CLINICO-PATHOLOGIC FEATURES AND EARLY SURGICAL OUTCOME OF ASTROCYTOMAS IN ELDORET, KENYA

By

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A thesis submitted to the School of Medicine in partial fulfillment for the award of the degree of Master of Medicine in General Surgery of Moi University

DECLARATION

Student's Declaration

I declare that this thesis is my original work and has not been presented to any other university/institution for consideration.

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DEDICATION

This work is dedicated to my mother, Prof. Miriam Bageni Mwita for her immense sacrifice and unwavering support in ensuring a good education for her children.

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LIST OF ABBREVIATIONS

CNS:	Central nervous system
CSF:	Cerebrospinal fluid
CT scan:	Computed tomography scan
EGFR:	Epidermal growth factor receptor
GBM:	Glioblastoma Multiforme
GFAP:	Glial fibrillary acidic protein
HICs:	High income countries
HLA:	Human leucocyte antigen
KPS:	Karnofsky performance status
LAMICs:	Low- and middle-income countries
MRI:	Magnetic resonance imaging
MTRH:	Moi Teaching and Referral Hospital
PTEN:	Phosphatase and tensin homolog
PXA:	Pleomorphic Xanthoastrocytoma
SEGA:	Subependymal giant cell astrocytoma
WHO:	World health organization

OPERATIONAL DEFINITION OF TERMS

High grade astrocytomas (HGA): A group of CNS neoplasms arising from astrocytes and that exhibit a high grade of anaplasia and tendency to infiltrate adjacent tissues. They include anaplastic astrocytoma and Glioblastoma multiforme.

Ki-67: Nuclear protein associated with cellular proliferation and is therefore utilized as a marker of proliferation in brain tumors.

Low grade astrocytomas (LGA): A group of CNS neoplasms arising from astrocytes that exhibit a lower grade of anaplasia. They include pilocytic astrocytoma, PXA, SEGA and diffuse astrocytoma.

Primary brain tumor: Tumors that originate within the tissues of brain or its protective lining.

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ABSTRACT

Background: Astrocytomas are a group of primary central nervous system tumors arising from astrocytes and accounting for up to 37.8% of all brain tumors seen in hospital-based studies from Africa. Despite being one of the commonest types of brain tumors, their patterns and short-term outcomes remain poorly studied in Kenya and the greater East African region.

Objective: To describe the clinico-pathologic features as well as surgical and functional outcome of astrocytomas in Eldoret, Kenya.

Methods: Prospective, descriptive census study involving consecutive patients with a histological diagnosis of astrocytoma seen in three hospitals located in Eldoret, Kenya. Clinical, MRI, pathological and treatment characteristics were recorded and patients followed up for 12 weeks after discharge from hospital. The study endpoints were early post-treatment complications and functional status.

Results: Thirty-one patients constituting 25% of all patients with brain tumors were recruited over a one-year period. Females constituted 51.6% (N=16) of the sample. Headache (83.9%) and focal neurological deficits (64.5%) were the commonest presenting features. Compared to high grade tumors, low grade astrocytomas had a higher median duration of illness (90 Vs. 45 days; p-value=0.433), lower median functional status (KPS score 40 Vs. 50; p-value=0.663), lower median tumor size (18.86 Vs. 104.34 Cm^3; p-value=0.022) and lower median MRI scores (9 Vs. 17; p-value=0.0001). Glioblastoma multiforme (71%) was the predominant histological subtype followed by diffuse astrocytoma (22.6%). The median Ki-67 proliferative index was 6% for pilocytic astrocytoma, 1.6% for diffuse astrocytoma and 60% for glioblastoma multiforme. All patients underwent surgery. Systemic surgical complications occurred in 6.5% of patients while regional complications occurred in 38.7%. Hematoma formation was the commonest regional surgical complication. The median length of hospital stay was 20 days for low grade tumors and 12 days for high grade tumors (p=0.828). In-hospital mortality was 19.4% and increased to 25.8% at 12 weeks. The KPS score at discharge was 50 and improved to 60 at 12 weeks. Only 9.7% (N=3) of patients had acceptable functional status at 12 weeks follow up.

Conclusions: In this study, headache, focal neurological deficits and reduced functional status are the commonest presenting features of astrocytomas while Glioblastoma multiforme is the commonest histological subtype. MRI can be used to differentiate low grade astrocytomas from high grade astrocytomas.Further, the encountered astrocytomas are highly proliferative and in the short-term, both their surgical and functional outcome are suboptimal.

Recommendations: Clinicians in peripheral facilities should be sensitized on early referral for patients with persistent neurological symptoms. Owing to increased proliferative activity, patients with astrocytoma in Eldoret should be followed up closely to detect recurrence early. More research is needed in order to determine the underlying mechanisms for the high proliferative activity of astrocytomas in this region as well as determine the reasons for the observed surgical and functional outcomes.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Within the human body, the nervous system is responsible for controlling and coordinating the functions of all the other systems that make up the human. It is divided into the central and peripheral nervous systems. The latter consists of peripheral nerves that are responsible for transmitting sensory and effector impulses to and from the central nervous system (CNS). The CNS therefore is responsible for integrating and interpreting sensory impulses as well as generating effector impulses in response to sensory stimuli. It is made up of the brain and the spinal cord. Both these structures are surrounded by a protective lining known as the meninges.

The CNS is composed of two main types of cells. Neurons (nerve cells) are responsible for impulse transmission and they form the functional unit of this system. Glial cells are supportive cells that provide protection and nutrition to the CNS. There are four main types of glial cells within the CNS: oligodendrocytes, ependymal cells, microglia and astrocytes (Mescher, 2013). The oligodendrocytes are responsible for electrical insulation within the CNS. Ependymal cells aid in the production and movement of cerebrospinal fluid (CSF) within the CNS, microglia are responsible for defense and immune related activities while astrocytes provide neurons with structural and metabolic support.

Among the many disorders that may affect the CNS, neoplastic disorders are of particular importance to the surgeon. Neoplasms of the CNS may be either benign or malignant and may arise from any of the glial cells within the CNS, the meninges or other tissues surrounding the nervous system (Gasco et al., 2012). Collectively, these are known as primary brain tumors. The CNS may also be afflicted by tumors that arise from other

organs. These are known as secondary or metastatic brain tumors. Overall, although tumors of the brain constitute a small proportion of all human neoplasms, they are among the top ten leading causes of tumor related deaths (Ostrom et al., 2013; S. Preston-Martin, 1996).

The clinical presentation of brain tumors is due to a combination of two factors. First is the focal compression of surrounding brain tissue by tumor and second is the subsequent rise in intracranial pressure due to the tumor itself, peritumoral brain edema and obstructed flow of cerebrospinal fluid leading to hydrocephalus. While the clinical presentation does not often differ by histology, tumor growth rate and location are the main determinants of clinical presentation (Gasco et al., 2012). More than half the patients with brain tumors present with headache that is worse in the morning, accompanied by nausea and often relieved by vomiting. Other presenting features include seizures, cranial nerve deficits, gait disturbances, focal neurological deficits as well as personality changes and hallucinations. The initial study employed for diagnosis is computed tomography (CT) scan. It can show changes in brain density suggestive of tumor. However, magnetic resonance imaging (MRI) is the gold standard modality for diagnosis. It can also be used for presurgical planning and monitoring of response to therapy (Ishita Pant et al., 2015).

A definitive diagnosis for brain tumors can only be achieved by histologic examination of brain tumor tissue. Tumors that arise from within the brain parenchyma are termed intra-axial tumors while those arising from without the brain parenchyma are termed extra-axial tumors. The majority of primary brain tumors are intra-axial arising from glial cells. Collectively, they are called gliomas. Histologic classification is based on cell type and is determined according to criteria devised by the world health organization (WHO).

To determine the degree of malignancy of a tumor, the WHO system also grades brain tumors based on microscopic features such as degree of cellularity, pleomorphism, presence of mitoses, endothelial proliferation and necrosis. There are four grades with higher grades depicting more aggressive and malignant tumors. Tumor proliferation is an additional yet important parameter to determine. Its usefulness is in the prognostication of astrocytomas and it is determined by measuring the number of mitoses present in a tumor sample (Skjulsvik et al., 2014). However, the histological grading of brain tumors is affected by a high degree of inter-observer and intra-observer variability which means that its reliability is poor. To help mitigate against this limitation, there are measurable biomarkers of tumor proliferation that can be utilized. They include the antigen Ki-67, mitosin, survivin, DNA topoisomerase IIa and PHH3 (Habberstad et al., 2011; Johannessen & Torp, 2006). Of these, Ki-67 is the most widely studied and utilized marker of proliferation.

The mainstay of treatment for brain tumors is surgery. The goals of therapy include obtaining tissue for a histological diagnosis and reduction of mass effect with the aim of restoring neurological function. In addition, surgery aims to achieve long-term oncological control of disease (as measured by disease free and overall survival) (Jakola et al., 2011). Surgery involves either tumor resection or biopsy. The choice of surgical procedure to be performed is determined by tumor location and size, tumor sensitivity to chemotherapy and/or radiotherapy as well as pre-operative functional status. Further, it is helpful to consider the adverse effects of surgery when determining the choice of procedure to be done (Moiyadi & Shetty, 2012).

The commonest type of primary brain tumor is astrocytoma which arises from astrocytes. Although they may occur in the spinal cord, they are predominantly tumors of the brain.

They represent more than 50% of all primary tumors of the brain and 76-80% of all tumors arising from glial tissue (CBTRUS, 2012). In Africa, astrocytomas account for 27.9-37.8% of all brain tumors encountered in surgical practice (Mwang'ombe & Ombachi, 2000; Zalata et al., 2011). Within the Kenyan context, astrocytomas constitute the bulk of brain tumors encountered in clinical practice in both adult and pediatric populations (Mwang'ombe & Ombachi, 2000; Wanyoike, 2004). However, their clinical and pathological characteristics as well as therapeutic outcomes remain inadequately reported particularly in the western region of the country. This study aimed to fill this gap.

1.2 Problem Statement

In Kenya, astrocytomas account for up to 37.8% of all brain tumors encountered in tertiary hospitals (Mwang'ombe & Ombachi, 2000; Wanyoike, 2004). In Eldoret (which provides neurosurgical care to much of western Kenya), more than 10% of all the elective neurosurgical cases done annually are due to brain tumors, majority of which are astrocytomas. However, their clinical pattern, pathological characteristics and short-term outcome are not adequately documented.

1.3 Justification

Astrocytomas account for more than one third the proportion of primary brain tumors seen in tertiary institutions in Kenya (Mwang'ombe & Ombachi, 2000; Wanyoike, 2004). With the recent introduction of neurosurgical services in Eldoret, this study provides clinicians with baseline data on the clinical and pathological pattern of astrocytomas within the local setup in addition to guiding clinicians, policy makers and patients when making decisions regarding treatment and its risks.

1.4 Research Question

What are the clinico-pathologic features and outcome of astrocytomas among neurosurgical patients seen in Eldoret?

1.5 Objectives

1.5.1 Broad objective

To describe the clinico-pathologic features and outcome of astrocytomas among neurosurgical patients seen in three hospitals in Eldoret, Kenya.

1.5.2 Specific objectives

1. To describe the clinical and MRI features of astrocytomas among neurosurgical patients seen in three hospitals in Eldoret, Kenya.
2. To determine the histological subtypes and proliferative activity of astrocytomas among neurosurgical patients seen in three hospitals in Eldoret, Kenya.
3. To determine the surgical outcome of astrocytomas among neurosurgical patients seen in three hospitals in Eldoret, Kenya.
4. To determine the functional outcome of astrocytomas among neurosurgical patients seen in three hospitals in Eldoret, Kenya.

CHAPTER TWO: LITERATURE REVIEW

2.1 Neuropathology, classification and grading of astrocytomas

Astrocytomas are tumors in which neoplastic cells are thought to arise from astrocytes. Since astrocytes are developmentally derived from the dorsal and ventral neuro-epithelium of the neural tube (Collins, 1998) these tumors are thought to be of neuro-epithelial origin. Most astrocytes and their precursors maintain their capability for division and are therefore susceptible to transformation. This explains their frequent occurrence among brain tumors (Wechsler-Reya & Scott, 2001). However, the resemblance of neoplastic cells to astrocytes does not mean that they are of astrocytic lineage and their true origin remains unclear. However, molecular studies may have a role to play in resolving this issue (Louis et al., 2001).

Macroscopically, low grade tumors are avascular, firm and fibrous in consistency with calcification in 15% of tumors (Kaye, 2005). Microscopically, they exhibit increased cellularity but no mitoses, necrosis or endothelial proliferation. Occasionally, they may infiltrate adjacent tissue. Higher grade tumors have a vascular macroscopic appearance with necrotic centers. Histologically, they have few astrocytes that appear normal and exhibit marked cellular pleomorphism, extensive endothelial proliferation and numerous mitotic figures. Cells also exhibit extensive necrosis. Any part of the brain may be affected but lower grade tumors have a predilection for the cerebellum while higher grade tumors occur more frequently in the cerebrum (Kaye, 2005).

The histological classification of astrocytomas has a rich history and was first probably attempted by Rudolph Virchow who introduced the term glioma around the year 1864 (Tonn et al., 2006). However, it was Bailey and Cushing who first proposed a

classification system based on the cell of origin of the tumor (Kaye, 2005). This system was later improved on by Kernohan who suggested that the degree of differentiation of the cell of origin of the tumor was related to its biologic progression (Susan Preston-Martin et al., 2006). As such, he proposed a four-tier tumor grading system in which the clinical grade increases with poorer differentiation of the tumor. However, this system lacked reliability and was not widely applied as the standard of histological assessment of brain tumors.

Subsequently, the WHO devised a system for tumor classification and grading that reflected tumor aggressiveness, had better inter-observer reliability and correlated with overall survival (Kleihues et al., 1993; Louis et al., 2007; Susan Preston-Martin et al., 2006). As such, Grade I tumors are well circumscribed and distinct from surrounding parenchyma and are curable with complete surgical excision. They include pilocytic astrocytoma, pleomorphic xanthoastrocytoma (PXA) and sub-ependymal giant cell astrocytoma (SEGA). They account for 5.1% of all gliomas with more than 90% survival at 10 years (CBTRUS, 2012; Kennedy & Bruce, 2014). These are slow growing neoplasms which preferentially develop in children and young adults with limited potential for malignant progression (Sarkar et al., 2009). They are often curable by complete tumor resection. Grade II tumors are more diffuse and infiltrative making complete surgical resection more difficult and increasing chances of tumor recurrence. This group comprises low-grade (diffuse) astrocytoma which accounts for 9.1% of all gliomas and has a survival of more than 5 years (CBTRUS, 2012; Kennedy & Bruce, 2014). Grade III tumors also have diffuse growth patterns but exhibit anaplastic features. It includes anaplastic astrocytoma which accounts for 6% of all gliomas and has a survival of 2-5 years (CBTRUS, 2012; Kennedy & Bruce, 2014). Grade IV tumors

(glioblastoma multiforme) have rapidly dividing poorly differentiated cells with vascular proliferation and areas of necrosis. Overall survival is often less than one year (Buczkowicz et al., 2014; Smith et al., 2001). Together, Grade II, III and IV tumors are the commonest primary tumors of the cerebral hemispheres in adults and share a number of characteristics. First, they exhibit diffuse infiltration of adjacent and distant brain structures (Louis et al., 2007). Secondly, they show a tendency for progression into more malignant phenotypes through sequential acquisition of genetic alterations (Sarkar et al., 2009). Thirdly, although they may occur at any site within the CNS, they show a predilection for the cerebral hemisphere (Kunwar et al., 2001; Ostrom et al., 2013). Fourth, their incidence is higher in adults compared to children (CBTRUS, 2012; Kunwar et al., 2001).

It has been reported that the WHO system is not only reproducible but has appropriate correlation of histopathologic features to clinical behavior and response to therapy (Kleihues et al., 1995). However, the system overlooks other important prognostic factors that are independent of histology such as age, duration of disease, surgical resection and clinical performance status. In addition, grading of rare or newly defined tumor entities is difficult with this system necessitating continuous re-assessment to determine the biological behavior of the tumor. Histologic variation within the same tumor makes classification and grading difficult such that multiple, image guided stereotactic biopsies complemented by good communication between neuro-pathologist and neurosurgeon are essential. Although histological classification and grading of astrocytomas has a prognostic value and is valuable in guiding therapy, there is still considerable variation in response to therapy and wide variations in overall survival pointing to another shortfall

in the classification system (Louis et al., 2001). These obstacles have been overcome with the advent of immunohistochemistry and the use of molecular biology techniques.

Immunohistochemical (IHC) staining of brain tumor samples for expression of differentiation and proliferation markers has become more common in recent years. This procedure helps to further characterize tumors and avoid the subjectivity of histologic techniques. The process involves the use of antibodies (conjugated to an enzyme) to detect specific antigens expressed by a tumor. Once the conjugated antibody binds the target antigen, a color signal is produced pointing to the presence of the antigen in question. This technique is still prone to inter-observer variability and its diagnostic value is diluted by the fact that some antigens are expressed on both normal and abnormal cells (Tonn et al., 2006).

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein expressed by astrocytes. IHC staining for this protein is used to determine cells of astrocytic lineage but cannot differentiate tumor from non-tumor (Sarkar et al., 2009). The proliferation marker Ki-67 is another commonly employed immunohistochemical test. It demonstrates the proliferative activity of a cell and is a useful supplement for diagnosis and prognosis of astrocytomas. In particular, it is of benefit when histopathology cannot differentiate between tumors e.g. where stereotactic biopsies have been performed and there is insufficient tissue for further histological evaluation. It is positive in 0-3.9% of pilocytic astrocytomas, 5-10% of anaplastic astrocytomas and 15-20% of glioblastomas (Sarkar et al., 2009). Still, it cannot be used as a diagnostic tool since there is wide variation and overlap in proliferative activity among astrocytoma subtypes (Shivaprasad et al., 2016). However, it has been reported to have value in prognosis such that tumors with higher proliferation have worse prognosis compared to those with lower proliferation

(Thotakura et al., 2014). P53 is a protein encoded by the gene TP53-the most frequently altered gene in cancer. This protein is responsible for regulating the process of gene transcription and its mutated form is present in most cancers. However, some studies show that it is inferior to Ki-67 for distinguishing between astrocytoma subtypes and it has a limited role in determining prognosis (Sengupta et al., 2012). Other biomarkers that have been studied include mitosin, survivin, PHH3 and DNA topoisomerase IIa (Habberstad et al., 2011). Mitosin is expressed during the active phase of the cell cycle. However, its value in prognosticating astrocytomas is at present unknown. Survivin promotes survival of tumor cells. It is not expressed in normal cells and it is thought to promote radiation resistance in GBM. PHH3 the phosphorylated form of core histone protein H3 which is present during mitosis. It has demonstrated value in prognostication of astrocytomas but is not as widely used as Ki-67 (Habberstad et al., 2011). Nuclear DNA topoisomerase IIa is an essential enzyme for DNA replication and repair. Proliferative signals may upregulate its genetic expression although its value lies in the prognostication of mainly high grade astrocytomas (Habberstad et al., 2011).

Molecular biology techniques have also played a role in supplementing histopathology. As such, a gain on chromosome 7 is associated with shorter patient survival in anaplastic astrocytoma (Kunwar et al., 2001). A gain on the q arm of chromosome 7 and a loss of the q arm on chromosome 10 are associated with increased risk of death regardless of histological grade (Wiltshire et al., 2004). Amplification of the EGFR oncogene however, reveals conflicting results. One study has shown it to be an independent predictor of prolonged survival in patients older than 60 years with glioblastoma multiforme (Smith et al., 2001) while another study demonstrates no correlation with survival (Olson et al., 1998). Of note however, the latter study included tumor subtypes

other than glioblastoma multiforme. Nonetheless, these disparate results suggest that individual genes are a part of a larger signaling system and therefore individual gene assessment may be inappropriate (Olson et al., 1998). Overall, it is anticipated that these molecular advances will allow prediction of response to a specific mode of therapy for each individual patient.

2.2 Epidemiology of astrocytomas

Brain tumors in general account for less than 2% of all malignancies among adults. However, they are the second leading cause of death due to neurologic disease after stroke while in children they are the second most common malignancy after the leukemias (S. Preston-Martin, 1996). Overall, the age-adjusted incidence of brain tumors in high income countries (HICs) is 6-11 cases per 100,000 persons in men and 4-11 cases per 100,000 persons in women. Of all primary brain and spinal cord tumors, astrocytomas account for approximately 23% (CBTRUS, 2012) while among those of neuro-epithelial origin (gliomas) they account for 76-80% regardless of tumor behavior (CBTRUS, 2012; Ohgaki & Kleihues, 2005).

According to population based studies from HICs, the incidence of astrocytomas differs by age, sex, race and histological subtype (CBTRUS, 2012; Ohgaki & Kleihues, 2005; Ostrom et al., 2013). The benign forms of astrocytomas have a higher incidence among pediatric age groups while the more malignant subtypes occur more frequently among the elderly. As such, pilocytic astrocytoma accounts for 10.4-17.5% of astrocytomas among children aged 0-19 years while glioblastoma accounts for only 2.6-2.9% in this age group (Ostrom et al., 2013). Among adults and the elderly, anaplastic astrocytoma and glioblastoma account for a larger proportion of astrocytomas. Overall, men are more affected than women with a male to female ratio of 1.09-1.7:1 (Ohgaki & Kleihues,

2005). In addition, Caucasians are more affected than people of African and Asian descent. However, this racial difference is thought to be due to underlying differences in socioeconomic status rather than differences in genetic susceptibility (Ohgaki & Kleihues, 2005).

The occurrence of brain tumors may be affected by reproductive, environmental, genetic or immunologic factors. Data from brain tumor registries show that females have a lower risk of developing brain tumors and that post-menopausal women have a higher occurrence of brain tumors compared to pre-menopausal women (CBTRUS, 2012). Environmental exposures such as ionizing radiation, cell phone use, exposure to electromagnetic fields, hair dyes, head trauma, tobacco and alcohol use, dietary intake of N-nitroso compounds, calcium, maternal folate supplementation and anti-oxidant intake have been suggested as risk factors for brain tumors (DeAngelis, 2001; Fisher et al., 2007). Of these, only exposure to ionizing radiation has been consistently shown to be associated with brain tumor development. However, most epidemiological studies on this issue are retrospective in nature, employ unreliable measurements of exposure and have small sample sizes hence the equivocal observations (Fisher et al., 2007).

Genetic factors have also been investigated for association with brain tumors. In northern Sweden, Malmer and colleagues demonstrated that first degree relatives of patients with astrocytomas are more likely to develop astrocytomas (Malmer et al., 1999). However, the absolute numbers involved in this study were small and environmental exposures were not controlled for. Other studies have investigated the association between genetic polymorphisms (in cytochrome P450 enzymes, DNA repair pathways and cell cycle regulation) and the risk of developing a brain tumor (Fisher et al., 2007). However, they were case-control studies with small sample sizes in which confounding by genes with

functions similar to those being studied was not controlled for. As such, although they suggest a possible association with glioma risk their findings are inconsistent (Fisher et al., 2007).

Interestingly, immunologic factors have been found to have a protective effect on the occurrence of brain tumors. Schoemaker and colleagues have shown that asthma, hay fever, eczema and other allergies are associated with a reduced incidence of brain tumors (Schoemaker et al., 2006). In addition, autoimmune conditions and infection with viral agents such as *Varicella-Zoster* virus have also been reported to be protective (Fisher et al., 2007). It is thought that the anti-inflammatory effects of cytokines involved in allergic and autoimmune disease as well as increased tumor immunosurveillance are responsible for these observations. Nonetheless, other studies have reported that HLA B13 and B7 are associated with a higher incidence of glioblastoma multiforme and that defects related to HLA presentation of antigen to T-lymphocytes may explain the poor results observed in clinical trials investigating T-cell based immunotherapy (Tang et al., 2005).

Mortality due to brain tumors is higher in men than in women (4-7 per 100,000 persons per year in men and 3-5 per 100,000 persons per year in women) (Ohgaki & Kleihues, 2005). This difference holds true for astrocytomas as well. It has been suggested that the ratio between incidence and mortality of brain tumors reflects the level of success in managing the disease (Susan Preston-Martin et al., 2006). However, owing to a lack of population-based studies from LAMICs, direct comparison with HICs would be inappropriate. Nonetheless, some authors suggest that the regional differences in mortality reflect differences in the success of managing brain tumors (Ohgaki & Kleihues, 2005). As a measure of disease outcome following therapy, survival rates differ between the different types of astrocytomas. In HICs, the 5-year survival rate for

pilocytic astrocytoma ranges from 84.1% to 97.2% with mean survival for this tumor subtype being 142 months (Ostrom et al., 2013). The more malignant glioblastoma has a 3-year survival rate of 7.7% (Ahmadloo et al., 2013).

2.3 Clinical and MRI features of astrocytomas

The clinical, radiological and treatment characteristics of astrocytomas are important in in characterizing astrocytomas. Indeed, it has been suggested that the histopathological interpretation of brain tissue samples by pathologists is aided by having an idea of the clinical, radiological and surgical features of the tumor. However, majority of studies report on clinical features for brain tumors in general with few studies focusing on astrocytomas specifically. Among children, features of raised intracranial pressure have the most frequent clinical presentation. In studies form North Africa, up to 67% of pediatric patients will present with headache, vomiting and altered sensorium (El-Gaidi, 2011). Progressive increase in head circumference is noted in those aged less than one year (El-Gaidi & Eissa, 2010). Ocular symptoms (reduced visual acuity and squint) occur in 28% of patients while in both infants and older children motor symptoms occur in about 19% (El-Gaidi, 2011; El-Gaidi & Eissa, 2010). Among African adults, raised intracranial pressure (83%), visual impairment (83%) and hemiparesis and hemiplegia (75%) are the commonest symptoms. (Idowu & Apemiye, 2009). Seizures are an important manifestation of brain tumors as well. They occur in tumors in which the epicenter is cortical, particularly in diffuse astrocytomas (Sarkar et al., 2009).

Magnetic resonance imaging is of particular importance in the diagnosis of astrocytomas and is often the first step towards an accurate diagnosis. Through assessment of intracranial lesions for characteristics of their margins, signal, contents, mass effect, bony changes (scalloping) and location a fairly accurate diagnosis of the type of brain tumor

can be reached. Concordance between MRI and pathological findings is 100% for high grade astrocytomas and 85% for low grade astrocytomas (Ishita Pant et al., 2015). In addition, MRI may provide important clues beyond those obtainable by histopathology alone. For example, in under sampled glioblastoma mulitiforme on biopsy, a ring enhancing lesion on MRI may represent the infiltrative edge of the glioblastoma while a cystic lesion with a mural enhancing nodule is suggestive of pilocytic astrocytoma (Perry, 2003). Certain MRI features are specific for astrocytomas in general and others for particular astrocytoma subtypes (Ishita Pant et al., 2015). Generally, astrocytomas are intra-axial parenchymal or ventricular tumors. Low grade astrocytomas have well defined margins and present no calcification or scalloping. In addition, they may be either supratentorial or infratentorial. High grade tumors have ill-defined margins and may also be either supratentorial or infratentorial. Macroscopic aspects of tumors can be accurately assessed on MRI and quantified for objectivity. Studies that have attempted this assign scores to each of these aspects (edema, mass effect, contrast enhancement, margins, signal homogeneity, necrosis, hemorrhage and flow void), compute an overall score and correlate this with tumor grade. Overall, there has been good correlation between scores and tumor grade suggesting a role for MRI in predicting outcome (Pierallini et al., 1997).

2.4 Management strategies for astrocytomas

Management of astrocytomas involves three modalities: surgery, radiotherapy and adjuvant treatment. The principal therapeutic option is surgery. This is often supplemented by radiotherapy and chemotherapy with more than 90% of patients in some reports undergoing both (Kunwar et al., 2001). Combination of radiotherapy with chemotherapy after surgery has also been found to result in prolongation of survival (Jakola et al., 2011).

The main goal of surgery is complete tumor excision however, in instances where there is extensive tumor infiltration or location in eloquent areas of the brain, incomplete resection or stereotactic biopsy is the only option (Kaye, 2005). Patient age and ability to withstand surgery also determine therapy e.g. in one study involving elderly patients, increasing age showed a trend towards less aggressive surgical options (Barnholtz-Sloan et al., 2008). In most instances, total resection of tumors is higher in low grade tumors and those in non-eloquent areas of the brain (Bianco et al., 2013; El-Gaidi & Eissa, 2010).

Radiotherapy is an effective addition to surgery for higher grade tumors and has been shown to increase survival (Jakola et al., 2011). However, its use is debated in lower grade tumors where complete tumor excision is possible and disease progression is slow (Kaye, 2005). Increasing the total radiation dose has a slight survival advantage but higher doses are associated with a higher incidence of complications. Attempts have been made to enhance the efficacy and safety of radiotherapy. The use of radiosensitizers and interstitial brachytherapy however, has not been met with the anticipated success unlike stereotactic radiosurgery which has been shown to be both safe and effective (Young et al., 2015).

Until recently, nitrosourea agents were the most commonly employed chemotherapeutic agents. However, the orally administered alkylating agent temozolomide has had increased uptake in recent years. It penetrates the CNS and is well tolerated with a predictable myelotoxicity and confers increased survival when used after surgery and radiotherapy (Kaye, 2005). Other management options for astrocytomas include hyperthermia and immunotherapy which have shown poor results while photodynamic and gene therapy show promise.

2.5 Surgical and functional outcome of management for astrocytomas

The aims of surgery for astrocytomas are twofold: achievement of long-term oncological control of disease (as measured by disease free and overall survival) and ensuring minimal therapy related toxicity (which in the context of surgery entails the immediate post-operative outcomes). Age, histological grade and clinical performance/functional status are the most reliable predictors of survival across studies (Olson et al., 1998; Wiltshire et al., 2004). Younger age and higher clinical performance predict longer survival (Kunwar et al., 2001) while the more malignant variants of astrocytomas have shorter survival times (El-Gaidi, 2011). The inclusion of radiotherapy and chemotherapy add to the survival benefit of surgery (Barnholtz-Sloan et al., 2008). With regard to radiotherapy, radiation dose has been reported to have an impact on survival in patients with glioblastoma (Ahmadloo et al., 2013).

Other factors that impact on long-term survival have also been reported. At the molecular level, gains on chromosome 7 are associated with shorter patient survival regardless of age (Kunwar et al., 2001). Among patients with high grade astrocytomas, EGFR gene amplification has been shown to be an independent predictor of survival among patients older than 60 years with glioblastoma while PTEN gene mutation is a prognostic factor in anaplastic astrocytoma (Smith et al., 2001). Losses on the q arm of chromosome 10 have been shown to increase the risk of death from astrocytoma regardless of tumor grade (Wiltshire et al., 2004). Overall, these studies seem to focus on the more prevalent high grade astrocytomas and it is unclear whether these observations hold true for all tumor subtypes. Further, their interaction with the various therapeutic options particularly surgery, is unknown.

The extent of surgical tumor resection is among the most studied predictor of astrocytoma outcome. In a recent meta-analysis, the impact of surgical resection on overall outcome was studied (Almenawer et al., 2015). Compared to biopsy only, any form of tumor resection (total or subtotal) was associated with better progression-free and overall survival. In another review, increased extent of resection had a survival benefit regardless of histological grade of astrocytoma (Hardesty & Sanai, 2012). Even in eloquent areas of the brain, tumor resection has better overall survival compared to biopsy only (Bianco et al., 2013).

In as much as tumor resection has a survival benefit, the extent of resection must be weighed against the possible complications of this approach and as such mortality and complication rates following surgery are a measure of its toxicity as a therapeutic approach (Moiyadi & Shetty, 2012). In HICs, the use of neuronavigation, intra-operative MRI and ultrasound scans, stimulation mapping techniques and fluorescence-guided surgery help to minimize surgical morbidity (Hardesty & Sanai, 2012). As such, even in series involving tumors in eloquent areas, total resection can be achieved in 74% of patients with no post-operative complications to report (Krieg et al., 2013). In studies from LAMICs, tumor resection has an overall morbidity of 38% and mortality of 3.6% (Moiyadi & Shetty, 2012). This may be attributed to a lack of technological adjuncts.

Surgical complications may be classified into regional or systemic. Regional complications include wound related complications (infection, dehiscence, CSF leak, hematoma formation), new/worsening seizures, peritumoral edema and wrong site surgery (Graus et al., 2013; Moiyadi & Shetty, 2012; Wong et al., 2012). Systemic complications include coagulopathy, hemodynamic instability, metabolic complications and medical complications such as pneumonia, renal dysfunction and cardiac arrhythmia

(Moiyadi & Shetty, 2012; Wong et al., 2012). Overall, regional complications have a reported prevalence of 14-82% compared to 7-10.7% for systemic complications (Chang et al., 2003; Ciric et al., 1987; Moiyadi & Shetty, 2012). However, the definitions for these categories differ from study to study and as such the variation in complication rates is not entirely surprising.

A recent systematic review reported on the frequency of adverse events following surgery for intracranial neoplasms (Wong et al., 2012). Majority of the studies were from HICs. Among patients with gliomas, venous thromboembolism had variable rates of 3-26% while new/worse neurological deficit occurred in 7-20% of patients and post-operative peri-tumoral edema in 7.7-9.5% of patients. Other complications were reported for brain tumors in general without specification for glial tumors or astrocytomas in particular. The risk for post-operative seizures varied from 1-12% and was higher in the first 48 hours following surgery. Surgical site infection (SSI) varied from 0.5-4% while post-operative hematoma had rates ranging from 1.1-4.4%. CSF leakage had a complication rate of 1-24%. Medical complications had rates of 6-7%. These rates are comparable to a study from India (Moiyadi & Shetty, 2012). Nonetheless, direct comparison would be inappropriate because ascertainment methods for each complication differ across studies. In addition, these studies examined a heterogeneous group of tumor entities.

A number of studies have attempted to determine the factors associated with post-operative complications following surgery for brain tumors and astrocytomas in particular. In a recent Spanish study, the influence of age, sex, neurological status, tumor location, type of resection and time from disease onset to surgery were assessed (Graus et al., 2013). However, only age was found to be a predictor of complications with older age associated with higher complication rates. In another study, age less than 18 years

was associated with higher morbidity, while pre-operative altered sensorium and emergency surgery were associated with higher mortality. Pre-operative neurological abnormality was associated with higher systemic complications (Moiyadi & Shetty, 2012). Overall, a majority of the literature on the pattern and outcome of astrocytoma is centered on HICs. With the exception of the Indian sub-continent, there is a paucity of information from other LAMICs.

CHAPTER THREE: METHODOLOGY

3.1 Study Design

This was a prospective, descriptive census study of consecutive patients with a confirmed histological diagnosis of astrocytoma seen over a one-year period (August 2015-July 2016). The study involved follow-up of individual patients for a period of 12 weeks after surgical treatment. The choice of follow-up period was determined based on two premises. The first was the need to have sufficient time elapse after surgery for the investigator to attribute outcome to surgery. The second premise was that 12 weeks was clinically sufficient to have a meaningful and measurable change in patient status attributable to surgical treatment.

3.2 Study Site

The study was carried out in three tertiary hospitals in Eldoret town, the headquarters for Uasin Gishu County. Moi Teaching and Referral Hospital (MTRH) is the second national hospital in Kenya while Mediheal and St.Luke's hospitals are private facilities within Eldoret town. All three hospitals serve western Kenya as well as parts of eastern Uganda and South Sudan and they all have access to a histopathology laboratory as well as MRI facilities. Although there are other hospitals within Eldoret town, it is only these three hospitals that had the capacity to manage astrocytomas at the time of the study.

3.3 Study Population

The study population consisted of all patients seen at MTRH, Mediheal and St. Luke's hospitals with a confirmed histological diagnosis of astrocytoma. Given the descriptive nature of the study and the relatively small number of patients seen per year with astrocytoma in the three hospitals, it was possible to study all patients seen within the

study period and therefore determination of a sample size was not necessary. Nonetheless, a target of 30 or more patients was sought.

3.4 Eligibility Criteria

3.4.1 Inclusion criteria

All patients with a histological diagnosis of astrocytoma and who gave informed consent/assent were recruited into the study.

3.4.2 Exclusion criteria

Patients with recurrent tumors after treatment were excluded. This criterion was based on two premises. The first was that lower grade astrocytomas may sometimes undergo malignant transformation into higher grade tumors. The second was that tumor recurrence portends worse surgical and functional outcomes and therefore inclusion into this study would confound the observed results.

3.5 Study Procedure and Data Management

Eligible participants for this study were identified in the neurosurgical units (neurosurgical clinics and wards) in the three study hospitals. Patients with clinical and MRI features consistent with any form of astrocytoma were considered for inclusion and informed consent/assent obtained after explanation of the nature and aims of the study. For patients who were neurologically impaired and unable to give consent/assent, the same was sought from their legal guardians/caretakers. The procedure followed for recruiting eligible patients is shown in figure 1. After obtaining informed consent/assent a data abstraction form (Appendix I) was used to collect the data necessary for achieving

study objectives. All data were subject to double entry and access was restricted to the investigator and his supervisors.

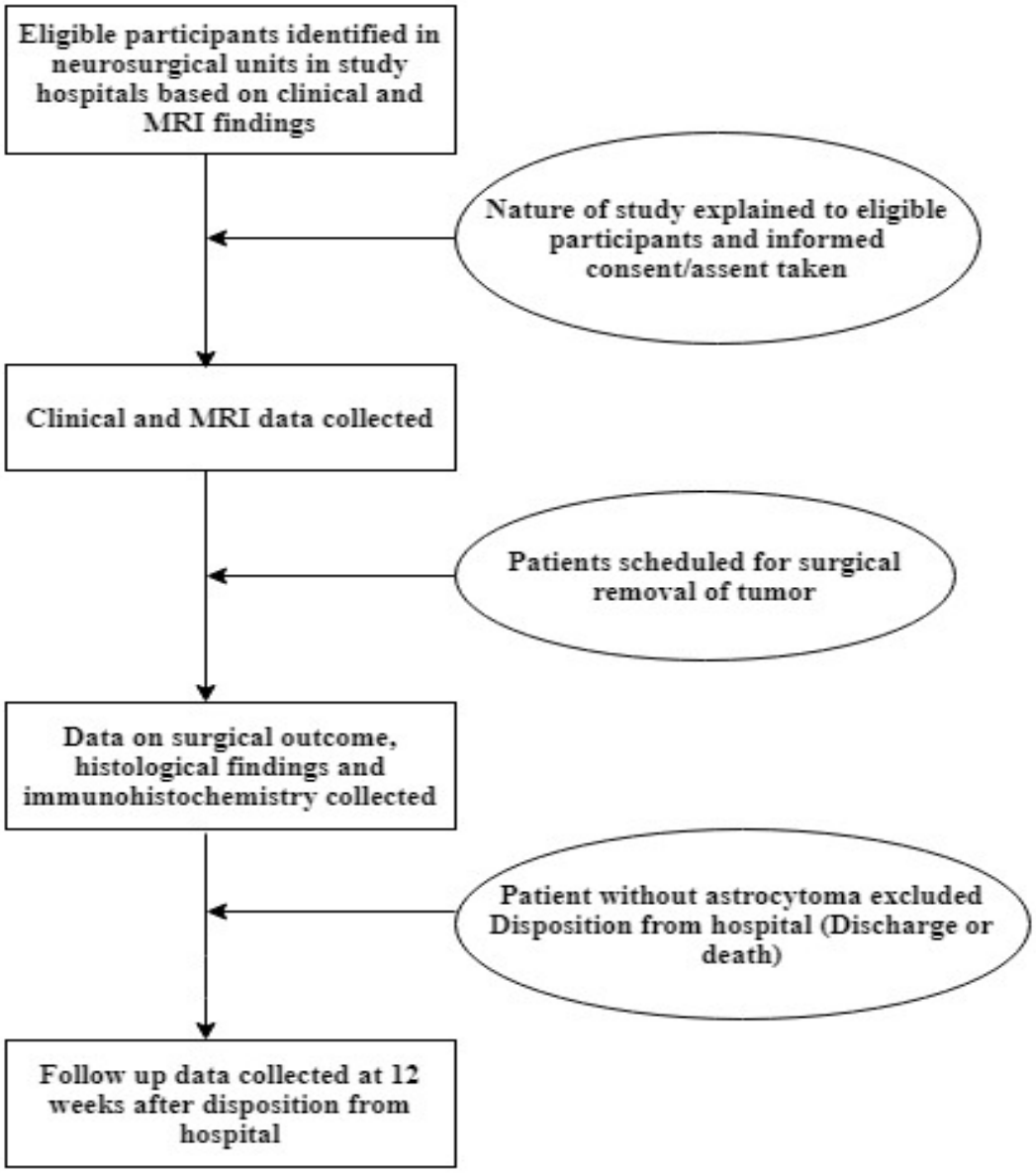

Figure 1. Flow diagram of study recruitment procedure

In order to achieve the first study objective, data on demographic, clinical and MRI characteristics were collected. Demographic characteristics included age and sex and a

note on their referring facility. Clinical characteristics included data on presenting symptoms, duration of illness and functional status. The Karnofsky performance status score (KPS) was used to assess functional outcome (Appendix II). It was developed for use in assessing the functional status of cancer patients and as a tool for monitoring response to therapy. It measures a patient's ability to carry out activities of daily living and their degree of dependence on nursing care. It ranges from 0-100 with scores less than 80 termed dependent (Ooi & Mazlina, 2013). Each patient had an MRI scan which was used to assess tumor size, location and various other macroscopic features. These were assessed by the investigator with the help of a radiologist. Axial, coronal and sagittal T1 and T2-weighted images with contrast enhancement were obtained. As previously described (Pierallini et al., 1997), edema, mass effect, contrast enhancement, borders, signal homogeneity, necrosis, hemorrhage and flow void were assessed by assigning a score ranging from 1 to 3 depending on whether they were mildly, moderately or strongly present (Appendix III). A total score (ranging from 8 to 21) was then calculated for each patient and recorded in the data abstraction form. Higher scores were indicative of higher-grade tumors while lower scores were indicative of lower grade tumors.

In order to achieve the second study objective, data on the histological subtypes, grading and proliferative indices of astrocytomas were recorded. Following surgery, brain tumor specimens were preserved in formalin solution and embedded in paraffin wax blocks using an automated tissue processor. Although tissues were processed in different laboratories, over two thirds of specimens were reviewed by one reference pathologist with agreement between primary and reference pathologists. For hematoxylin and eosin (H&E) staining, the blocks were sectioned using a microtome, deparaffinized and stained. Each H&E slide was assessed for cellularity, presence of mitoses, endothelial

thickening and necrosis. The diagnostic criteria for each histological subtype of astrocytoma as well as the system utilized for grading are depicted in table 1.

Table 1. Diagnostic criteria for histological subtypes and grading of astrocytomas

Histological subtype	Histopathological features (WHO[a] Grade)
Pilocytic astrocytoma	Compact, densely fibrillated areas consisting of bipolar or multipolar cells with round to oval nuclei (Fibrillary pattern) and microcyst development. No anaplasia or mitotic activity (WHO Grade I)
Diffuse astrocytoma	Fibrillary pattern of cells with moderately increased cellularity, enlarged and hyperchromatic nuclei. No anaplasia or mitotic activity (WHO Grade II)
Anaplastic astrocytoma	Fibrillary pattern with markedly increased cellularity, presence of mitotic activity, nuclear pleomorphism and atypia but no necrosis or endothelial proliferation (WHO Grade III)
Glioblastoma multiforme	Similar to anaplastic astrocytoma but with necrosis and endothelial proliferation (WHO Grade IV)

[a]World Health Organization

The proliferative activity of tumor specimens was determined using the Ki-67 proliferative index after staining for the Ki-67 antigen. For immunostaining, 3-micrometer (μm) thick sections placed on adherent glass slides were deparaffinized and microwaved for antigen retrieval. The Envision FLEX system (K8000; Dako Denmark A/S) was used for immunostaining. After retrieval, peroxidase blocking was done (100μL of reagent for up to 5 minutes) followed by primary antibody staining (100μL of reagent for up to 20 minutes). Thereafter, horse-radish peroxidase (HRP) was applied as the labeled polymer (100μL of reagent for up to 20 minutes) followed by substrate working solution (200μL of DAB+ solution for up to 10 minutes). The process was completed by hematoxylin counter staining for 5 minutes. After each stage, washing was done using phosphate buffer solution. The Ki-67 proliferative index was determined as

the number of immunoreactive nuclei per 1000 tumor cells visualized under high power (X40) on the microscope. Photographs of H&E as well as immunohistochemistry slides were taken at low (X10) and high power (X40) using a Samsung Galaxy smartphone (Samsung, S.Korea) and processed on the Photos application available on the Windows 10 operating system (Microsoft Corp., Seattle,WA).

In order to achieve the third study objective, data on intra-operative characteristics such as duration of surgery and extent of surgical resection and post-operative outcome such as functional status, regional and systemic complications, length of hospital stay and peri-operative mortality were recorded. The overall presence or absence of both regional and systemic complications were assessed and recorded at the time of discharge by reviewing patient case notes for indicators that any of the complications occurred. Regional complications assessed included operative site hematoma; worsening/new onset seizures, wrong site surgery as well as wound related complications i.e. wound dehiscence, CSF leak and surgical site infection. Systemic complications such as venous thromboembolism, pneumonia and renal failure were also assessed. Data on the choice of therapy i.e. surgery, radiotherapy or chemotherapy alone or in combination were also recorded.

In order to achieve the fourth study objective, data on functional status after surgical therapy were collected. For all patients, functional status was assessed by the investigator at discharge from hospital, and at 12 weeks after discharge from hospital. Patients were asked to return for re-evaluation. In the event that they were unable to return, they were contacted by telephone. Functional status at these time points was assessed using the KPS score.

3.6 Data Analysis

Data on patient demographics, clinical presentation and pathological characteristics (histological subtypes and proliferative indices) of the tumors as well as frequency of complications (including mortality) were stratified according to tumor grade and summarized using descriptive statistics on SPSS Statistics software v23 (IBM, 2015). Owing to the small sample size, continuous variables were summarized as medians with interquartile ranges (IQR) while categorical variables were summarized as proportions. For comparison of continuous variables, the Kruskal Wallis test was used while Fishers exact test was utilized for categorical variables. Where individual cells contained numbers less than 3, data variables were combined to facilitate comparison. A p-value of <0.05 was considered significant. Results are displayed on tables and figures.

3.7 Ethical Consideration and Funding

Approval to conduct the study was sought and obtained from the Moi University/MTRH Institutional Research and Ethics Committee (IREC) (Appendix IV). Informed consent/assent was sought from eligible patients and confidentiality assured (Appendix V). There was no coercion or inducement and all patients were made to understand that they were free to withdraw from the study without compromising the quality of their care. Permission to access patient records was sought from the administration in all three hospitals (MTRH, St. Luke's hospital and Mediheal hospital) (Appendix VI). This study was supported by a science, technology and innovation grant awarded to the investigator by the National Commission for Science, Technology and Innovation (NACOSTI) (Appendix VII).

CHAPTER FOUR: RESULTS

4.1 Sample Description

A total of 121 patients with brain tumors were seen during the study period. Of these, 31 (25%) had histologically confirmed astrocytoma and were recruited into the study. Overall, their ages ranged from 4 to 73 years with a median of 51 years (IQR 27-59 years). Majority of patients (36%) were between the ages of 50 and 59 years. Only seven patients (22.6%) were above 60 years of age while only three patients (9.7) were below the age of 20 years. The age distribution for the sample of patients included in this study is depicted in figure 1.

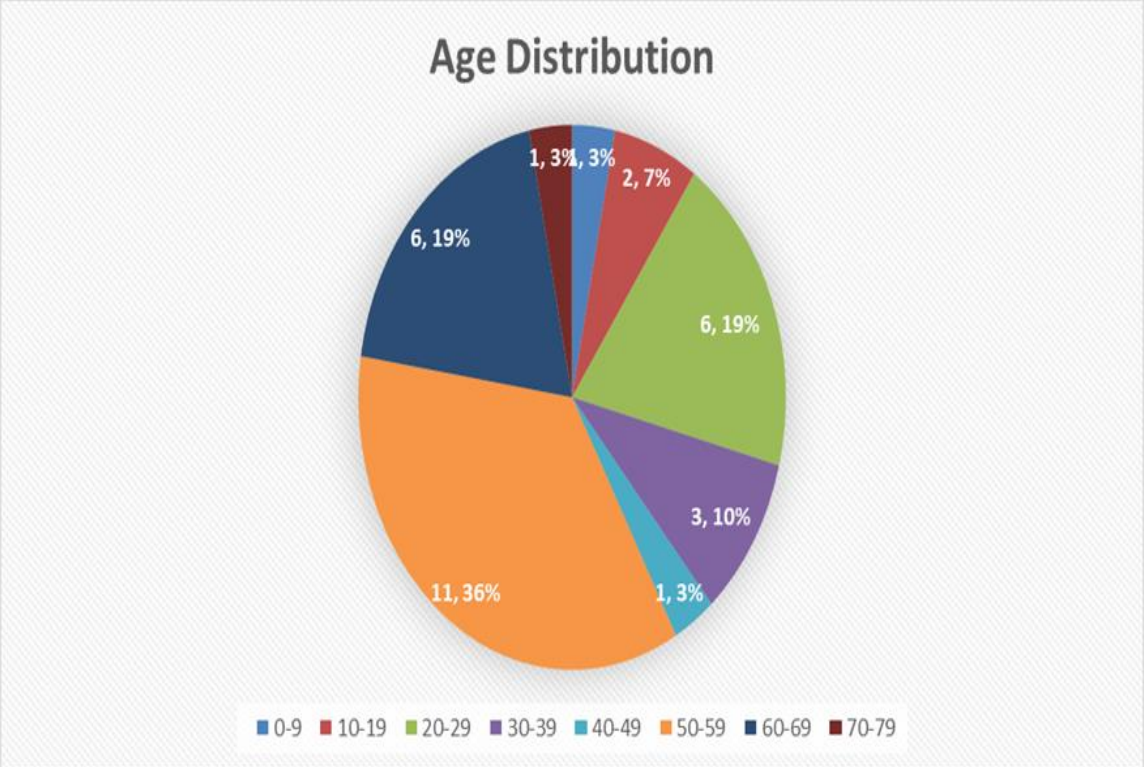

Figure 2. Pie chart of age distribution (N=31)

There were 16 female patients (51.6%) and 15 male patients (48.4%). Majority of these patients (64.5%) were referred from Level IV facilities from across western Kenya. Table 1 gives a description of the study sample stratified by tumor grade.

Table 2. Sample description

Patient characteristic	**LGA (N=9)**	**HGA (N=22)**	**Total (N=31)**	**P-value**
Median age (IQR[a])	22(18-27)	54 (45-60)	51 (27-59)	0.007
Sex				
Female	3 (33.3%)	13 (59.1%)	16 (51.6%)	0.252
Male	6 (66.7%)	9 (40.9%)	15 (48.4%)	
Referral facility				
Level II	0	1 (4.5%)	1 (3.2%)	0.657
Level III	1 (11.1%)	1 (4.5%)	2 (6.5%)	
Level IV	6 (66.7%)	14 (63.6%)	20 (64.5%)	
Level V	2 (22.2%)	6 (27.3%)	8 (25.8%)	

[a] Interquartile Range

4.2 Clinical and MRI features of astrocytomas at presentation

Overall, the median duration of illness was 60 days (IQR 21-180 days) while median time from diagnosis to surgery was 10 days (IQR 3-14 days). Headache (83.9%) and focal neurological deficits (64.5%) were the commonest presenting features. Other features included vomiting (32%), seizures (22.6%) and personality changes (29%). The median KPS score at presentation was 50 with only one patient (3.2%) having a non-dependent performance status. Majority of tumors involved the cerebrum (90.3%). The median tumor volume was 100 Cm^3 (IQR 45.28-134.64 Cm^3). The overall MRI score ranged from 8 to 19 with a median score of 16. Table 2 summarizes these features stratified by tumor grade.

Table 3. Clinical and MRI features of astrocytomas at presentation

Clinical features at presentation	**LGA (N=9)**	**HGA (N=22)**	**Total (N=31)**	**P-value**
Median duration of illness in days (IQR[a])	90 (30-210)	45 (21-120)	60 (21-180)	0.433
Median time from diagnosis to surgery (IQR)	11 (7-15)	9(3-14)	10 (3-14)	0.396
Presenting features				
Headache	6 (66.7%)	20 (90.9%)	26 (83.9%)	N/A
Vomiting	1 (11.1%)	9 (40.9%)	10 (32.3%)	
Seizures	4 (44.4%)	3 (13.6%)	7 (22.6%)	
Personality changes	0	9 (40.9%)	9 (29%)	
Focal neurological deficits	6 (66.7%)	14 (63.6%)	20 (64.5%)	
Median GCS[b] (IQR)	14 (11-15)	15 (12-15)	15(12-15)	0.571
Median KPS score (IQR)	40 (40-60)	50 (40-50)	50(40-50)	0.663
MRI[c] features at presentation				
Tumor location				
Supratentorial	6 (66.7%)	22 (100%)	28 (90.3%)	N/A
Infratentorial	3 (33.3%)	0	3 (9.7%)	
Median tumor volume in Cm^3 (IQR)	18.86 (10.7-120)	104.34 (81-150)	100 (45.28-134.64)	0.022
Median MRI score (IQR)	9 (8-10)	17 (16-18)	16(11-18)	0.0001

[a]Interquartile Range [b]Glasgow Coma Scale [c]Magnetic Resonance Imaging

4.3 Histological subtypes and proliferative index

Glioblastoma multiforme was the commonest histological subtype (71%) followed by diffuse astrocytoma (22.6%). Pilocytic astrocytoma occurred in only 6.5% of patients while there were no subjects with anaplastic astrocytoma. Out of a total of 31 included patients, 11 did not have Ki-67 immunostaining done owing either to poor tissue processing or preservation. For the remaining 20 patients, the Ki-67 proliferative index ranged from 2-10% for pilocytic astrocytoma, 1-8% for diffuse astrocytoma and 24-95% for glioblastoma multiforme. Table 3 gives a summary of the pathologic characteristics stratified by tumor grade. The histopathological features and immunohistochemical findings for pilocytic astrocytoma are depicted in figure 1 while those for diffuse astrocytoma and glioblastoma multiforme are shown in figures 2 and 3 respectively.

Table 4. Histological subtypes and proliferative index of astrocytomas

	LGA (N=9)	**HGA (N=22)**	**Total (N=31)**
Histological subtype and grade			
Pilocytic astrocytoma (Grade I)	2 (22.2%)	0	2 (6.5%)
Diffuse astrocytoma (Grade II)	7 (77.8%)	0	7 (22.6%)
Glioblastoma multiforme (Grade IV)	0	22 (100%)	22 (71%)
Median Ki-67 Proliferative Index (Range)[a]			
Pilocytic astrocytoma	6% (2-10%)	N/A	6% (2-10%)
Diffuse astrocytoma	1.6% (1-8%)	N/A	1.6% (1-8%)
Glioblastoma multiforme	N/A	60% (24-95%)	60% (24-95%)

[a]Based on 20 patients for whom Ki67 immunostaining was available

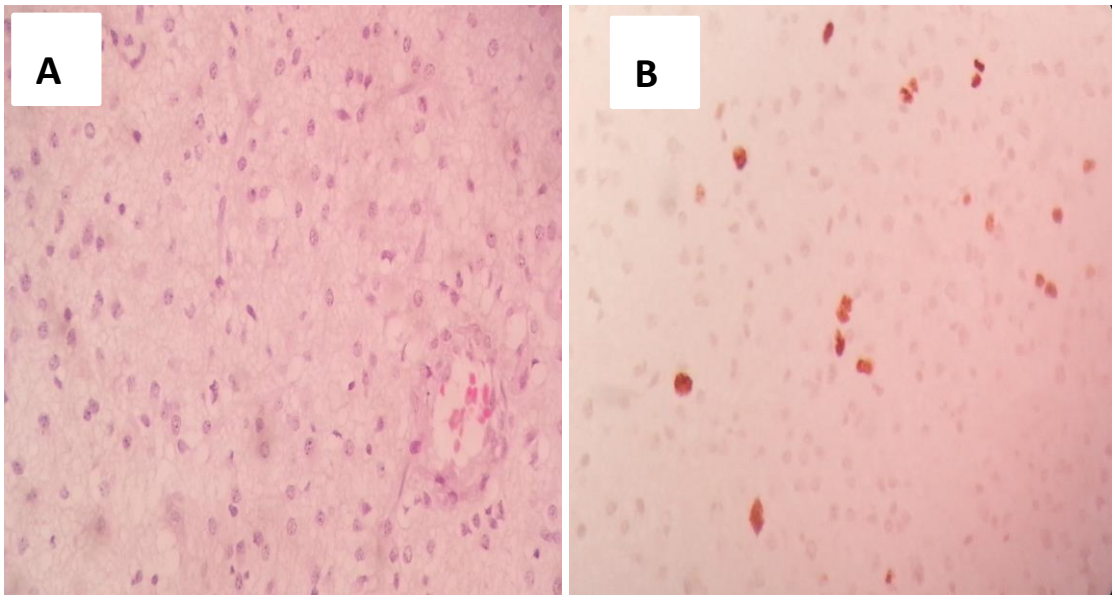

Figure 3: Photomicrographs of pilocytic (Grade I) astrocytoma. (A) H&E stain (X40) showing mild cellularity, fibrillar background and microcysts. (B) Ki-67 immunostain (X40) showing few immuno-labelled nuclei

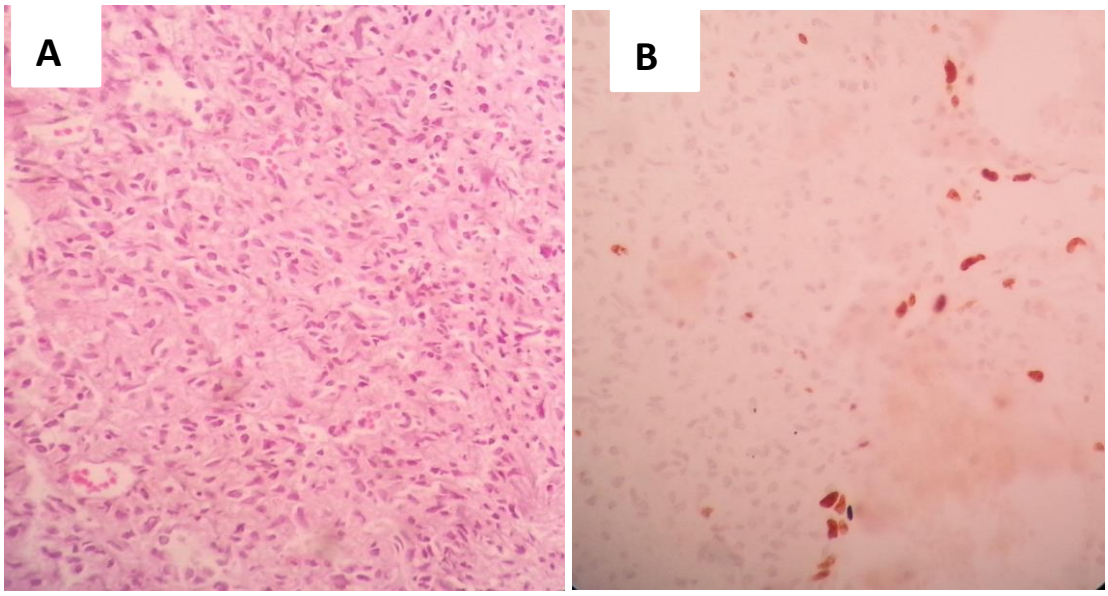

Figure 4: Photomicrographs of diffuse (Grade II) astrocytoma. (A) H&E staining (X40) showing moderate cellularity and fibrillar background. (B) Ki-67 immunostain (X40) showing few immuno-labelled nuclei

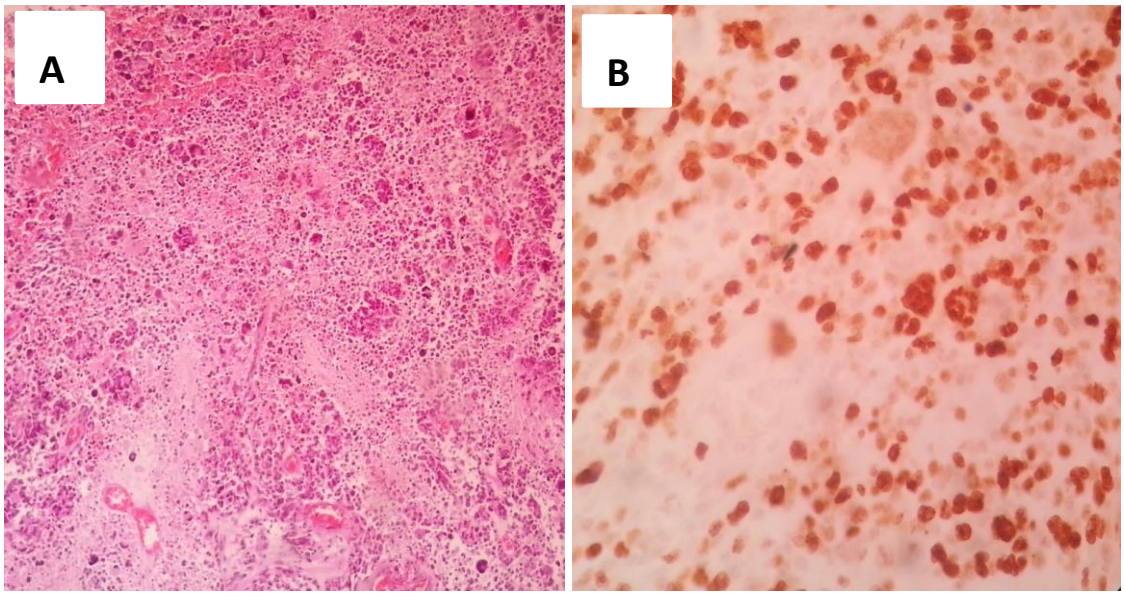

Figure 5: Photomicrographs of glioblastoma multiforme (Grade IV astrocytoma). (A) H&E stain (X40) showing high cellularity, mitoses and zonal necrosis. (B) Ki-67 immunostain (X40) showing numerous immuno-labelled nuclei

4.4 Surgical Outcome

All patients underwent surgery. Gross total tumor resection was achieved in 21 (67.7%) patients while 9 (21%) had subtotal resection. One patient underwent biopsy only. Among patients with high grade tumors, 36.4% were given chemotherapy and 22.7% had radiotherapy by 12 weeks of follow-up. Overall, the median length of hospital stay was 12 days (IQR 7-21 days). Systemic surgical complications occurred in 2 patients (6.5%) while 12 (38.7%) had regional surgical complications. Of the 12 patients who developed regional surgical complications, one (8.3%) had CSF leak, two (16.7%) had wound dehiscence, one (8.3%) had new onset/worse seizure activity while five (41.7%) had wound hematoma. Figure 1 depicts the distribution of regional surgical complications while table 4 summarizes surgical outcome stratified by tumor grade.

Table 5. Surgical outcome of astrocytomas

Outcome measure	LGA (N=9)	HGA (N=22)	Total (N=31)	P-value
Median length of hospital stay (IQR[a])	20 (7-23)	12 (9-21)	12 (7-21)	0.828
Systemic complications	0	2 (9.1%)	2 (6.5%)	N/A
Regional complications	2 (22.2%)	10 (45.5%)	12 (38.7%)	N/A

[a]Interquartile Range

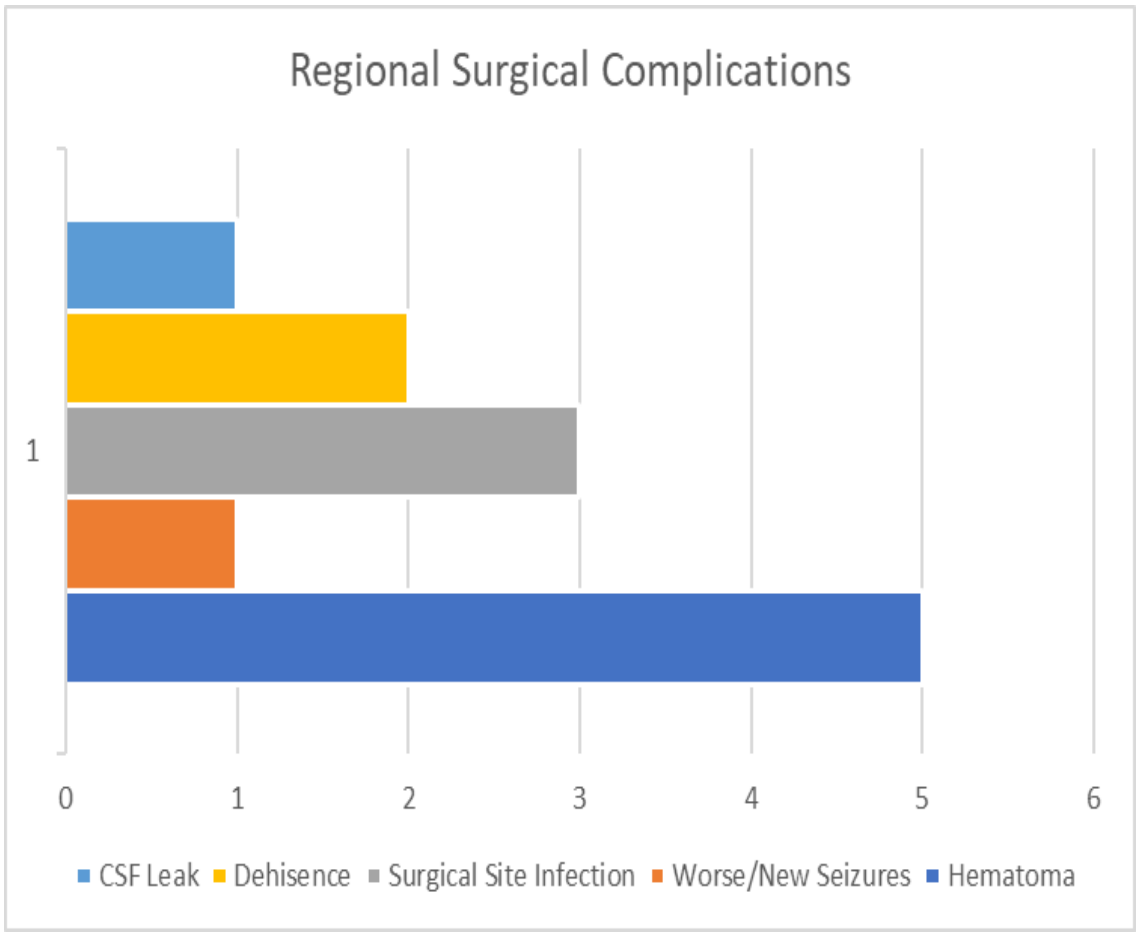

Figure 6. Bar graph depicting regional surgical complications (N=12)

4.5 Functional outcome

The median KPS score at discharge was 50 with 3 patients (9.7%) having non-dependent performance status (KPS >80). At 12 weeks follow up the median KPS score had improved to 60 with 3 patients (9.7%) having non-dependent performance status. In patient mortality was 19.4% (6 patients) and by 12 weeks of follow-up mortality stood at 25.8% (8 patients). Table 5 depicts functional outcome stratified by tumor grade.

Table 6. Functional outcome of astrocytoma

Outcome measure	**LGA (N=9)**	**HGA (N=22)**	**Total (N=31)**	**P-value**
Median KPS[a] score at discharge (IQR[b])	60 (50-60)	50 (50-60)	50 (50-60)	0.408
Median KPS score at 12 weeks (IQR)	60 (0-60)	55 (50-60)	60 (0-60)	0.896

[a]Karnofsky Performance Status [b]Interquartile Range

CHAPTER FIVE: DISCUSSION

5.1 Sample description

Within the local (and regional) context, this study is among the first attempts at characterizing astroctyomas and their outcomes. The proportion of brain tumor patients with astrocytomas (25%) is slightly lower than that seen in other studies from Africa (Mwang'ombe & Ombachi, 2000; Wanyoike, 2004; Zalata et al., 2011). Overall, the median age in this study was 51 years. However, when stratified by tumor grade, the median age for LGAs was 22 years and that for HGAs was 54 years. This difference was statistically significant and only differs slightly with other studies in which LGAs are predominantly tumors of childhood and young adulthood while HGAs occur in a much older age group (Ostrom et al., 2013). While this is biologically plausible, the small sample size and the fact that this was a hospital-based study may explain these differences. HGAs occurred in more males than females. This is in keeping with other studies with similar findings although the explanation for this observation has not as yet been elucidated (Ahmadloo et al., 2013; Graus et al., 2013; Jakola et al., 2011). Majority of patients were referred from level IV and V facilities from the Western Kenya region. Since the introduction of a devolved system of health governance in Kenya, many level IV and V facilities have been equipped with CT scan machines and this may account for this observation.

5.2 Clinical and MRI Features

The median duration of illness in this study (60 days) was longer compared to studies from HICs but shorter compared to similar studies from LAMICs (Idowu & Apemiye, 2009). Since most patients presented with a history of headache or focal neurological

deficits, it is possible that the wide differential diagnosis for these features delayed a definitive diagnosis and extended the duration of illness. Lack of financial resources and seeking help from traditional healers may also explain the delayed presentation for specialized therapy (Idowu & Apemiye, 2009). When stratified for tumor grade, LGAs had a longer duration of illness at presentation compared to HGAs although this difference was not statistically significant. LGAs have an indolent onset and are more likely to present with seizures (DeAngelis, 2001). In our setup, this scenario is likely to be treated as epilepsy before its refractory nature prompts neuroimaging and identification of a focal lesion.

The clinical presentation of intracranial tumors can vary depending on tumor size and location (Young et al., 2015). Headache and focal neurological deficits were the commonest presenting features of astrocytomas in this study. This observation stood despite stratification by tumor grade. However, seizures are noted to be more prevalent in patients with LGAs compared to those with HGAs (DeAngelis, 2001). Functional status as assessed by the KPS score is also a key presenting feature of astrocytomas. In this study, the mean KPS score was 50 for HGAs and 40 for LGAs. Only one patient had non-dependent functional status (KPS score >80) at presentation. This may be attributed to the longer duration of illness such that by the time a definitive diagnosis is arrived at, a patient's functional status would have deteriorated as a result of disease progression. Further, particularly for high grade tumors, brain edema due to tumor may explain the poor functional status and neurological deficits found at presentation (Young et al., 2015).

All patients had an MRI scan done prior to surgery. A number of features were assessed so as to help in arriving at a radiological diagnosis and in planning for treatment. Each

feature assessed was scored and an overall score given. Lower overall scores suggest LGAs while higher ones suggest HGAs (Pierallini et al., 1997). The median score for LGAs in this study was 9 while that for HGAs was 17. This difference was statistically significant and is in keeping with what was suggested by Pierallini and colleagues (Pierallini et al., 1997). Although the MRI scores in this study were arrived at using weaker machines than those used in Pierallini's study (0.3T and 0.5T vs. 0.5T and 1.5T), the overall result is similar. Neuro-imaging is of particular importance when assessing astrocytic tumors. MRI has been shown to correlate well with histological grade of astrocytoma and it can therefore be used to assess macroscopic features that cannot be assessed by histology alone e.g. effect of tumor on surrounding tissues (Ishita Pant et al., 2015; Pierallini et al., 1997). Further, sampling error during biopsy/tumor resection may lead to underestimation of tumor grade. MRI features suggestive of higher-grade tumor may help in arriving at a more accurate grading and help in predicting clinical outcome.

5.3 Histological subtypes and proliferative activity of astrocytomas

A histological diagnosis was arrived at in each of the recruited patients. Of the 31 patients recruited into this study, GBM and diffuse astrocytoma were the commonest histological subtypes. This is in keeping with both hospital and population based brain tumor registries in which GBM is among the commonest brain tumors (CBTRUS, 2012; DeAngelis, 2001; Fisher et al., 2007; Ohgaki & Kleihues, 2005). The occurrence of the other subtypes i.e. PXA and SEGA is uncommon (Ostrom et al., 2013). Anaplastic astrocytoma (AA) also occurs quite commonly but was not encountered in this study. However, since both AA and GBM exhibit features of poorly differentiated tumor cells, it may be difficult to tell them apart. Further, AA is likely to progress into the more malignant GBM (Sarkar et al., 2009).

In this study, Ki-67 immunostaining was performed in order to determine the proliferative activity of astrocytomas. Higher proliferative indices were noted for pilocytic astrocytoma (6%) and glioblasoma multiforme (60%) compared to similar studies (Ambroise et al. (2011); Hsu et al., 1997; Shivaprasad et al., 2016). Such variation may be attributed to differences in fixatives used, antigen retrieval and interpretation methods. However, higher proliferative indices in pilocytic astrocytomas have been noted in other studies and attributed to glial proliferation although this observation has no impact on long-term prognosis (Ambroise et al., 2011). Nonetheless, a high proliferative index for LGA warrants close follow-up and neuroimaging to detect recurrence and malignant transformation early. Tumors with similar histology may have genetic differences that are reflected in variant proliferative indices (Thotakura et al., 2014). For HGA, higher proliferation demonstrates more aggressive tumor and it is possible that the higher proliferative indices observed for glioblastoma in this study point to underlying genetic differences that confer worse prognosis in this region. All in all, these findings should be interpreted with caution since this was a small hospital-based study.

5.4 Surgical and functional outcome of astrocytomas

The Standard therapy for astrocytic tumors involves surgery. For LGAs, this is adequate therapy and has been shown to have favorable 5-year survival rates (Ostrom et al., 2013). Owing to the possibility of recurrence or transformation into a more malignant variant, patients with diffuse astrocytoma require regular follow-up with MRI so as to pick recurrence early enough (Sarkar et al., 2009). After surgery, patients with HGA require a combination of chemotherapy and radiotherapy. These have been shown to prolong survival. In this study, all patients underwent surgery and the majority had gross total

tumor resection. However, of the 22 patients with HGA who required chemotherapy only 36.4% got it and only 22.7% got radiotherapy within the study follow up period. Reasons for this are varied. First, access to radiotherapy was a problem since at the time of the study, the nearest radiotherapy machine was in Nairobi (300 Kilometers from Eldoret). Secondly, it is possible that for a majority of patients, the long turnaround time for histology results delayed the starting of chemo-radiation.

In this series, 6.5% of patients experienced systemic complications and 38.7% experienced regional complications. These figures are higher than those reported in similar studies (Graus et al., 2013; Jakola et al., 2011) and poor functional status at presentation as well as lack of neuro-navigational equipment may explain these findings. However, even in some areas without such equipment, lower complication rates have been reported (Moiyadi & Shetty, 2012) and other factors may be at play. Majority of the patients with these complications had HGA. However, it is not possible to determine the role of tumor grade in these findings owing to the small sample studied. Tumor size in this study was larger compared to studies in the west (Jakola et al., 2011). This complicates surgery and predisposes to surgical complications. Overall, in-hospital mortality was 19.4% (N=6) and this increased to 25.8% (N=8) at 12 weeks of follow up. Similarly, these figures are higher than those reported in other studies. Further, it is not possible to study the effects of tumor grade on these outcomes owing to the small sample size obtained.

Functional status is as important a presenting feature as it is an outcome (Young et al., 2015). There was no difference in overall KPS score at presentation and at discharge. However, overall functional status had improved by 12 weeks of follow-up. Nonetheless, majority of patients were still dependent with only 9.7% of patients having a score of 80

and above. This observation may be attributed to a number of reasons. First, majority of patients had had their illness for quite some time and therefore it is unlikely that a dramatic change in condition would be observed within the time frame available for the study. Indeed, some authors recommend surgery for patients with favorable scores only (Chaichana et al., 2011; Young et al., 2015), which was not the case in this study. Secondly, majority of the patients had HGA which is an aggressive and infiltrating tumor with generally poor short and long term outcomes (Marina et al., 2011; Moiyadi & Shetty, 2012).

5.6 Study limitations

As a limitation, while this study highlights the pattern of astrocytomas in Eldoret and gives baseline data on their outcomes it did not assess the long-term outcome of astrocytomas and therefore the impact of surgical therapy on survival could not be assessed. Further, owing to the small sample size, it was not possible to determine the impact of the various clinical, MRI and pathological features on outcome.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Among patients seen with astrocytomas in Eldoret, headache, focal neurological deficits and reduced functional status are the commonest presenting features while Glioblastoma multiforme is the commonest histological subtype. MRI can be used to differentiate low grade astrocytomas from high grade astrocytomas. The tumors encountered are highly proliferative and in the short-term, both surgical and functional outcome remain suboptimal.

6.2 Recommendations

Based on the observations of this study, the following are recommended:

1. Clinicians in lower level healthcare facilities should be encouraged to refer patients with persistent neurological symptoms for timely work-up and diagnosis.
2. Owing to the highly proliferative nature of astrocytomas in Eldoret, closer follow up of patients after treatment is warranted in order to detect tumor recurrence earlier.
3. Further research is needed in order to:
    a. Determine the underlying mechanisms for the high proliferative activity of astrocytomas seen in Eldoret.
    b. Determine the reasons for the suboptimal surgical and functional outcome observed in patients with astrocytomas in Eldoret.

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APPENDICES

Appendix I: Data Collection Form

Serial No._____

PATIENT CHARACTERISTICS

1. Age (in years) _____
2. Sex
   a) Male ☐
   b) Female ☐
3. Referral facility
   a) Level I ☐
   b) Level II ☐
   c) Level III ☐
   d) Level IV ☐
   e) Level V ☐

CLINICO-PATHOLOGIC CHARACTERISTICS

4. Duration of illness_____
5. Presenting symptoms and signs
   a. Headache
      i. Yes ☐
      ii. No ☐
   b. Vomiting
      i. Yes ☐
      ii. No ☐
   c. Seizures
      i. Yes ☐
      ii. No ☐
   d. Personality changes
      i. Yes ☐
      ii. No ☐
   e. Focal neurological deficits
      i. Yes ☐
      ii. No ☐
6. Glasgow coma scale score_____
7. Functional status (KPS score) _____
8. Tumor location
   a. Cerebrum ☐
   b. Brainstem ☐
   c. Cerebellum ☐
9. Tumor size (Cm^3) _____
10. MRI score_____
11. Histopathological subtype (Grade)
    a. Pilocytic astrocytoma (Grade I) ☐
    b. Pleomorphic xanthoastrocytoma (Grade I) ☐
    c. SEGA (Grade I) ☐
    d. Diffuse astrocytoma (Grade II) ☐
    e. Anaplastic astrocytoma (Grade III) ☐
    f. Glioblastoma multiforme (Grade IV) ☐
12. Immunohistochemistry staining
    a. Ki-67 Proliferative index_____

MANAGEMENT

13. Surgery
    a. Yes ☐
    b. No ☐
14. If yes
    a. Duration of surgery_____
    b. Extent of resection _____
15. Radiotherapy
    a. Yes ☐
    b. No ☐
16. Chemotherapy
    a. Yes ☐
    b. No ☐

OUTCOME

17. Length of hospital stay (in days) _____
18. Systemic surgical complications
    a. Venous thromboembolism ☐
    b. Pneumonia ☐
    c. Renal failure ☐
19. Regional surgical complications
    a. New/worse neurological deficit ☐
    b. Worse/new seizures ☐
    c. Operative site hemorrhage ☐
    d. Wound dehiscence ☐
    e. Surgical site infection ☐
    f. CSF leak ☐
    g. Wrong site surgery ☐
20. Functional status at discharge (KPS score) ___
21. Functional status at 12 weeks (KPS score) ___

Appendix II: Karnofsky Performance Status Scale

Able to carry on normal activity and to work; no special care needed	100	Normal no complaints; no evidence of disease
	90	Able to carry on normal activity; minor signs or symptoms of disease
	80	Normal activity with effort; some signs and symptoms of disease
Unable to work; able to live at home and care for most personal needs; varying amounts of assistance needed	70	Cares for self; unable to carry on normal activity or to do active work
	60	Requires occasional assistance but is able to care for most of his/her personal needs
	50	Requires considerable assistance and frequent medical care
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly	40	Disabled; requires special care and assistance
	30	Severely disabled; hospital admission indicated although death not imminent
	20	Very sick; hospital admission necessary; active support treatment necessary
	10	Moribund; fatal processes progressing rapidly
	0	Dead

Appendix III: MRI score form

Tumor characteristic	Score		
	1	2	3
Edema			
Mass effect			
Contrast enhancement			
Borders			
Signal homogeneity			
Necrosis			
Hemorrhage			
Flow void			
Total score			

Appendix IV: Informed Consent Forms

ENGLISH CONSENT FORM:

My name is Clifford Chacha Mwita. I am a qualified doctor, registered by the Kenya Medical Practitioners and Dentists Board (Registration number A7400). I am currently pursuing a Master's degree in General Surgery at Moi University. I would like to recruit you into my research study on the clinical pattern and surgical outcome of astrocytomas.

INFORMATION ABOUT ASTROCYTOMAS

Astrocytomas are a form of brain tumor with star-shaped cells. They often increase in size but some may grow fast within a short period of time. General symptoms of an astrocytoma are a result of growing pressure inside the skull and include headache, vomiting and mental status changes. In young children, the growing pressure of an astrocytoma inside the skull may enlarge the head. Seizures are more common with slow-growing astrocytomas. Diagnosis is based on a complete examination, which may include a magnetic resonance imaging (MRI) scan or a computed tomography (CT) scan. Several treatment approaches are used and surgery is usually done to make a diagnosis and to improve symptoms. However, it may sometimes be enough to cure some types of astrocytomas. Surgery may be followed by radiotherapy and chemotherapy.

All your information will be kept confidential. Treatment does not depend on your participation in this study and will be offered according to the results of all the necessary tests and investigations. There will be no rewards for participating in the study.

This study has been approved by the Institutional Research and Ethics Committee (IREC) of Moi University/Moi Teaching and Referral Hospital. If you need further clarifications please contact IREC using the address below.

The Chairman IREC,
Moi Teaching and Referral Hospital,
P. O. Box 3, Eldoret.
Tel: 0787723677

YOUR CONSENT:

Adults (above 18 years of age)

I have been adequately informed that I am being recruited into a study on the clinical pattern and outcome of astrocytomas. The investigator has also informed me that my participation in this study is voluntary and will not affect treatment should I opt to pull out. I have been assured that my confidentiality will be respected. No benefits/rewards have been promised to me as a result of my participation in the study.

Sign: …… Witness signature…….

Name: …… Witness name…….

Date: …… Date: …….

YOUR ASSENT:

Minors (7-17 years of age)

I have been adequately informed that I am being recruited into a study on the clinical pattern and outcome of astrocytomas. The investigator has also informed me that my participation in this study is voluntary and will not affect treatment should I opt to pull out. I have been assured that my confidentiality will be respected. No benefits/rewards have been promised to me as a result of my participation in the study.

Sign: …… Witness signature…….

Name: …… Witness name…….

Date: …… Date: …….

YOUR CONSENT:

Minors (below 18 years of age)

I have been adequately informed that my son/daughter is being recruited into a study on the clinical pattern and surgical outcome of astrocytomas. The investigator has also informed me that his/her participation in this study is voluntary and will not affect his/her treatment should he/she opt to pull out. I have been assured that his/her confidentiality will be respected. No benefits/rewards have been promised to him/her as a result of his/her participation in the study.

Patient's PARENT/GUARDIAN:

Sign: …… Witness signature: …….

Name: …… Witness name: …….

Date: …… Date: …….

SWAHILI CONSENT FORM:

Jina langu ni Clifford Chacha Mwita. Mimi ni daktari aliyesajiliwa na bodi ya madaktari ya Kenya (nambari A7400) na pia ni mwanafunzi wa Upasuaji katika chuo kikuu cha Moi. Ningependa kukuhusisha katika utafiti ninaofanya kuhusu saratani ya ubongo ya astrocytoma na matokeo yake baada ya upasuaji.

MAELEZEO KUHUSU ASTROCYTOMA

Astrocytoma ni aina ya saratani ya ubongo. Ni ugonjwa ambao unaenea pole pole kwa muda mrefu lakini pia kuna uwezekano mkubwa wa kuenea haraka na ndani ya muda mfupi tu. Dalili za ugonjuwa huu husababishwa na kule kuenea kwa saratani ndani ya kichwa na mgonjwa hupata dalili kama vile kuumwa na kichwa, kutapika na kuruka akili. Kwa watoto wachanga, ukubwa wa kichwa huongezeka. Ili kutambua chanzo cha ugonjwa huu, mgonjwa anahitaji picha ya CT au MRI. Tiba yake ni kufanyiwa upasuaji ili kutoa saratani ndani ya ubongo. Isitoshe, kuna uwezekano kwa kuhitaji miale ya kuchoma saratani baada ya upasuaji au kupewa dawa zaidi.

Utahusishwa kikamilifu katika matibabu yako na kuelezewa matokeo ya uchunguzi wo wote utakaofanyiwa. Maswala yote kuhusu ugonjwa wako na matibabu yake yatahifadhiwa vizuri. Utakua na uhuru wa kujiondoa kwenya utafiti huu wakati wo wote na iwapo utaamua kujiondoa, uamuzi huo hautaadhiri matibabu yako kwa namna yo yote ile. Hapatakuwa na malipo/zawadi yo yote ya kuhusishwa na utafiti huu.

Utafiti huu umeidhinishwa na kamati ya kusimamia utafiti (Institutional Research and Ethics Committee-IREC) katika chuo kikuu cha Moi na hospitali ya rufaa na mafunzo ya Moi (MTRH). Ikiwa una maswali zaidi tafadhali wasiliana na IREC kupitia anwani ifuatayo.

Mwenyekiti IREC,
Moi Teaching and Referral Hospital,
S. L. P. 3, Eldoret.
Simu: 0787723677

IDHINI YAKO:

Wenye miaka 18 na zaidi

Nimeelezewa kikamilifu kuhusu utafiti wa saratani ya ubongo ya astrocytoma na matokeo yake ya upasuaji. Mtafiti pia amenihakikishia kuwa maswala yote kuhus matibabu yangu yatahifadhiwa vizuri na kuwa iwapo sitataka kushiriki kwenye utafiti huu, uamuzi wangu hautaadhiri matibabu yangu kwa namna yo yote ile. Nimeelewa ya kwamba hakuna malipo/zawadi yo yote ya kuhusishwa na utafiti huu.

Sahihi: ……..…… Sahihi ya shuhuda…..……

Jina: ….…… Jina la shuhuda…..……

Tarehe: …… Tarehe…….…….

IDHINI YAKO:

Wenye miaka kati ya 7 na 17

Nimeelezewa kikamilifu kuhusu utafiti wa saratani ya ubongo ya astrocytoma na matokeo yake ya upasuaji. Mtafiti pia amenihakikishia kuwa maswala yote kuhus matibabu yangu yatahifadhiwa vizuri na kuwa iwapo sitataka kushiriki kwenye utafiti huu, uamuzi wangu hautaadhiri matibabu yangu kwa namna yo yote ile. Nimeelewa ya kwamba hakuna malipo/zawadi yo yote ya kuhusishwa na utafiti huu.

Sahihi: ……..…… Sahihi ya shuhuda…..……

Jina: ….…… Jina la shuhuda…..……

Tarehe: …… Tarehe…….…….

IDHINI YAKO:

Wenye miaka chini ya 18

Nimeelezewa kikamilifu kwamba mwanangu anashiriki katika utafiti wa saratani ya ubongo ya astrocytoma na matokeo yake ya upasuaji. . Mtafiti pia amenihakikishia kuwa maswala yote kuhusu matibabu yake yatahifadhiwa vizuri na kuwa iwapo mwanangu atakataa kushiriki kwenye utafiti huu, uamuzi wake hautaadhiri matibabu yake kwa namna yo yote ile. Nimeelewa ya kwamba hakuna malipo/zawadi yo yote ya kuhusishwa na utafiti huu.

MZAZI ama MLINZI:

Sahihi: ……..…… Sahihi ya shuhuda….……

Jina: ….…… Jina la shuhuda….……

Tarehe: …… Tarehe…….…….

Appendix V: IREC approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2015/113
Approval Number: 0001437

22nd July, 2015

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
22 JUL 2015
APPROVED
P.O. Box 4606-30100 ELDORET

Dr. Clifford Chacha Mwita,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Mwita,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Clinico-Pathologic Features and Outcome of Astrocytomas in Eldoret, Kenya."

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1437*** on 22nd July, 2015. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 21st July, 2016. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc Director - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

Appendix VI: Administrative approvals

MTRH Approval

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
Ref: ELD/MTRH/R.6/VOL.II/2008

P. O. Box 3
ELDORET

22nd July, 2015

Dr. Clifford Chacha Mwita,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Clinico-Pathologic Features and Outcome of Astrocytomas in Eldoret, Kenya".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR. JOHN KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM

St Luke's Hospital Approval

ST. LUKE'S ORTHOPAEDICS AND TRAUMA HOSPITAL
Exceptional Commitment to Quality

Nandi Road
Opp. School of Dentistry
P.O. Box 3705 - 30100
Eldoret

Tel: +254 20 61005
+254 707 611 625
+254 737 419 145
Email:info@stlukesorthopaedics.com
Website:www.stlukesorthopaedics.com

10th October, 2015

Clifford Chacha Mwita,
C/O Department of Surgery & Anesthesiology,
Moi University School of Medicine,
PO BOX 4606-30100,
ELDORET, KENYA

Dear Clifford Mwita,

RE: APPROVAL TO CONDUCT RESEARCH IN ST.LUKE'S ORTHOPAEDIC AND TRAUMA HOSPITAL

The above refers.

Following your letter dated the 28th of August 2015, and the contents therein noted, I am pleased to inform you that your request to conduct your study in St. Luke's hospital entitled "*Clinico-pathologic features and outcome of astrocytomas in Eldoret, Kenya"* has been approved. This therefore allows you to proceed with data collection. You are kindly asked to submit a copy of the final report to the Director once done.

ST. LUKE'S ORTHOPAEDICS TRAUMA HOSPITAL
10 OCT 2015
P. O. Box 3705 ELDORET - 30100

Sincerely,

Dr. L.K. Lelei
DIRECTOR
St. Luke's Orthopaedics and Trauma Hospital

Mediheal Hospital Approval

CLIFFORD CHACHA MWITA,
C/O DEPARTMENT SURGERY AND ANESTHESIOLOGY,
MOI UNIVERSITY SCHOOL OF MEDICINE,
PO BOX 4606,
ELDORET, KENYA

THE MEDICAL DIRECTOR,
MEDIHEAL HOSPITAL AND FERTILITY CENTER,
PO BOX 7905-30100,
ELDORET, KENYA

28/8/2015

Dear Sir/Madam,

RE: REQUEST FOR APPROVAL TO CONDUCT RESEARCH

I am a postgraduate student pursuing a master's degree in General Surgery at Moi University. As part of my thesis I am conducting a study on the clinico-pathologic features and outcome of astrocytomas in Eldoret, Kenya for which I have secured ethical approval. In summary, the study aims to describe the clinical presentation, radiological and pathological features and immediate outcome of astrocytomas among patients seen in hospitals in Eldoret town.

I humbly request that I be allowed to interact with and collect data from any patients diagnosed with and managed for astrocytoma in your facility. During the conduct of the study, I shall be under the close supervision of Dr. F. Koech who is also my supervisor. Find attached a copy of the ethical approval.

I look forward to a favorable response.

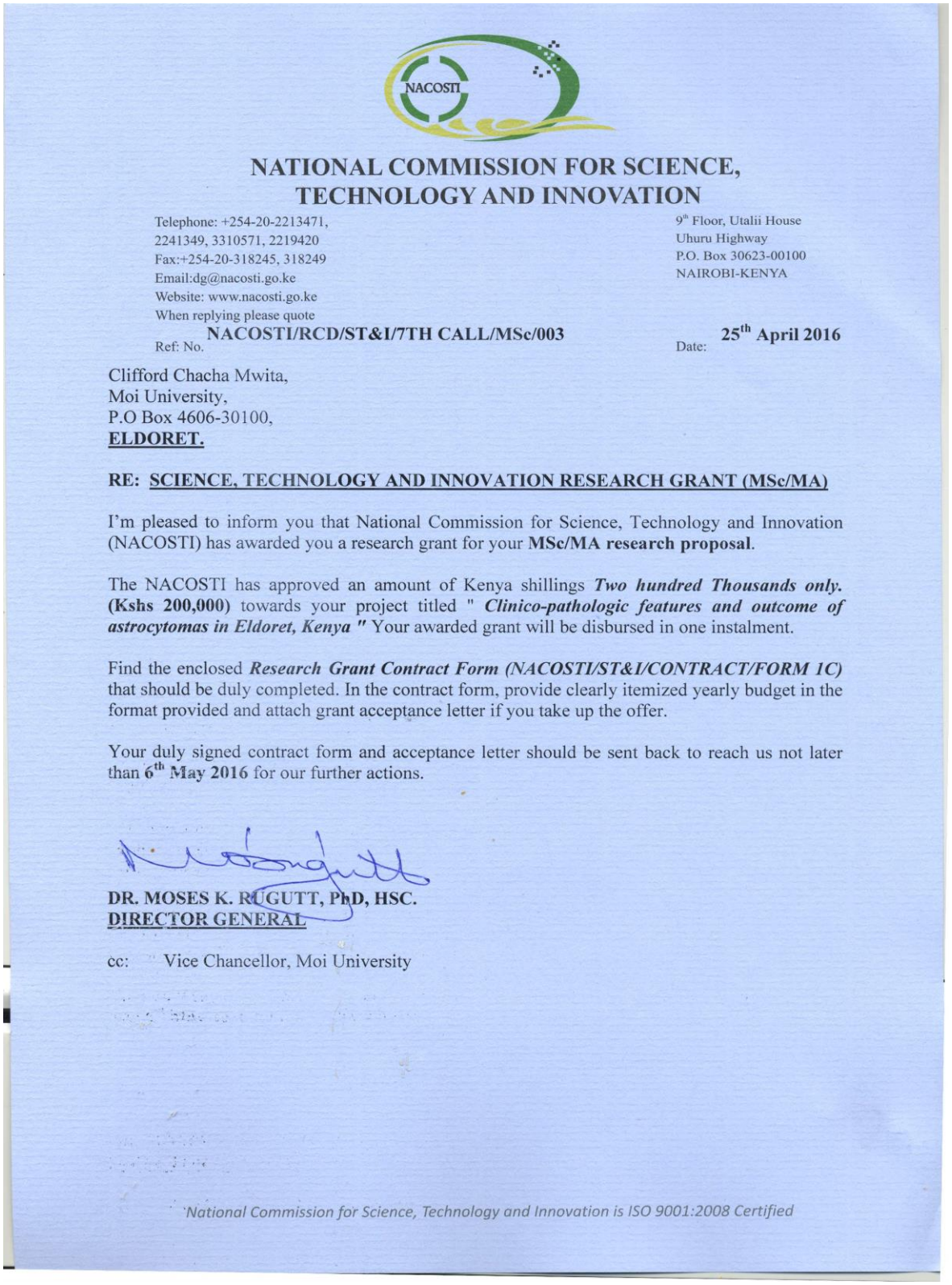
Sincerely,

Clifford C. Mwita

MEDIHEAL HOSPITAL AND FERTILITY CENTRE
P.O. Box 7905 - 30100
ELDORET, Kenya
Tel: 053 2060071 / 2

Allowed
1/9/15

Appendix VII: NACOSTI Approval



NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471, 2241349, 3310571, 2219420
Fax:+254-20-318245, 318249
Email:dg@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/RCD/ST&I/7TH CALL/MSc/003**

Date: **25th April 2016**

Clifford Chacha Mwita,
Moi University,
P.O Box 4606-30100,
ELDORET.

RE: SCIENCE, TECHNOLOGY AND INNOVATION RESEARCH GRANT (MSc/MA)

I'm pleased to inform you that National Commission for Science, Technology and Innovation (NACOSTI) has awarded you a research grant for your **MSc/MA research proposal**.

The NACOSTI has approved an amount of Kenya shillings ***Two hundred Thousands only.*** **(Kshs 200,000)** towards your project titled " ***Clinico-pathologic features and outcome of astrocytomas in Eldoret, Kenya "*** Your awarded grant will be disbursed in one instalment.

Find the enclosed ***Research Grant Contract Form (NACOSTI/ST&I/CONTRACT/FORM 1C)*** that should be duly completed. In the contract form, provide clearly itemized yearly budget in the format provided and attach grant acceptance letter if you take up the offer.

Your duly signed contract form and acceptance letter should be sent back to reach us not later than **6th May 2016** for our further actions.

DR. MOSES K. RUGUTT, PhD, HSC.
DIRECTOR GENERAL

cc: Vice Chancellor, Moi University

National Commission for Science, Technology and Innovation is ISO 9001:2008 Certified