Symptoms at first presentation (6-13) Pain, Difficulty in comprehension, difficulties in movement, decreased sensitivity of the senses, abnormal growth, abnormal excretion, disorders of nutrition, disorders of respiration
    - Reasons for delay- Symptoms not serious, healthcare access, delay by primary physician, problems of financing, Lack of family support
    - Availability of CT scan
    - Ct scan evaluation- Site, Laterality, pattern, Contrast uptake,
    - MRI evaluation- T1 characteristics, T2 characteristics, Flair, Diffusion weighted, Gado (Contrast) scan,
    - Histological findings, Histological diagnosis, IDH1

Ordinal Data

- Duration between the start of symptoms and presentation to tertiary center (9) less than 2 weeks/between 2 weeks and 3months/more than 3 months

3.12 Training of Key Personnel

The scientific concept and general aims of the study were presented to the neurosurgical unit at a meeting organized by the principal investigator after consent from the supervisors and the unit.

3.12.1 Clinicians

The principal investigator was responsible for initial examination of the patient and any equivocal signs elicited was confirmed by the consultant in charge of the clinic on that day.

Operative details were left to the discretion of the consultant in charge of theatre on the day.

The consultant pathologist was responsible for pathological diagnosis, storage and staining processes at the histopathological lab.

3.13 Ethical Considerations

Since the staining was done retrospectively, patient management did not have the benefit from knowledge of the glial markers for patients until the study had achieved its sample size, tissue slides made. However, the patient was not disadvantaged from not having this information compared with the current where these markers were not available.

Informed consent from both the patient and primary caretaker was taken, however some patients were incapable of consent and this was obtained from relatives.

Also, some nominal was missing in the retrospective arm and some participants were expected to have recall bias.

4.0 CHAPTER FOUR: RESULTS

4.1 Descriptive Statistics

Thirty-three patients were recruited into the study. There were 23 prospective cases and 10 retrospective cases during the period. Of the 33 cases 22 Cases were eligible for IDH-1 characterization. Twenty-one were eventually done omitting one case of glioblastoma whose block could not be traced for the IDH-1 characterization after storage. Of the 21 cases, 5(24%) had IDH-1 mutation. There were 2 Glioblastomas and one each of diffuse astrocytoma, anaplastic astrocytoma and anaplastic oligodendrogliomas. The other Gliomas, Pilocytic astrocytoma, SEGA, ependymoma, pediatric astrocytoma and gangliogliomas were not IDH-1 characterized as this is no prognostic. The overall outcome survival was 45% at 3 months, with 8(24%) confirmed dead and 10(30%) lost to follow-up.

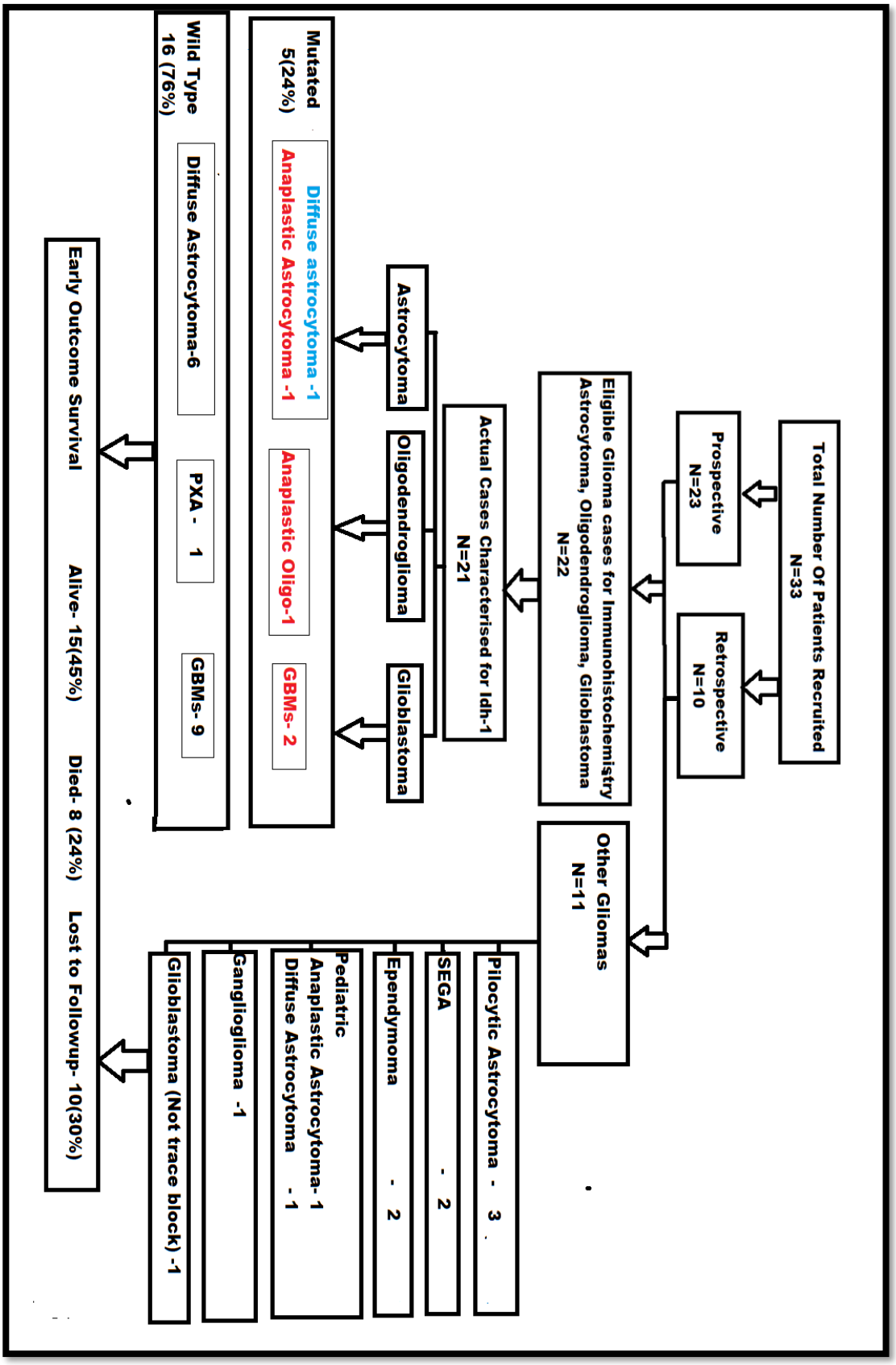
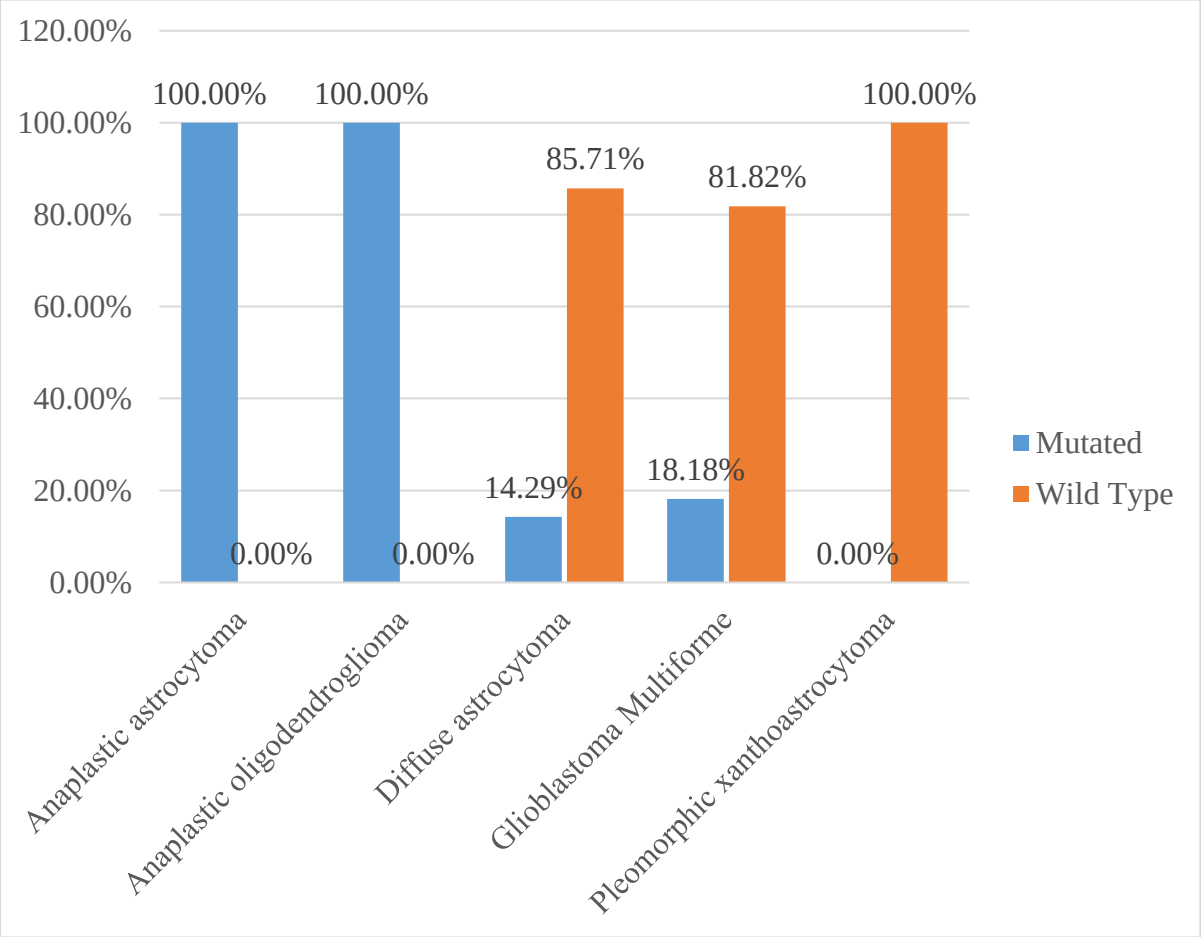


Figure 3: Flowchart of recruited patients and Outcome at 3 months.

4.1.1 Prevalence of IDH-1 mutations amongst the different types of Gliomas

In our sample, 1/6 (14.3%) of diffuse astrocytomas were IDH-1 mutant, whilst in Glioblastomas, 2/9 (18.2%) were IDH-1 mutant. In the other tumors, only one of each tumor was characterized. Anaplastic astrocytoma and anplstic oligodendroglioma had one tumor each with IDH mutation and Pleomorphic xanthoastrocytoma had one tumor with IDH-1 wildtype characteristic.

Figure 4 Clustered column chart showing the prevalence of IDH-1 mutation amongst different types of gliomas



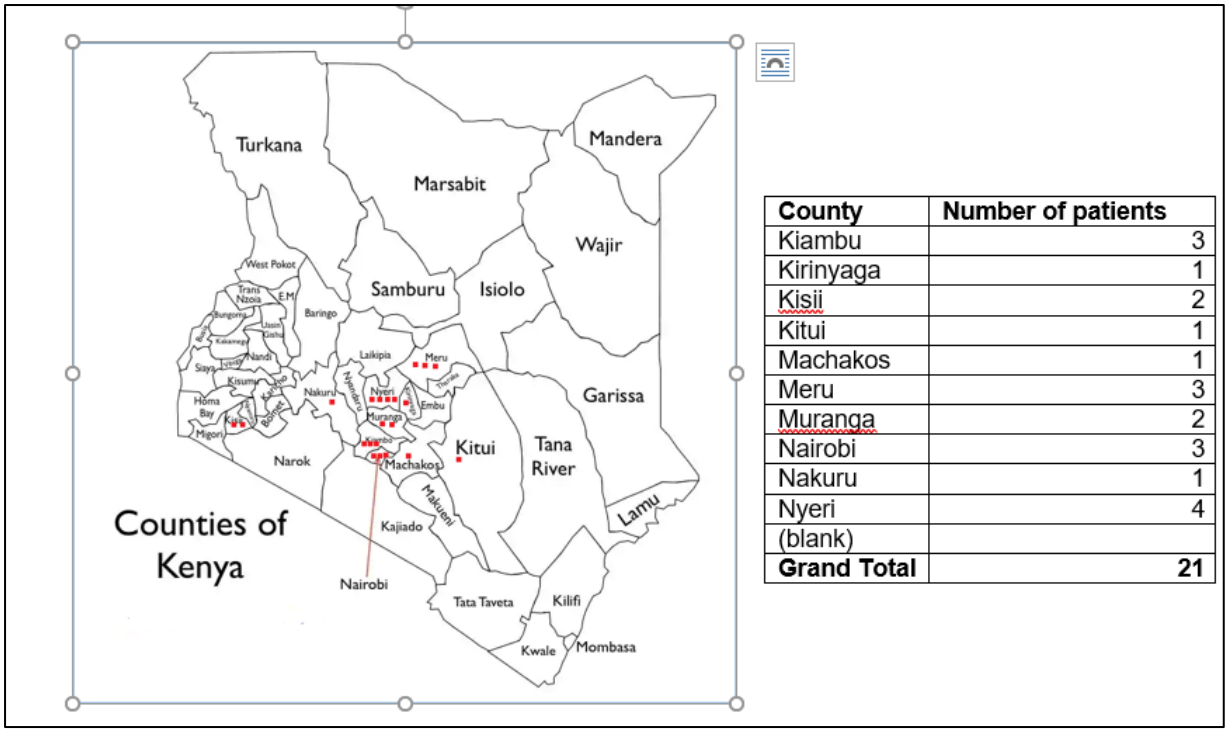
4.2 Demographic Data

4.2.1 Age and Sex Distribution

There were 13 male patients and 8 female patients, giving a male to female ratio of 1:1.6. The mean age of the patients was 43.5 years (SD ±20.62) with a range of 18-70 years.

4.2.2 County of Origin

Most of the patients came from counties that geographically border Nairobi where the hospital is located. This is shown in figure 4.



County	Number of patients
Kiambu	3
Kirinyaga	1
Kisii	2
Kitui	1
Machakos	1
Meru	3
Muranga	2
Nairobi	3
Nakuru	1
Nyeri	4
(blank)	
Grand Total	**21**

Figure 5:Visual representation of counties of origin
Each Dot represents one patient

4.3 Clinical Signs and Symptoms

Fourteen patients (63%) presented with headaches as the primary complaint, followed by 4 patients (18%) who presented with seizures. Three patients (13.6%) of the participants presented with memory loss followed by 1 patient (4.55%) who had aphasia.

Of the 14(63%) patients whose primary complaint was a headache, 6 (27%) had secondary complaints of seizures, 2(9.09%) had hemiparesis, visual loss and vomiting each. One patient presented with memory loss (4.55%) as a primary complaint.

Thirteen patients (59%) of the patients presented between 2 weeks and 3 months of development of symptoms. This was followed by 6 patients (27%) of patients who presented 3 months after developing their symptoms. Only 3 patients (14%) presented with 2 weeks of developing their symptoms.

Six patients (27%) subjectively had no delay in presenting to the tertiary facility. However, 5 patients (22.7%) said the primary physician had caused the delay. Three patients (3) 13.6% had thought that the symptoms were not serious whilst 2 patients (9.09%) had found difficulty in financing. Other reasons for delay included lack of healthcare access, lack of family support, alternative medicine and thought it was another ailment.

Twenty patients (90.9%) of the patients had a Karnofsky score of 50 and above. Fifteen patients (68.1%) had a karnofsky score of 70 and above.

Table 5: Clinical presentation of Glioma patients

Clinical Presentation				
Symptoms	Primary Symptom	N=22	Secondary Symptom N=14	
	Headache	14 (63.64%)	Seizures	6 (27.27%)
			Visual Acuity Loss	2 (9.09%)
			Vomiting	2 (9.09%)
			Hemiparesis	2 (9.09%)
			Memory Loss	1 (4.5%)
	Aphasia	1 (4.55%)		
	Seizures	4 (18.8%)		
	Memory Loss	3 (13.64%)		
Duration of Symptoms		N=22		
A	Less than 2 weeks	3 (13.64%)		
B	Between 2 weeks and 3 months	13(59.09%)		
C	Greater than 3 months	6(27.27%)		
Reasons for Delay			N=22	
	Aversion to doctors and formal medical establishment		1(4.5%)	
	Finance		2(9.09%)	
	Healthcare access		1(4.5%)	
	Lack of family support		1(4.5%)	
	No delay		6(27.27%)	

	Primary physician delay	5(22.73%)
	Sought alternative healthcare	1(4.55%)
	Symptoms not serious	3(13.64%)
	Thought it was another ailment	1(4.5%)
	N/A	1(4.5%)
Karnofsky Performance		
	70-100%	15(68.1%)
	50-69%	5 (22.73%)
	<50%	2 (9.09%)

4.4 Imaging Findings

Availability of imaging

Twenty- one patients (95.45%) of the patients had an initial CT (Both non-enhanced and contrast enhanced) scan done before surgery. Sixteen patients (72.7%) had a T1WI, 15 patients (68.1%) had a T2WI and MRI Gadolinium image. Fourteen patients (63.6%) had an MRI Flair image whilst 9(40.3%) had an MRI DWI and 7(31.8%) had an MRI ADC image.

The mean tumor volume was 126.6 $cm^{3.}$ Sd(±98.9cm3) Range (18.0 cm3 -383). 53% of gliomas had ring enhancement on contrast.85% of gliomas had surrounding edema.

Table 6:Imaging Characteristics of Tumors

Availability of Imaging Modality		N=22
	NECT	21 (95.4%)
	CECT	21 (95.4%)
	T1WI	16 (72.7%)
	T2WI	15 (68.1%)
	MRI Gadolinium	15 (68.1%)
	MRI Flair	14 (63.6%)
	MRI DWI	9 (40.3%)
	MRI ADC	7 (31.8%)
Edema		N=21
	Present	18 (85.7%)
	Nil	3 (14.3%)
Contrast Enhancement		N= 18
	Ring Enhancing	10 (55.5%)
	Patchy/Mild	5 (27.8%)
	No contrast enhancement	3 (16.7%)

Nine patients (45%) had tumors in the frontal lobe, followed 5 patients (23%) with tumors in the parietal lobe, 3(15%) in the temporal lobe, 2 (10%) in the occipital lob and finally one tumor (5%) r in the cerebellar hemisphere.

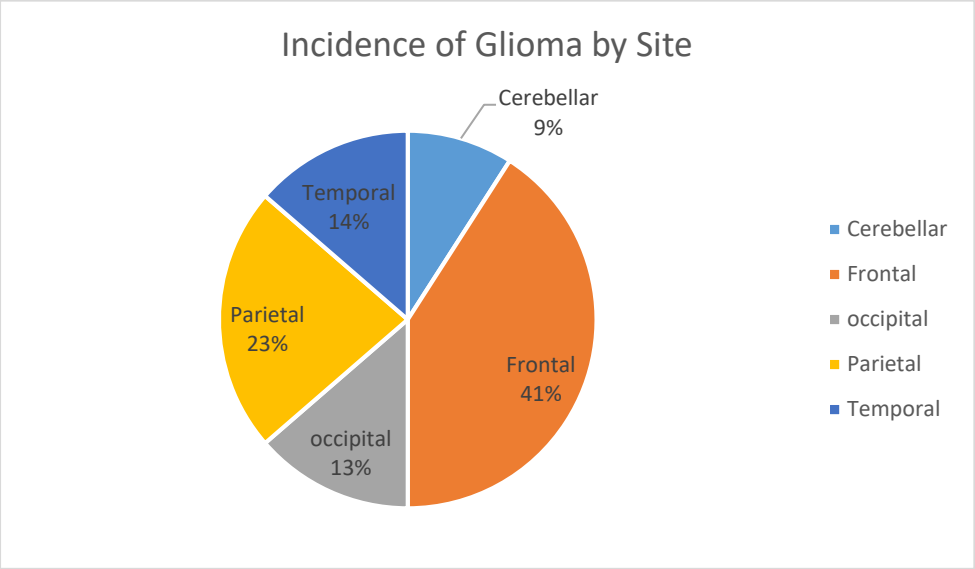


Figure 6 Incidence of gliomas by site

Sixteen patients (76%) had well defined margins compared to 24% which had ill-defined margins.

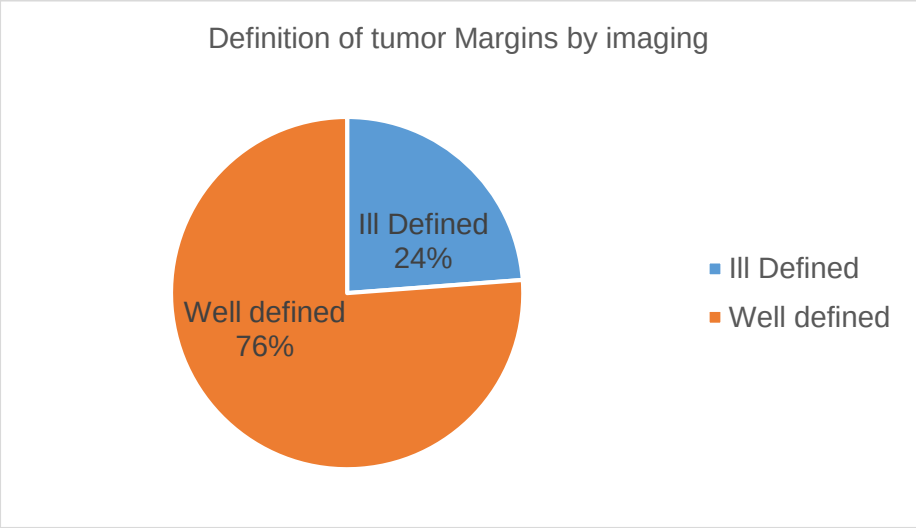


Figure 7 Definition of margins by imaging

4.5 Associations

4.5.1 Univariate Analysis

4.5.1.1 Predictors of Mortality

Sex, Karnofsky score, and volume of tumor did not predict early (3 month) post-operative deaths.

Low grade gliomas were 6 times more likely to be alive at 3 months postoperatively compared to high grade gliomas.

Lack of edema also predicted a better prognosis at 3 months.

IDH-1 mutated predicted a significant prognosis at 3 months that was significant.

Table 7: Univariate analysis of predictors of Early postoperative Mortality

	Odds Ratio	P value
Sex	0.6 (0.07-4.6)	0.6
Karnofsky performance score	1.0 (0.95-1.06)	0.9
WHO grade	6.2 (1.4-2.6)	**0.0005**
Volume of Tumor	0.99 (0.98-1.009)	0.5
Absence of Edema	1.35 (0.25-7.1)	**0.0005**
IDH-1	1.85e+08 (3.81e+07 8.988e+08)	**0.0005**

4.5.2 Multivariate Analysis

4.5.2.1 Independent predictors of Mortality

When subjected to multivariate analysis, the independent predictors of mortality 3 months were IDH-1 wild type tumor, high grade tumors and presence of edema on imaging.

Table 8:Multivariate analysis of Independent predictors of early Postoperative mortality

Predictor		Unadjusted Odds Ratio (95% confidence Interval)	P value	Adjusted Odds Ratio, (95% Confidence interval)	P value
IDH Status					
	Mutated	1 (referent)			
	Wild type	1.85e+08 (3.81e+07-8.988e+08)	< **0.00005**	1.975e+09 (1.829e+07-2.133e+11)	< 0.00005
Sex					
	Female	1 (referent)			
	male	1.1 (0.1-9.9)	0.9		
Karnofsky performance		1.0 (0.9-1.0)	0.8		
WHO Grade	Low grade	1 (referent)			
	High grade	1.257e+09 (1.864e+08-8.482e+09)	< **0.00005**	4.378e+09 (2.973e+08-6.447e+10)	< 0.00005
Tumor Volume		1.0 (0.98-1.01)	0.7		
Edema					
	Present	1 (referent)			
	Nil	4.254e+07 (5.686e+06-3.183e+08)	< **0.00005**	1.3 (1.3- 1.3)	< 0.00005

5.0 CHAPTER FIVE: DISCUSSION

IDH-1 mutations have revolutionized our thinking into glioma prognosis and will change how gliomas are managed in the future. For high grade gliomas, IDH mutation and MGMT methylation are considered the hallmark characteristics of long time survival. For low grade gliomas, the prognostic distinction between grade 2 and 3 has been blurred by use of IDH 1 to the point that it is proposed that these tumors should be known as 'lower' grade gliomas to distinguish them from Glioblastoma. This has a great implication in future management of this patients. The rate of IDH-1 mutated types in our sample was 24%. This study represents the first attempt in sub-Saharan Africa to characterize and follow-up patients with gliomas using IDH-1 as a prognosticating tool.(31–33)

We managed 33 Glioma patients in an 18- month period. This compares well with facility data from previous studies where Mwangombe in a 10 year period from 1984 to 1993 managed 97 gliomas, whilst Boore in 2005 managed 19 gliomas. The latest study prior to our study was done by Mureithi and he managed 39 gliomas over a 2 year period. This could be due to the fact that gliomas are rare tumors accounting for 3% of all brain tumors. However, this figure is severely depressed when we use the use the annual adjusted incidence rate of Tumors of Neuroepithelial Tissue as described by the CTBRUS report as 3.96(3.89-4.03)/100,000 population. Projections for our population (50 Million in 2017) in Kenya, we would expect that the incidence of tumors of neuroepithelia tissue to be 1,980 tumors.(34) (13)

Of the 33 gliomas managed, only 22 were eligible for IDH-1 characterization. These tumors were adult astrocytic tumors, Oligodendroglial tumors and Glioblastoma Multiforme. The other types of gliomas have different pathogenesis and IDH-1 mutations are rarely found expressed in theis tumors. Of the diffuse astrocytomas, 14.29% were IDH-1 mutant. This rate is much lower than what has been found internationally. In Morocco, this rate was 63.2% in France it was 63% and in the USA the rate was 78%. This shows that a larger proportion of diffuse astrocytomas in our sample were of the IDH-1 wildtype and potentially more aggressive. Closer follow-up of this patients is required. We may even consider adjuvant therapy with radiation and temozolomide, but this has to await long term follow-up data. Of the Glioblastoma, 18.2% were IDH-1 mutated and therefore considered secondary glioblastomas that evolved from a lower grade tumor. The majority 81.8% were IDH-1 wild type and considered primary Glioblastomas. This rate is comparable to rates in international samples of glioblastoma.(35–37)

IDH-1 mutation in our sample was found to be an independent predictor of early post-operative mortality. In multivariate analysis, there is several thousand increased odds of dying within 3 months post-op for individuals with wild type tumor. IDH-1 mutations delays tumorigenesis and sensitizes tumor cells to Endoplasmic Reticulum stress and apoptosis. This increased apoptosis is mediated by interaction of RNA gene miR183 which is upregulated in IDH mutant tumors. MiR183 downregulates the apoptotic suppressor semaphorin3E. This apoptotic tendency improves the prognosis of these patients. This phenomenon has been shown in other populations. (36,38–40)

Current research endeavors are geared at non-invasive measurement of IDH-1 status and IDH-1 mutant inhibitors. Use of using (18)F-fluoro-ethyl-tyrosine positron emission tomography to predict which tumors are IDH wildtype or mutated is currently being validated. This will enable prognostication of patients before surgery and sampling is done. In the past 2 years, two mutant IDH (mutIDH) inhibitors, Enasidenib (AG-221), and Ivosidenib (AG-120), have been FDA-approved for IDH-mutant tumors including gliomas. Their efficacy is still under study. This two areas of research will further increase the utility of IDH-1 characterization.(41,42)

The other independent predictors of early postoperative mortality included WHO grade and presence of edema. The WHO grade is based on the Ste Anne-Mayo system that categorizes tumors according to their histomorphological characteristics. This are nuclear pleomorphism, mitoses, endothelial proliferation and tumor necrosis. Prior to the 2016 WHO blue book, this was the primary method of prognostication. However, the 4 tiered system, grade 1-4 was not prognostically relevant leading to a two tiered grading system, Low and High Grade, which was prognostically relevant. For all glioma entities however, contrast enhancement was a key criterion indicating tumor progression into high grade malignancy. This is because contrast enhancement is an indicator of the onset of tumor angiogenesis with endothelial proliferation, key histological features of a high grade tumor. For our sample no contrast enhancement was protective against early post-operative mortality.(43)

Many of our patients were geographically from areas surrounding the hospital with large swathes of the country undeserved. This shows that there is still a large underserved population with gliomas in the country. Some confounders would be that some patients are managed in other centers in the country and abroad. However, our hospital is the largest referral hospital in the country, and it would be expected that larger majority of the patients would be seen at KNH. Another confounder is that the incidence of gliomas may be lower in our population than what

is quoted in literature. It could also be that the short natural history of some gliomas prevent patients from far off areas from reaching the hospital in time for management. (44,45)

Of the patients that were managed at our hospital, 59% presented between 2 weeks and 3 months from the onset of presentation with 27% being 3 months or more. Physician delay to refer by the primary physician was cited as a major reason for their delay in presentation. This however is understandable as a large majority of patients presented with headache as their first symptom with only a few patients presenting with seizures or focal neurological deficit which would elicit more aggressive measures.

The usual cut off for surgical management of patients with gliomas is a karnofsky perfomance score of 70. 68% of our patients had a karnofsky performance score of 70 and above and this has been shown to have better prognostic outcomes than patients with a kanorfsky of below 70. In earlier studies done a KPS below 70 was shown to be a poor prognostic factor. However, in our study on the univariate and multivariate analysis, Karnofsky performance score was not a predictor of early post-operative mortality.

On imaging most tumors occurred in the frontal lobe followed by the parietal lobe, temporal, occipital and finally cerebellar. This compares well with other studies that have found that frontal lobe is the commonest site for glioma occurrence. Low grade gliomas that occur in the frontal lobe have been shown to have better prognosis due to better resection margins. It is not known whether this is the case in High grade gliomas. In our tumor absence of edema was a good prognostic marker against early(3 month) post-operative mortality..(46,47)

5.1 Conclusions

- KNH has a low incidence of gliomas.
- Most of the patients come from areas neighboring the hospital
- The primary physician is the cause of delay in some patients
- The prognostic factors for early postoperative death (3-months) are IDH-1 mutated state, tumor grade and absence of edema of imaging characteristics.
- The prevalence of secondary glioblastomas (IDH-1 Mutated) is higher than in other populations
- The prevalence of IDH-1 wild type diffuse astrocytomas is higher than other populations.

5.2 Recommendations

- The burden of disease in the underserved populations requires to be investigated
- Establishment of centralized tumor registry should be undertaken
- Programs needed to build capacity of primary physicians to reduce cases of delay in referral.
- IDH-1 characterization should be considered as it has clear prognostic implications in the early post-operative course of the patient

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APPENDICES

Appendix I: Consent form (English)

Filling-in	Use black pen. Start with the right-hand sided box.
Instructions:	Encircle pre-filled boxes. Fill in M for missing answers

Participant no.	Study day: Screening
Hospital Number	
Participant First Name	

TITLE OF THE STUDY:

Assessment of isocitrate dehydrogenase markers, clinical presentation and imaging characteristics in patients under management of glial neoplasms

Introduction

Thank you for considering participating in this study. This form will give you the information you need to decide on whether you want to participate in the study or not. Refusal to participate in the study will not affect the management of your condition. Feel free to ask any question whether related to the study or not.

Purpose of the study

The purpose of this study is to find out the molecular signatures of the illness that you have. Molecular signatures are small proteins produced uniquely by the disease you have and help in knowing how the disease will develop. In some cases, it will predict how well you will respond to medication given after surgery. Molecular signatures have only been recently introduced by the world health organization as the future of management of brain illnesses as you may have. This study therefore attempts to characterize those molecular signatures to help improve the management of the disease here in Kenya.

The management of your condition has been standardized for the purposes of the study. A standard medical form will be filled, and your imaging will be evaluated using a standardized protocol. Surgical planning and removal of your disease will be at the discretion of the operating consultant. Pieces of the disease will be taken to other doctors who will examine

them under a microscope and do additional test to determine this molecular signature. The implications of the results will be explained to you by the principle investigator.

You will be managed in the ward and observed for any complications until discharge. You will then be followed up in the standard manner with referrals to the oncologist for other treatment that may be required.

The benefits of examining your tumor for molecular signatures is better management of patients in the future.

You are free to ask any questions about the study at any point and can withdraw from the study in writing at any point without attaching any reason.

Withdrawal from the study will not affect the management of your condition including the follow-up in our clinics. Participation in the study does not entail financial benefits.

Confidentiality

All information that identifies you to the data collected will be held in confidence. Standardized medical forms will be left in your file for future reference. The data extracted will be kept in lockable cabinets in the department of surgery and password enable computers accessible only to the principal investigator, academic supervisors and any support staff the principal investigator may deem necessary in conducting the study.

Cost and payment

I understand that participation is volunteering and that I will meet all the hospital, theatre and imaging expenses.

Ethical consideration

This study has been reviewed and approved by the Kenyatta National Hospital-University of Nairobi ethical review Committee (KNH-UON ERC). It fulfills all conditions set. Do you have any questions? Do you agree? NEXT OF KIN

The study described above has been explained to me. I have had a chance to ask questions. I am aware that participating in this study is voluntary and my declining will not result in victimization whatsoever. Having understood the above Signature……. or Thumb print…… Date…… Signature of investigator…… Name of investigator……..

Withdrawal Priviledge

I understand that I will be free to withdraw from the study at any stage. Useful contacts: 1) KNH/UON/ERC. Telephone: 020726300 ext.: 44102

Email: uonknh_erc@uonbi.ac.ke, P.O Box 20723code 00202 CONSENT BY THE PATIENT

I……..of…… hereby give consent to be included in this study. The nature of the study has been explained to be by Dr…… He has NEITHER coerced me NOR has he forced me to be part of this study. I understand that there will be NO monetary gain in return. Date……Signed…….. I Dr…… confirm that I have explained to the patient the nature of the study. Date…… Signed…….

Appendix II: Consent form (Swahili)

Kiswahili Consent

Utafiti : Tathmini ya alama za ishara za dehydrogenase, uwasilishaji wa kliniki na sifa za picha za wagonjwa chini ya usimamizi wa saratani ya ubongo.

Utangulizi

Asante kwa kuzingatia kushiriki katika utafiti huu. Fomu hii itakupa habari unayohitaji kuamua kama unataka kushiriki katika utafiti. Kukataa kushiriki katika utafiti hauathiri usimamizi wa hali yako. Jisikie huru kuuliza swali lolote ikiwa linahusiana na utafiti. Kusudi la utafiti Kusudi la utafiti huu ni kujua saini za molekuli za ugonjwa unao. Ishara za molekuli ni protini ndogo zinazoonyesha vile ugonjwa unao utajiendeleza. Katika baadhi ya matokeo inatabiri jinsi vile dawa zitapambana na ugonjwa baada ya upasuaji. Ishara za molekuli zimeanzishwa hivi karibuni na shirika la afya duniani. Kwa hiyo utafiti huu unajaribu kutambua saini hizi za ugonjwa wa ubongo ili kusaidia kuboresha matibabu ya ugonjwa huu nchini Kenya.

Katika utafiti huu, fomu ya matibabu ya kawaida itajazwa na picha zako ziangaliwe na madaktari. Mpango wa upasuaji na uondoaji wa ugonjwa wako zitaendezwa na daktari wa ubongo anayeitwa neurosurgeon. Vipande vya ugonjwa huo vitachukuliwa kwa madaktari wengine ambao watawachunguza chini ya darubini na pia kufanya mtihani wa ziada kuamua aina ya ugonjwa ambayo unayo.Utasimamiwa katika kata na kuzingatiwa kwa matatizo yoyote mpaka kutolewa.

Utakuwa kufuatiwa kwa namna ya kawaida na kuruhusu kwa oncologist kwa matibabu mengine ambayo yanahitajika.Faida za kuchunguza tumor yako kwa saini za molekuli ni usimamizi bora wa wagonjwa katika siku zijazo.Wewe ni huru kuuliza maswali yoyote kuhusu utafiti wakati wowote na unaweza kujiondoa kwenye maandishi kwa maandishi wakati wowote bila kuunganisha sababu yoyote.Kuondoka kwenye utafiti hautaathiri usimamizi wa hali yako ikiwa ni pamoja na kufuatilia katika kliniki zetu. Kushiriki katika utafiti hauhusisha faida za kifedha.**Usiri**

Taarifa zote zinazokutambulisha data zilizokusanywa zitafanyika kwa ujasiri. Fomu za matibabu zilizosimamiwa zitasalia katika faili yako kwa kutaja baadaye. Takwimu zilizochukuliwa zimehifadhiwa katika makabati yaliyohifadhiwa katika idara ya upasuaji na

nenosiri huwezesha kompyuta kupatikana tu kwa uchunguzi mkuu, wasimamizi wa kitaaluma na wafanyakazi wowote wa msaada ambaye uchunguzi mkuu anaweza kuonekana kuwa muhimu katika kufanya utafiti.

Gharamana Malipo

Ninaelewa kuwa kushiriki ni kujitolea na kwamba nitakutana na gharama zote za hospitali, ukumbi wa michezo na picha.

Kuzingatia Maadili

Utafiti huu umepitiwa na kupitishwa na Kamati ya Kenyta ya Taifa ya Kenyatta ya Kitaifa ya Ukaguzi wa Maadili (KNH-UON ERC). Inatimiza hali zote zilizowekwa. Je! Una maswali yoyote? Unakubali?

Kutenda Kin

Utafiti ulioelezwa hapo juu umeelezewa kwangu. Nimekuwa na nafasi ya kuuliza maswali. Ninafahamu kuwa kushiriki katika utafiti huu ni kwa hiari na kushuka kwangu hautafanya uonevu wowote. Baada ya kuelewa Ishara iliyo juu ..... Chapisha kuchapa ..... Tarehe ..... Saini ya uchunguzi ..... Jina la uchunguzi ..... .

Ufunzo Wa Kutawa

Nitaelewa kuwa nitakuwa huru kujiondoa kwenye utafiti wakati wowote. Mawasiliano muhimu: 1) KNH / UON / ERC. Namba ya: 020726300 ext .: 44102 Barua pepe: uonknh_erc @ uonbi.ac.ke, P.O Sanduku 20723code 00202

Katika Patient

Mimi .... ya ..... hapa kutoa idhini ya kuingizwa katika utafiti huu. Hali ya utafiti imeelezewa kuwa na Dk ..... Yeye anaye na NINI alinikandamiza NOR amenimlazimisha kuwa sehemu ya utafiti huu. Ninaelewa kuwa hakutakuwa na faida ya fedha kwa kurudi. Tarehe ..... Imewekwa .... Mheshimiwa ..... kuthibitisha kwamba nimemwelezea mgonjwa hali ya utafiti. Tarehe ..... Ilisainiwa .....

Appendix III: Demographic, History and Clinical Examination Screening Tool

Filling-in Use black pen. Start with the right-hand sided box.
Instructions: Encircle pre-filled boxes. Fill in M for missing answers

	Study day: Screening
Participant no.	
Participant First Name	

Demographic Data

1. Sex

Male	Female
1	2

2. Date of Birth

Dav Month Year

3. Age

Years

4. County of Origin

5. Literacy

Below primary school ☐

Primary school ☐

Secondary school ☐

Tertiary education ☐

Medical History

Symptoms at first presentation (encircle, you can choose more than one)

6. Pain (headache, facial-pain, discomfort)	Yes	No
7. Difficulties in comprehension (reasoning, memory, consciousness/seizures)	Yes	No
8. Difficulties in movement	Yes	No
9. Decrease in sensitivity of the senses (visual, hearing, touch, taste)	Yes	No
10. Abnormal growth (excess or inadequate compared to peers)	Yes	No
11. Abnormal excretion (disinhibited/ incontinent)	Yes	No
12. Disorders of nutrition (nausea, vomiting, wasting	Yes	No
13. Disorders of respiration	Yes	No

9. Duration between the start of symptoms and presentation to tertiary center(encircle only one)

a) Less than 2 weeks
b) Between 2 weeks and 3 months
c) More than 3 months

10. Description of symptoms in patients own words

_____.

_____.

Reasons for delay (encircle, you can choose more than one)

11. Symptoms not serious	Yes	No
12. Healthcare access	Yes	No
13. Delay caused by primary clinician	Yes	No
14. Problems of financing	Yes	No
15. Lack of family support	Yes	No
16. Sought alternative healthcare	Yes	No
17. Aversion to doctors or formal medical establishment	Yes	No
18. Thought it was another ailment	Yes	No

19. Reasons for delay in the patient's own words

_____.

_____.

_____.

Filling-in	Use black pen. Start with the right-hand sided box.
Instructions:	Encircle pre-filled boxes. Fill in M for missing answers

	Study day: Screening
Participant no.	
Participant First Name	

General Examination

Systolic **Diastolic**	**20. Blood Pressure in mm/Hg**	
	21. Respiratory Rate /min	
	22. Temperature °C	
	23. Heart Rate /min	

24. Pallor	Yes	No
25. Jaundice	Yes	No
26. Wasting	Yes	No
27. Lymphadenopathy	Yes	No

Filling-in Use black pen. Start with the right-hand sided box.
Instructions: Encircle pre-filled boxes. Fill in M for missing answers

	Study day: Screening
Participant no.	
Participant First Name	

Neurological Examination

. Glasgow Coma Scale	Response	Score
Eye Opening Response	Spontaneously	4
	To Speech	3
	To Pain	2
	No Response	1
Best Verbal Response	Oriented in Time, Place and person	5
	Confused	4
	Inappropriate words	3
	Incomprehensible Sounds	2
	No Response	1
Best Motor Response	Obeys Commands	6
	Moves to localized pain	5
	Flexion withdrawal from pain	4
	Abnormal flexion(decorticate)	3
	Abnormal extension(decerebrate)	2
	No response	1

E M V	Total

Gait

28. Hemiplegic	Yes	No
29. Diplegic	Yes	No
30. Neuropathic	Yes	No
31. Myopathic	Yes	No
32. Choreiform	Yes	No
33. Ataxic	Yes	No

34. Parkisonian

Yes	No

35. Sensory

Yes	No

Filling-in	Use black pen. Start with the right-hand sided box.
Instructions:	Encircle pre-filled boxes. Fill in M for missing answers

36.

	Study day: Screening
Participant no.	
Participant First Name	

37. Sex

38. Mini-mental Exam

		Test	Score
Orientation	5	What is the date,year,season,date,day,month	
	5	Where are we: country, county, town, hospital, floor	
Registration	3	Name 3 objects.-1 sec to say each, then ask patient to recall all three. Repeat until the patient has learnt all three. Count and record trials	
	5	Serial 7s-one point for each correct. Stop at 5	
	3	Ask for the 3 objects repeated above	
Language	2	Name a pencil and a watch	
	1	Repeat the following' no ifs, ands or buts'	
	3	Follow a three stage command. Take a piece of paper in your right hand, fold it in half and put it on the floor	
	3	Read and obey the following: close your eyes. 'write a sentence' Copy a design'	
		Total	
		Folstein: A practical method for grading the cognitive state of Patients for the clinician	

Cranial Nerves

40. Olfactory(cranial I) Yes No
41. Optic nerve(cranial II) Yes No
42. Oculomotor(crania lII) Yes No
43. Trochlea(cranial IV) Yes No
44. Trigeminal(cranial V) Yes No
45. Abducen(cranial VI) Yes No
46. Facial(cranial VII) Yes No
47. Vestibulocochlear(cranial VIII) Yes No
48. Glossopharyngeal(crania IX) Yes No
49. Accessory(cranial XI) Yes No
50. Hypoglossal(cranial XII) Yes No

51.

Karnofsky Performance Scale		
General category	**%**	**Specific criteria**
• Able to carry on normal activity • No special care needed	100	Normal general status - No complaint - No evidence of disease
	90	Able to carry on normal activity - Minor sign of symptoms of disease.
	80	Normal activity with effort, some signs or symptoms of disease.
• Unable to work • Able to live at home and care for most personal needs • Various amount of assistance needed	70	Able to care for self, unable to carry on normal activity or do work
	60	Requires occasional assistance from others, frequent medical care
	50	Requires considerable assistance from others; frequent medical care.
• Unable to care for self • Requires institutional or hospital care or equivalent • Disease may be rapidly progressing	40	Disabled, requires special care and assistance
	30	Severely disabled, hospitalization indicated, death not imminent
	20	Very sick, hospitalization necessary, active supportive treatment necessary
• Terminal states	10	Moribund
	0	Dead

Appendix IV: Imaging Evaluation screening Tool

Filling-in	Use black pen. Start with the right-hand sided box.
Instructions:	Encircle pre-filled boxes. Fill in M for missing answers

	Study day: Screening
Participant no.	
Participant First Name	

Images **AVAILABLE**

IMAGE NUMBERS

	. CT Scan NECT	**Yes**	**No**	
	. CT Scan Enhanced	**Yes**	**No**	
	. MRI T1WI	**Yes**	**No**	
	. MRI Gado+	**Yes**	**No**	
	. MRI Flair	**Yes**	**No**	
	. MRI DWI	**Yes**	**No**	
CT-scan Evaluation Site	. MRI ADC	**Yes**	**No**	

58. Lobes

Yes	No

Frontal_____

Parietal_____

Temporal_____

Occipital_____

59. Skull base

Yes	No

Anterior Cranial Fossa_____

Middle cranial fossa_____

Posterior Cranial Fossa_____

Filling-in	Use black pen. Start with the right-hand sided box.
Instructions:	Encircle pre-filled boxes. Fill in M for missing answers

	Study day: Screening
Participant no.	
Participant First Name	

60. Infratentorial

Yes	No

Midbrain_____

Pontine_____

Medulla_____

Cerebellum_____

61. Laterality

Right_____

Left_____

Midline_____

Bilateral_____

62. Tumor size

A- Largest Length

B- Largest Width

C- Number of slices

(A*B*C)/2) =_____ cm^3

Filling-in	Use black pen. Start with the right-hand sided box.
Instructions:	Encircle pre-filled boxes. Fill in M for missing answers

	Study day: Screening
Participant no.	
Participant First Name	

63. Pattern

Definition of Margins

Well defined	
ILL-defined	

Parenchyma

Homogenous	
Heterogenous	

64. Surrounding Edema

Present	Notpresent

65. Contrast Uptake

Circle of Willis clearly visible on Contrast CT Scan	Yes	No
No contrast enhancement		
Patchy/Mild contrast enhancement		
Avid contrast enhancement		

Filling-in	Use black pen. Start with the right-hand sided box.
Instructions:	Encircle pre-filled boxes. Fill in M for missing answers

	Study day: Screening
Participant no.	
Participant First Name	

MRI evaluation

66. T1 Weighted Characteristics

Hypointense ☐

Hyperintense ☐

67. T2 Weighted characteristics

Hypointense ☐

Hyperintense ☐

68. FLAIR

Edema Area ☐

Larger than lesion ☐

Equal to Lesion ☐

Less than lesion ☐

69. Diffusion Weighted

Diffusion (Light bulb bright) Yes ☐ No ☐

70. **ADC Map** Dark ☐

Light ☐

71. T1 Weighted Contrast Scan

No contrast enhancement

Patchy/Mild contrast enhancement ☐

Avid contrast enhancement ☐

Appendix V: Immunohistochemistry Results

Filling-in Use black pen. Start with the right-hand sided box.
Instructions: Encircle pre-filled boxes. Fill in M for missing answers

	Study day:2nd week Followup
Participant no.	
Participant First Name	

Histological findings(Tick the histological findings and Grade)(48)

72. Nuclear atypia	Yes	No
73. Mitotic figures	Yes	No
74. Endothelial proliferation	Yes	No
75. Necrosis	Yes	No

76. Histological diagnosis.

Astrocytic tumors	**Grade 1**	**Pilocytic astrocytoma** **Subependymal giant cell astrocytoma**	
	Grade II	**Diffusely infiltrating astrocytoma**	
	Grade III	**Anaplastic astrocytoma**	
	Grade IV	**Glioblastoma Multiforme**	
Oligodendroglial Tumors	**Grade II**	**Oligodendroglioma**	
	Grade III	**Anaplastic oligodendroglioma**	
Oligoastrocytic tumors	**Grade II**	**Oligoastrocytoma**	
	Grade III	**Anaplastic oligoastrocytoma**	
Ependymal Tumors	**Grade I**	**Subependymoma** **Myxopapillary ependymoma**	
	Grade II	**Ependymoma**	
	Grade III	**Anaplastic ependymoma**	

Filling-in	Use black pen. Start with the right-hand sided box.
Instructions:	Encircle pre-filled boxes. Fill in M for missing answers

		Study day: 2nd week followup
	Participant no.	
Immunohistochemistry	Participant First Name	

77. KI67

Yes	No

78. Mitotic figures in percentage.

79. IDH-1

mutated	Wildtype

Appendix VI: Three Month follow-up Evaluation Form

Filling-in	Use black pen. Start with the right-hand sided box.
Instructions:	Encircle pre-filled boxes. Fill in M for missing answers

		Study day: 3month-followup
Immunohistochemistry	Participant no.	
	Participant First Name	

80. Patient alive — Yes | No

If Yes

81. Symptoms have improved (subjective) since surgery — Yes | No

82. Symptoms have remained the same — Yes | No

83. Symptoms have become worse — Yes | No

84. Chemotherapy Regime

Days after surgery____.

Cycles given____.

Complications — Yes | No

Which complications_____.

85. Radiotherapy Regime

Days after surgery_____.

Cycles given_____.

Complications — Yes | No

Which complications_____.

Appendix VII: Three Month follow-up Evaluation Form

Filling-in Use black pen. Start with the right-hand sided box.
Instructions: Encircle pre-filled boxes. Fill in M for missing answers

		Study day: 3month-followup
Immunohistochemistry	Participant no.	
	Participant First Name	

80. Patient alive — Yes | No

If Yes

81. Symptoms have improved (subjective) since surgery — Yes | No

82. Symptoms have remained the same — Yes | No

83. Symptoms have become worse — Yes | No

Appendix VIII: Immunohistochemistry Manual

MANUAL IMMUNOSTAINING PROTOCOL

1. Leave already sectioned slides overnight at 37°c or heat already sectioned slides at 100°c for 1 hour.
2. Cool on a cooling bench.
3. Dewax in xylene; 3 changes each 2minutes.
4. Hydrate in alcohol; 3 changes each 2minutes.
5. Let slides air dry and use a novapen to outline tissue section.
6. Put slides in a coupling jar filled with citric buffer pH 6.0 or EDTA buffer pH 8.0
7. Put in a microwave or steamer for 20 minutes checking at 5-minute intervals for the level of the buffer (if slides not covered add buffer).
8. Cool the slides for 10 minutes then rinse with distilled water.
9. Apply peroxidase block for 5 minutes (to prevent endogenous peroxidase reaction) then rinse with Tris/PBS buffer.
10. Apply protein block for 10 minutes (to ensure proteins do not react) then thoroughly with Tris/PBS buffer.
11. Apply primary antibody for 30 minutes then wash thoroughly with Tris/ PBS buffer.
12. Apply post primary conjugate for 30 minutes (to enhance staining) then wash thoroughly with Tris/PBS buffer.
13. Apply polymer for 30 minutes (to enhance binding of DAB) then wash thoroughly with Tris/PBS buffer.
14. Apply DAB chromogen diluted 1:20 with substrate buffer 2-5 minutes then wash with Tris/PBS buffer or distilled water.
15. Counterstain in hematoxylin for 1-2 minutes then wash with distilled water.
16. Differentiate in 1% acid alcohol then wash either distilled water.
17. Blue in scotts tap water then wash with distilled water.
18. Dehydrate with alcohol 3 changes each 2 minutes.
19. Clear with xylene 3 changes each 2 minutes.
20. Mount with DPX and examine.

    Nucleus stain blue, Positive cells stain brow

APPENDIX: IX TIMELINES

February 2017- March 2018	March-April 2018	August 2018- March 2019	March 2019- May 2019
Proposal development			
	Ethics committee and corrections		
		Data collection	
			Analysis and Write up

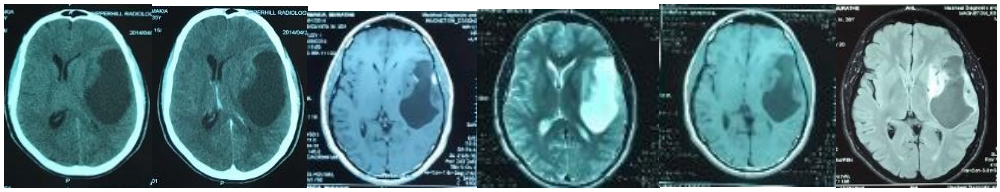
APPENDIX X: BUDGET

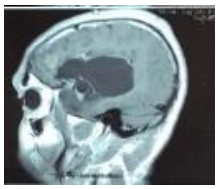
Unit Number	**Material**	**Unit Price (Ksh)**	**Total (Ksh)**
50	Data collection forms	@100	5000
	Stationary, internet, phone		10000
1	Statistician		50000
1	Research Assistant		50000
1	IDH-1 Antibodies		200000
	Total		315,000

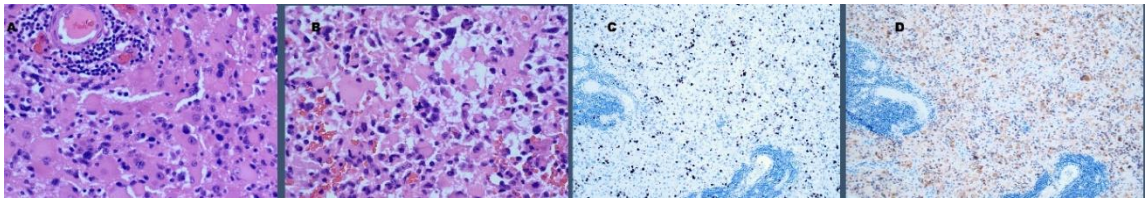
Appendix XI: Individual Case Studies

A 39-year-old with Kluver-Bucy syndrome who first complained of headaches and convulsions before developing hyperorality, hyperphagia, hypersexuality and violent episodes. Examination findings were of intermediate memory loss.

Imaging findings as shown below were of a large, left temporal, complex cystic lesion with nodular areas of enhancement.







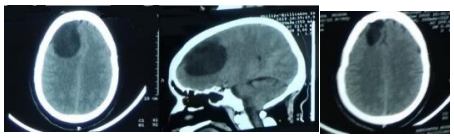
An example of an anaplastic astrocytoma grade III, IDH-1 mutant, with ATRX loss, that is cystic with small enhancing elements on imaging. The cyst is complex as it does not completely null on FLAIR imaging.

Status at 3 months- Alive

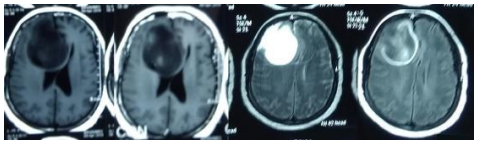
C N, 55 yr. old female on follow-up for grade 2 astrocytoma for 5 years earlier when a resection was done. Now presents with change in personality, left sided weakness and involuntary voiding

Images from 5 years prior

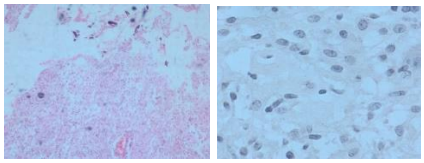
Preoperative



After 5 years, No chemoradiation given



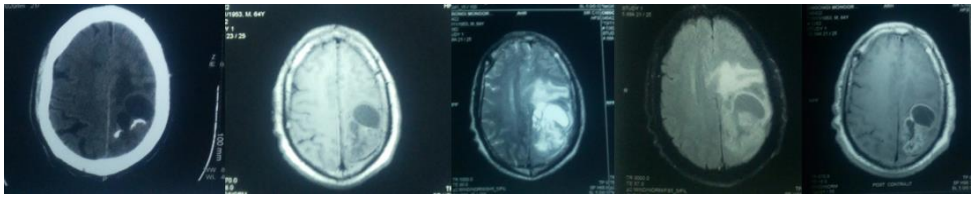
8 H&E ***9 IDH-1 wild type***



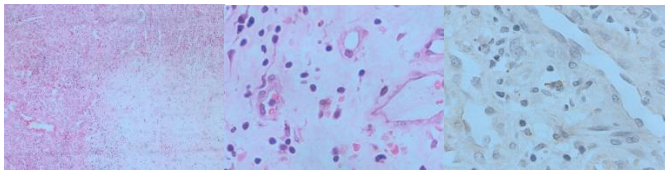
Tumor was still a diffuse astrocytoma grade 2

Status at 3 months- Alive

M O, 64 yr. old male with chronic headaches increasing in intensity with associated with diminution of visual acuity, and right hemiparesis. Also has reduced consciousness of 1 week's duration on examination Found a moribund patient, with reduced consciousness, signs of uncal herniation and rt sided facial and limb asymmetric weakness



10 H&E x10 ***H&E x40*** ***IDH-1 wildtype***



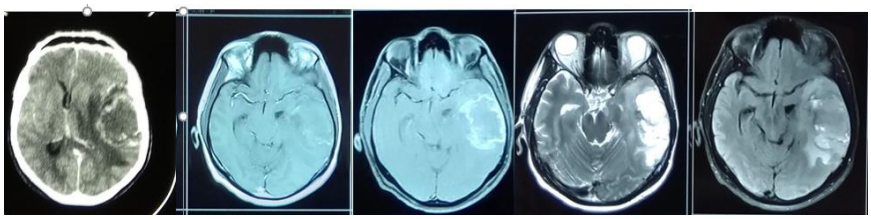
Tumor is a wild type Glioblastoma Multiforme

Status at 3 months- Died

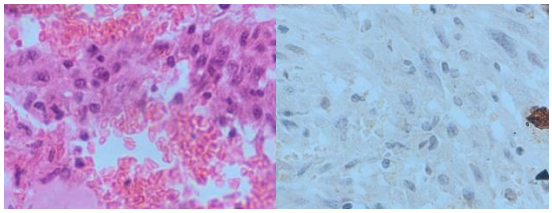
S K, frontoparietal headaches with memory lapses, no convulsions

On examination GCS 14/15 with disorientation of place, Had no cranial nerve palsies or motor weakness.

Postoperatively developed Steven-Johnson syndrome and did not undergo radiation.



11H&Ex40 ***IDH-1 Mutant***

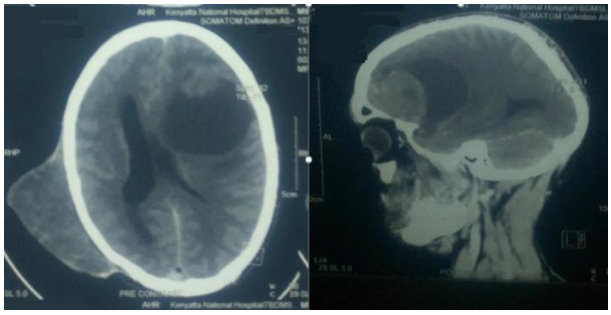


Glioblastoma IDH-1 Mutated

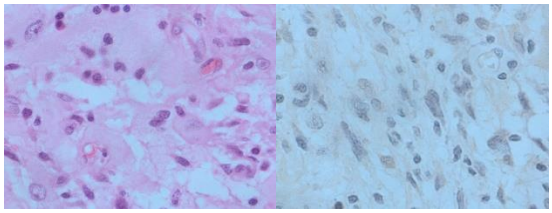
Status at 3 months- Died

K M, 31 males with NF 2 and scalp AVM, comes with deterioration of consciousness,

On examination, GCS 12 with anisocoria.



12 H&Ex40 ***IDH-1 wild type***



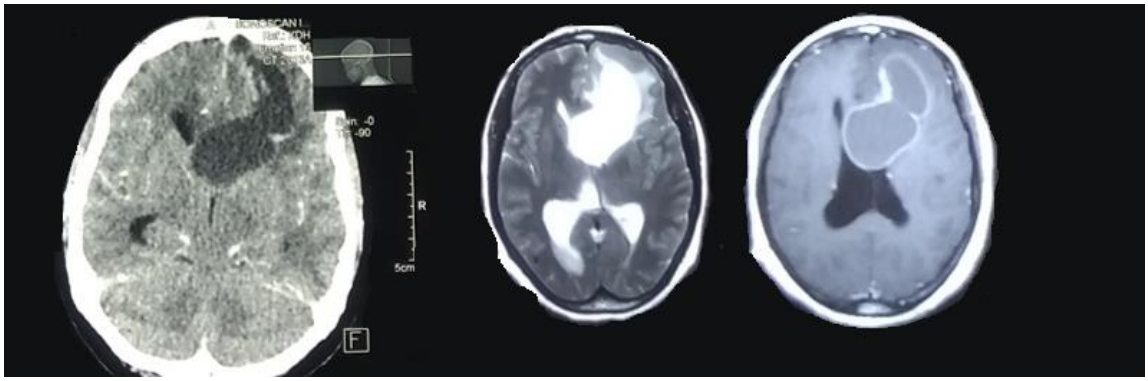
Diffuse Astrocytoma IDH-1 Wildtype

Status at 3 months- Alive

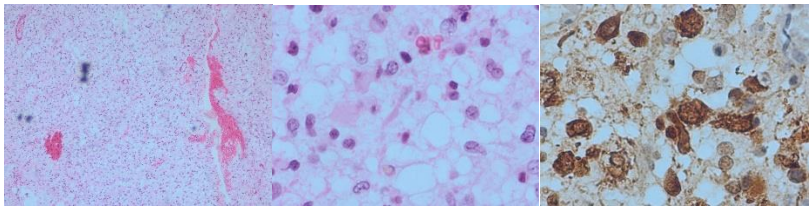
C N, 56 female complains of headaches, confusion and urinary incontinence

On examination GCS 15 with memory loss

Metastatic Workup was negative



13 H&E x10 ***H&Ex40*** ***IDH-1 Mutated***

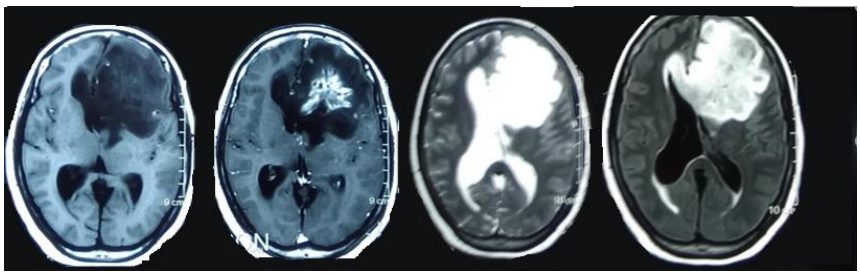


Diffuse astrocytoma, IDH-1 mutated

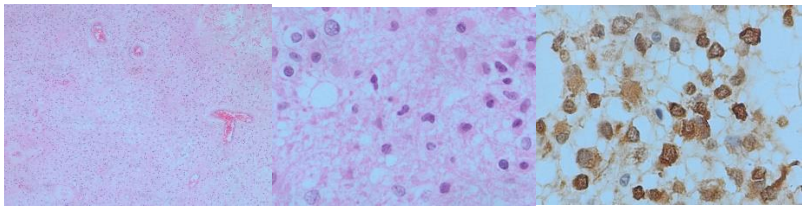
Status at 3 months- Alive

JN, 34 female presented with seizures 6 months prior but sought alternative care. When she was brought in by the husband, he reported changes in personality with an increase in paranoia and over religiosity

On examination GCS 15/15 with no focal deficits. She was mild mannered with anxiety.



14 H&E x10 ***H&E x40*** ***IDH-1 Mutated***

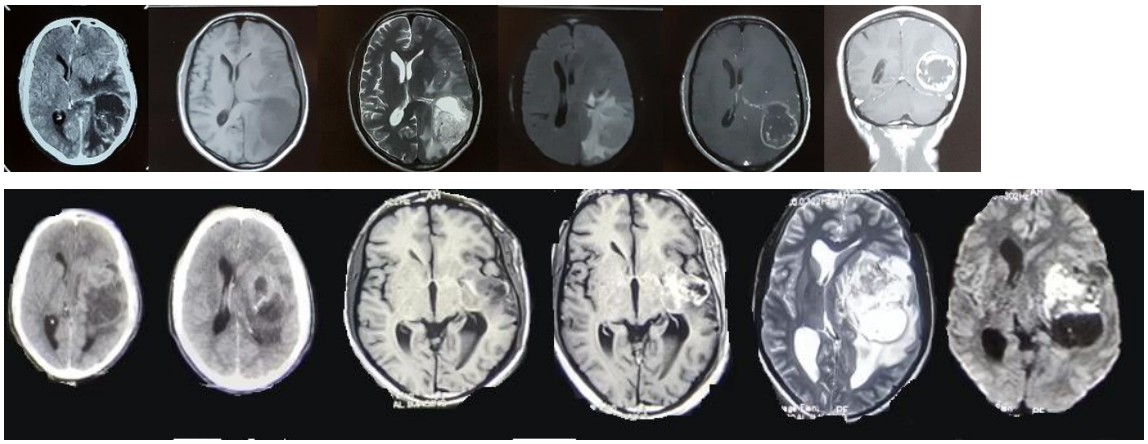


Anaplastic oligodendroglioma, IDH-1 Mutated

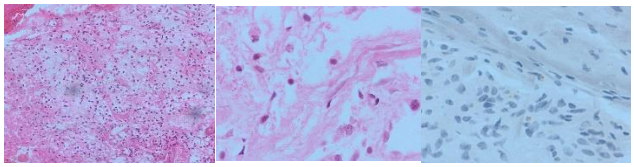
Status at 3 months- Alive

WG, 18-year-old male, presented with headaches and seizures

On examination GCS 15 with no focal deficits.



15 H&E x10 ***H&E x40*** ***IDH-1 wildtype***



Glioblastoma Multiforme, IDH-1 wild type

Status at 3 months- Alive