ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL BIOCHEMISTRY

EVALUATION OF TOTAL OXIDATIVE STRESS AND CREATINE KINASE (BB) LEVEL FOR SCREENING AND DIAGNOSIS OF BRAIN TUMOR AMONG BRAIN TUMOR PATIENTS ATTENDING TIKUR ANBESSA SPECIALIZED HOSPITAL.

BY

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Declaration Sheet

This is to certify that this master's thesis entitled "Investigation of Serum total Oxidative Stress and creatine kinase BB for screening and diagnosis of brain tumor among Brain tumor Patients attending Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia", a comparative cross sectional study was prepared by Fitalew Tadele Admasu, and Submitted to the department of Biochemistry for partial fulfillment of the requirements for the degree of Masters of science in Medical Biochemistry complies with the regulation of university and meet acceptance standards with respect to originality and quality.

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Abbreviation and acronyms

ACS……..…American Cancer Society

ADP……..…Adenosine Di Phosphate

ATP……..Adenosine Tri Phosphate

BT…….....Brain Tumors

CNS……..…Central Nervous System

CK…….Creatine Kinase

CPK……..…Creatine Phosphokinase

CK-BB…….Creatine Kinase Brain Isoenzyme

CK-MM ……..….Skeleton Muscle Type Creatine Kinase

CK-MB ……....Cardiac Muscle Type Creatine Kinase

CSF……..Cerebrospinal Fluid

CT……Computed Tomography

DNA……Deoxyribonucleic Acid

ELISA…….Enzyme Linked Immunosorbent Assay

PUFA……..Polyunsaturated Fatty Acid

MDA……..Malondialdehyde

ROS…….Reactive Oxygen Species

RNS……Reactive Nitrogen Species

OS……..Oxidative Stress

MRI……Magnetic Resonance Imaging

TOS……Total Oxidant Status

TNF…….Tumor Necrosis Factor

WHO……...World Health Organization

4-HNE……4- Hydroxynonenal

Abstract

Background: The exact cause of brain tumors is still unknown, however, it is an important cause of morbidity and mortality of both adults and children, often generating severe disabilities and producing high burden in the health care systems. A recently growing advanced laboratory research has shown that oxidative stress can facilitate brain tumor initiation, growth and metastasis by altering tumor cell biology and microenvironment. Scholars are searching for an early biomarker for diagnosis, prognosis and potential target for treatment by looking at tumor metabolism as almost all type of cancer need high energy input and substrate via aberrant metabolism for survival. Therefore, the present study focused on investigating and examining metabolic activity of creatine kinase brain isoenzyme and oxidative stress in brain tumor patients for a potential use as a Biomarker for screening and early diagnosis of brain tumor.

Aim of the study: To evaluate total oxidative stress and Creatine kinase (BB) level for screening and diagnosis of brain tumor among brain tumor patients attending Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia, from April 2018 - October 2018.

Material and methods: Hospital based comparative cross-sectional study was conducted on a total of 90 study participants (50 brain tumor patients and 40 healthy controls). The parameters measured were serum levels of total oxidative stress and creatine kinase BB. Purposive sampling technique was implemented to select study participant in the hospital. This was a collaborative work between Departments of Biochemistry, Neurology and Oncology center.

Results: The present study result demonstrated that total oxidative stress and activity of creatine kinase BB were significantly increased in the serum (10.34±3.23µmoleH2O2eqv./l, 35.12±31.25 µg/L) of brain tumor patients as compared to serum control groups (3.87±2.62 µg/L, 6.9467±2.91µmoleH2O2eqv./l) ($p<0.05$). Total oxidative stress level of malignant brain tumors (12.76 ±3.38µmoleH2O2eqv./l) were significantly higher than benign tumor types (8.86±2.03µmoleH2O2eqv./l) ($p < 0.05$) but creatine kinase BB level were not significantly different between benign(38±34.33 µg/L) and malignant(25.98 ±25.98µg/L) types of brain tumor ($p > 0.05$). Both serum level total oxidative stress ($p= 0.001$, OR= 1.535, CI=1.247-1.889) and creatine kinase BB ($p=0.001$) were significantly associated with brain tumor.

Conclusion: Catalytic activity of creatine kinase BB and serum total oxidative stress were significantly increased among brain tumor parents when compared to serum levels of apparently healthy control groups. The serum level of total oxidative stress and creatine kinase BB were not significantly different and correlated among different brain tumor sizes. Creatine kinase BB activity was significantly associated with brain tumor and may be simple non-invasive biomarker for diagnosis of suspected cases of brain tumor. Participants with increased total oxidative stress (brain tumor patients) have significantly high risk of brain tumor development compared to lower serum value (healthy controls).

Key words: Brain Tumor, Creatine Kinase Brain Isoenzyme, Oxidative Stress.

1. INTRODUCTION

According to American cancer society (ACS) cancer is defined as a group of disease characterized by uncontrolled growth and spread of abnormal cells. It affects worldwide countries of all income levels. It is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. In low and middle income countries morbidity and mortality due to cancer is expected to increase rapidly because of population growth, age, economic transition, increased exposure and access to international market, tobacco use, physical inactivity, excess body weight, and adoptive lifestyle behaviors which increase cancer risk. In 2012, an estimated 14.1 million new cancer cases and 8.2 million cancer deaths occurred worldwide(Torre et al., 2015).Cancer is an emerging public health problem in Africa because of the aging and growth of the population as well as increased prevalence of risk factors. United Nation's population estimates the population of Africa between 2010 and 2030 to increase by 50% overall (from 1.03 billion to 1.52 billion) and by 90% for those aged 60 years (from 55 million to 105 million), the age at which cancer most frequently occurs. About 715,000 new cancer cases and 542,000 cancer deaths were estimated to have occurred in 2008 in Africa and this number is expected to double in the next 20 years. Despite the increase in incidence and prevalence, cancer continues to receive a relatively low public health priority in Africa, because of limited economic resources and other communicable diseases and non-communicable public health problems(Jemal et al., 2012).In Europe and America, prostate and Lung cancer are the most commonly diagnosed cancer among males whereas; breast and cervical cancers are the most common cancer in females. In Africa and Asia, the leading cancers among men include prostate, lung, colorectal, liver, whereas breast and lung cancer are the most common cancer in females(Torre *et al*., 2016).

A brain tumor in adults is less frequent or is a rare disease. The incidence and prevalence of brain tumors is poorly understood, as there is a paucity of data on their incidence and prevalence across the globe. The world age-standardized incidence rate for all primary brain tumors ranged from 4.3 to 18.6 per 100 000 per year. More than any other cancer, brain tumors can have long lasting and life-altering physical, cognitive, and psychological impacts on a patient's life and survival is generally poor compared to many other cancers(Robles *et al*., 2014).

Brain tumors are a mixed group of mass or growth of abnormal cells originating from intracranial tissues and the meninges affecting different brain cell types and are an important cause of morbidity and mortality in both adults and children, often generating severe disabilities and producing high burden health care systems. There are two main types of brain tumors: malignant or cancerous tumors and benign tumors. Cancerous brain tumors are of two types based on their origin, tumors that start in the brain parenchyma (primary brain tumors) and tumors that start in other organs, such as the lung or breast, and spread to the brain (metastatic or secondary brain tumors). Benign tumors can be lethal due to their site in the brain, their ability to

infiltrate locally, and their propensity to transform to malignancy. Malignant tumors are cancerous which tend to grow aggressively and spread to surrounding tissues and despite treatment; they recur either in the same place or in another location and if left untreated, can ultimately lead to death. In adults, metastatic tumors to the brain are more common than primary brain tumors. Brain tumor differ from other tumors in that both malignant and benign tumors can result in significant morbidity and mortality due to their intracranial location(McKinney, 1999). Gliomas, metastases, meningiomas, pituitary adenomas, and acoustic neuromas account for 95% of all brain tumors(Lo and Brenner, 2015).

Brain tumor is a multifactorial disease but the causes remain largely unknown. However, established risk factors include gender, (brain tumors happen more frequently in males than in females), race, (Caucasians get more often than people of other races), age, in general brain tumors are most common in adults aged 70 or older and in children who are under 8 years old, exposure to ionizing radiation such as head X-rays, work in the nuclear industry, family history or genetic predisposition(such as neurofibromatosis) and exposure for some chemicals like formaldehydeVinyl chloride, Acrylonitrile(Rothman, 2013).

Symptoms of brain tumor vary widely depending on the location of the tumor in the brain and cell type involved. As the tumor grows, it tends to put pressure on nearby regions of the brain and affects the functions controlled by those regions. The presenting complaints of patients with intracranial neoplasm tend to be similar for primary brain tumors and intracranial metastases(Laws and thaper, 1993). The onset of symptoms usually is insidious, but acute episode can occur if bleeding into the tumor, or when an intraventricular tumor suddenly occludes the third ventricle. Chronic headache, altered mental status, ataxia, nausea, vomiting, weakness, gait disturbance, focal seizures, fixed visual changes difficulty or changes in speech, hearing, vision, or motor functions abnormalities are the most common manifestations of patient with brain tumor. Brain cancers may progress rapidly or slowly over a period of many years and are often difficult to treat, and complete cure is often unattainable.(Lo and Brenner, 2015).

There are more than 120 types of brain and central nervous system (CNS) tumors. Today, most medical institutions use the World Health Organization (WHO) classification system to identify brain tumors. Some tumor types are assigned a grade, ranging from Grade I (least malignant) to Grade IV (most malignant), which signifies the rate of growth. The classification and grade of an individual tumor help predict its likely behavior. Glioma account for 28% of primary brain tumors and make up 80% of malignant brain tumors(collins, 2018).

The 2016 WHO Classify Tumors of the Central Nervous System using integrated phenotypic and genotypic parameters (histological similarities dependent on light microscopic features, immunohistochemical expression of lineage-associated proteins and ultrastructural characterization).This classification adds newly recognized neoplasms, and has deleted some entities, variants and patterns of the tumor that no longer have diagnostic relevance and in a way

which facilitate clinical, experimental and epidemiological studies which will improve the lives of patients with brain tumors(Perry *et al*., 2016)

Creatine Kinase (CK) also known as creatine phosphokinase (CPK) or ATP: creatine N-phosphotransferase is a common cellular enzyme that plays a crucial role in the production and maintenance of cellular energy homeostasis of cells and tissue.CK is widely expressed in various excitable tissues and cell types that require large energy fluxes with highest activity found in the heart, brain, skeletal muscle, and other tissues(Teixeira and Borges, 2012). It catalyzes the reversible conversion of creatine and ATP into ADP and phosphocreatine. In tissues and cells that consume ATP rapidly, when the cell needs energy it causes the transfer of a high-energy phosphoryl group from phosphocreatine to ADT to form ATP which is the source of energy for cell metabolism.CK consists of two isoforms: M (muscle) and B (brain), and has three isoenzymes: CK-MM (skeleton muscle), CK-MB (cardiac muscle), and CK-BB (brain)(Kekelidze and Holtzman, 2001).

The ubiquitous brain-type cytosolic isoform of creatine kinase, BB-CK is widely distributed in brain, heart, smooth muscle, nervous system and other tissues, but exists primarily in the brain and retina and is associated with ion transport pumps in the brain(Teixeira and Borges, 2012).The brain uses the energy provided from CK activity to activate Na+-K+ ATPase ion pumps and ATP-gated K+ channels which is imperative for neural cells, as they manage the membrane potential and propagate action potentials which account for 50% of the brain's energy usage(Paisley, 2012).

Measuring serum CK is an important part of the evaluation, screening, and monitoring of the progression of known disease(Bayer et al., 1976).CK released from skeletal muscle accounts for almost all of the CK detected in the plasma of healthy individuals. Increased amounts of CK are released into the blood when there is cell and tissue damage. Total serum CK (CK isoenzymes) can elevate and detected in the sera of patients who have various types of tissue damage to the organs that contain CK activity. Creatine kinase level is assayed in blood tests as a marker of damage of CK-rich tissue and disease such as in myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, autoimmune myositides, stroke, convulsions brain injury, brain cancer, and acute kidney injury(Tsung, 1981).

CK-BB is not normally detected in the blood however it is found in the serum of patients with various types of disease and cancer. Brain cancer, head injury, stroke, electroconvulsive therapy, seizure, prostate cancer and small cell carcinoma of the lung are some of the reasons that this enzyme show higher than normal results in a blood test(Silverman *et al*., 1979).

The Human body is constantly exposed for exogenous factors such as ultraviolet rays, tobacco smoke, and drugs (including anticancer drugs) which are used in medical practice, and endogenous factors include those derived from activities of mitochondria or microsomes and peroxisomes in the electron transfer system and those from the enzyme NADPH present in

macrophages and neutrophils as a mechanism of protection against infection are important source of oxidative stress(Sircus, 2017).Oxidative stress refers to the imbalance between levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the protective "antioxidant" mechanisms. Various reducing substances in the human body control the status of oxidation-reduction (redox), and a continuing imbalance in favor of oxidation causes various problems when it exceeds the capacity of such control. These toxic oxidizing species and free radicals collectively called ROS and RNS induces peroxidation of cellular and vascular structures, oxidation lipids, proteins, carbohydrates, oxidative cleavage of DNA, and impairment of the mitochondrial electron transport chain(Abdul-Muneer *et al*., 2014).

It is clear that injuries to cells by oxidative stresses are too significant to be ignored(Sircus, 2017).Oxidative stress by active oxygen involve in carcinogenesis and as a driver of brain tumor through two possible mechanisms: by induction of gene mutations that result from cell injury and through its effects on signal transduction and transcription factors. Oxidized and injured DNA has the potential to induce genetic mutation. Telomeric chromosomal genes are highly susceptible to mutation by free radicals, and tumor suppressor genes such as p53 and cell cycle-related genes can be damaged. In addition, free radical oxidized lipids can react with metals to produce active substances (e.g., epoxides and aldehydes) or synthesize malondialdehyde, which can induce mutation. Active oxygen species through DNA damage can act on gene expression (DNA binding of transcription factors) and signaling at the cellular level(Noda and Wakasugi, 2001).

1.2. Literature Review

1.2.1, Brain tumor

Brain tumors are one of the most devastating diseases faced by modern medicine. Rare as compared to other cancers, they account around 1% of primary malignant cancers(Laws and thaper, 1993). A brain tumor is a group of abnormal cells growing in the brain or central spine canal. There are two basic kinds of brain tumors; primary brain tumors and metastatic brain tumors; primary brain tumor, begins when healthy cells in the brain changes and grow out of control and secondary or metastatic brain tumors which spread from another cancer in the body to the brain and are almost always malignant. Brain tumors are either malignant or benign. A malignant tumor (brain cancer), grows rapidly and often invades healthy areas of the brain. Benign brain tumors do not contain cancer cells and are usually slow growing. Whether a brain tumor is benign or malignant they are potentially life threatening as are enclosed in bony skull the brain can't expand to make room for the growing mass as a result the tumor will compress and displace normal brain tissue. Some brain tumor case a blockage of CSF which increase intracranial pressure and enlarge brain ventricle. The tumor can directly destroy healthy brain cells or can indirectly damage healthy brain cells by causing inflammation, brain swelling and pressure within the skull which collectively create mass effect which is accountable for most of the symptom(Bunyaratavej *et al*., 2010).

Although neurons are the most common type of cell in the brain they are not the common origin for brain tumor as they have very limited replicative capacity so have limited potential for neoplastic change. Glial cells, neuron supporting cells which fulfill many structural and metabolic functions like nourishment, protection and electrochemical stability give rise to many different benign and malignant brain tumors. Glioma account around 40-60 % of primary brain tumor and astrocytoma, oligodendroglioma, and malignant glioblastomamultiforme are the most common forms of glioma. Meningioma which rise from meninges are another common tumor which account around 20% of adult brain tumor and are almost always benign(Laws and thaper, 1993).

Most of the time brain tumor is diagnosed accidentally because of signs and symptoms a person is having or when scan is performed for a non-brain tumor purpose and requires high degree of suspicion from doctors. Imaging tests like x-rays, Magnetic resonance imaging (MRI) and computed tomography (CT) scans are used most often for the diagnosis of brain tumor. These scans will show a brain tumor in almost all cases and also give an idea about the type of tumor based on how it looks on the scan and where it is in the brain(Laws and thaper, 1993). Magnetic resonance angiography (MRA): special form of MRI may be done to look at the blood vessels in the brain. Magnetic resonance spectroscopy (MRS): which measures radio wave interactions with different chemicals in the brain highlights some features of brain tumors that are not clearly seen by MRI. But in most cases biopsy of the tumor is still needed to get an accurate diagnosis, know exactly what type of tumor it is and either the tumor is benign or malignant as imaging

scans can't tell exactly what type of tumor it is. CT angiography (CTA),Positron emission tomography (PET) are some additional tests which creates detailed images of brain blood vessels and help doctors in planning surgery. Blood and urine tests rarely are part of the actual diagnosis of brain tumors, but they may be done to check how well the liver, kidneys, and some other organs are working(Avenue and CareLine, 2016).

1.2.2, Reactive oxygen species generation and oxidative stress

A free radical is unstable, free atoms or chemical species containing unpaired electrons, therefore can extract an electron from a neighboring molecule to become stable. Most of the free radicals relevant to cell biology are unstable, short-lived and highly reactive species generated inside the human body. Since they are unstable and freely wandering inside the cells, they react quickly with other compounds in their vicinity in order to capture the required electron to form a pair and become stable(Liu and Wang, 2015). This inclination and reaction of free radicals with different structure of organelles inside a living cell lead to their structural damage and homeostatic disruption. Normally free radicals protect us from bacteria, viruses and other foreign substances. When our antioxidant defenses are adequate, damage caused by those free radicals is repaired without many consequences. However when excessive amount of free radicals generates and supersedes repair it can readily cross cellular membranes and damage proteins, lipids, enzymes and DNA that can alter downstream cell signaling and a cause a variety of diseases process like depressed immunity, easy susceptibility to viruses and bacteria, cardiovascular diseases, nerve degeneration, atherosclerosis, inflammatory condition, certain cancers, and the process of aging(V-Lobo *et al*., 2010).

In living organisms free radicals are produced continuously and spontaneously inside all metabolically active cells. Free radicals are end products of enzymatic which include those involved in the respiratory chain, phagocytosis, prostaglandin synthesis, and in the cytochrome P-450 system and nonenzymatic reactions occurring inside lysosomes, mitochondria, peroxisomes and other dynamic structures inside the cells. Apart from natural oxidants produced inside the body, plethora of exogenous agents initiate a cascade of oxidation reactions upon gaining entry into the human body and come in contact with tissue cells. Solar radiation, air pollutants such as carbon monoxide, ozone, tobacco smoke, benzene, chlorine, formaldehyde toluene, exposure to ionizing radiation, consumption of medicines, heavy metals in food and water, Inhalation of chemical solvents such as cleaning products, paints, cosmetics, perfumes, pesticides, processed foods containing artificial flavorings, food color agents, trans fats, high levels of lipid peroxides, are the most common exogenous source of free radical(V-Lobo *et al.*, 2010).

Free radicals are grouped into two broad categories; Reactive oxygen species (ROS) and reactive nitrogen Species (RNS). Reactive oxygen species comprises peroxides, hydroxyl radical, superoxide, oxybenzone, oxygen singlet, and hypochlorous molecules. Reactive Nitrogen species include nitric oxide, peroxynitrite, etc. Reactive oxygen species, hydroxyl radical is the most

powerful endogenous oxidants that directly attack cellular structures and DNA (Abdul-Muneer et al., 2014).

Oxidative stress is a condition of oxidative damage resulting when the critical balance between free radical generation and antioxidant defenses is unfavorable. Oxidative stress happens when free radicals take the upper hand and result in imbalance between free radical production and antioxidant defenses and is associated with excessive damage and uncontrolled distress in cellular homeostasis. Most cells can withstand this damage to a limited extent by recognizing and removal of oxidatively damaged particles and repairing the structures. The extent of tissue damage depends on the type of reactive species involved. Extensive oxidative damage can lead to the death of the cell and part or whole of the tissue(Sircus, 2017). Peroxidation of lipids in cell membranes can cause collapse of cell membranes and alter the function of membrane bound proteins, enzymes and receptors. Damage to structural proteins inside the cells may result in loss of cell architecture and lack of its ability to restore. DNA damage and the associated chromosomal defects may predispose individuals to oncogene activation and cancer. Oxidative stress can modify many intracellular signaling pathways including protein phosphatases, protein kinases and transcription factors suggesting the major effect of ROS are through their actions on signaling pathways rather than via non- specific damage of macromolecules; however exact mechanism by which redox status induces cells to proliferate or to die, and how oxidative stress can lead to evoking tumor formation are still not clear(Liu and Wang, 2015).

1.2.3. Oxidative stress and brain tumor

The human brain is one of the highly metabolically active organs and consumes about 15-20% of the total basal oxygen budget of our body to support ATP intensive neuronal and glial activity. This high metabolic rate is attributed to the high proportion of omega-three polyunsaturated fatty acids and high glucose in brain tissue. These phospholipids (omega-three polyunsaturated fatty acids) are highly susceptible to peroxidation and the increased glucose metabolism results in increased production of volatile reactive oxygen species in the mitochondria. Moreover, brain tissue contains high levels of active iron and copper which further increase the oxidative stress burden and make the brain tissue more vulnerability to oxidative stress(Cobleya et al., 2018).

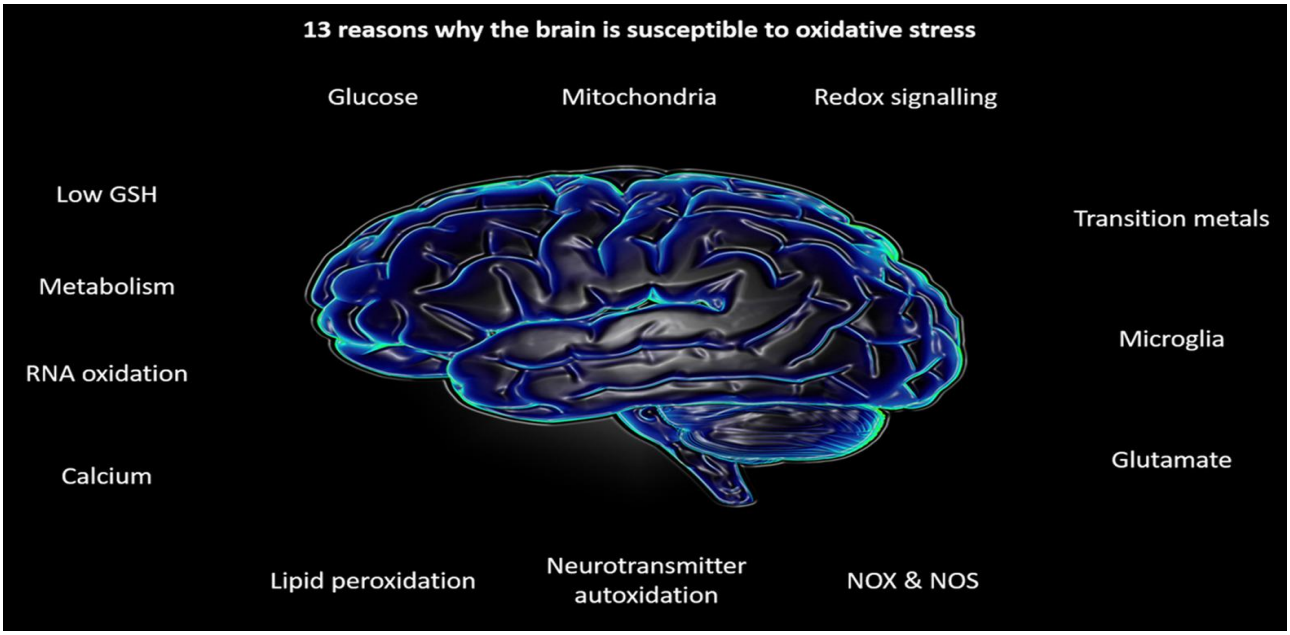


Figure1. The main sources of free radical(oxidative stress) in human brain(Cobleya et al., 2018)

Key mediators in the carcinogenesis of tumor include nuclear factor-kappaB, reactive oxygen and nitrogen species, and specific microRNAs and their collective activity is largely responsible for pro-tumorigenic response through changes in cell proliferation, cell death, cellular senescence, DNA mutation, DNA methylation and angiogenesis. Inflammatory mediators like cytokines, reactive oxygen and nitrogen species, cyclooxygenase-2 and nuclear factor- κB (NF-κB) can result a cellular condition which is favorable for tumor initiation, promotion and metastasis. Reactive oxygen and nitrogen species can cause reduction of cell-mediated cytotoxicity and potential immune evasion for tumors, fostering proliferation, survival and migration of tumors(Conti et al., 2010).

According to a study done in Turkey by Pinar Atukeren in 2010 on, The Impact of Redox Balance in Brain Tumors, states that redox balance in neural tissue has an important role in the pathophysiology of brain tumor from free radical generation. Particularly ROS are active in the brain and neuronal tissue and more specifically in the glial cells and neurons. The brain has high peroxidizable unsaturated fatty acids, high oxygen consumption, high content of iron which is a key in lipid peroxidation and a shortage of antioxidant defense systems that makes the brain more susceptible to oxidative stress and a unique target organ for metastatic tumor growth. Hydrogen peroxide free radical is of major concern in the brain since brain contains large quantities of iron and copper which catalyze the formation of hydroxyl radical and induce lipid peroxidation(Atukeren, 2010).

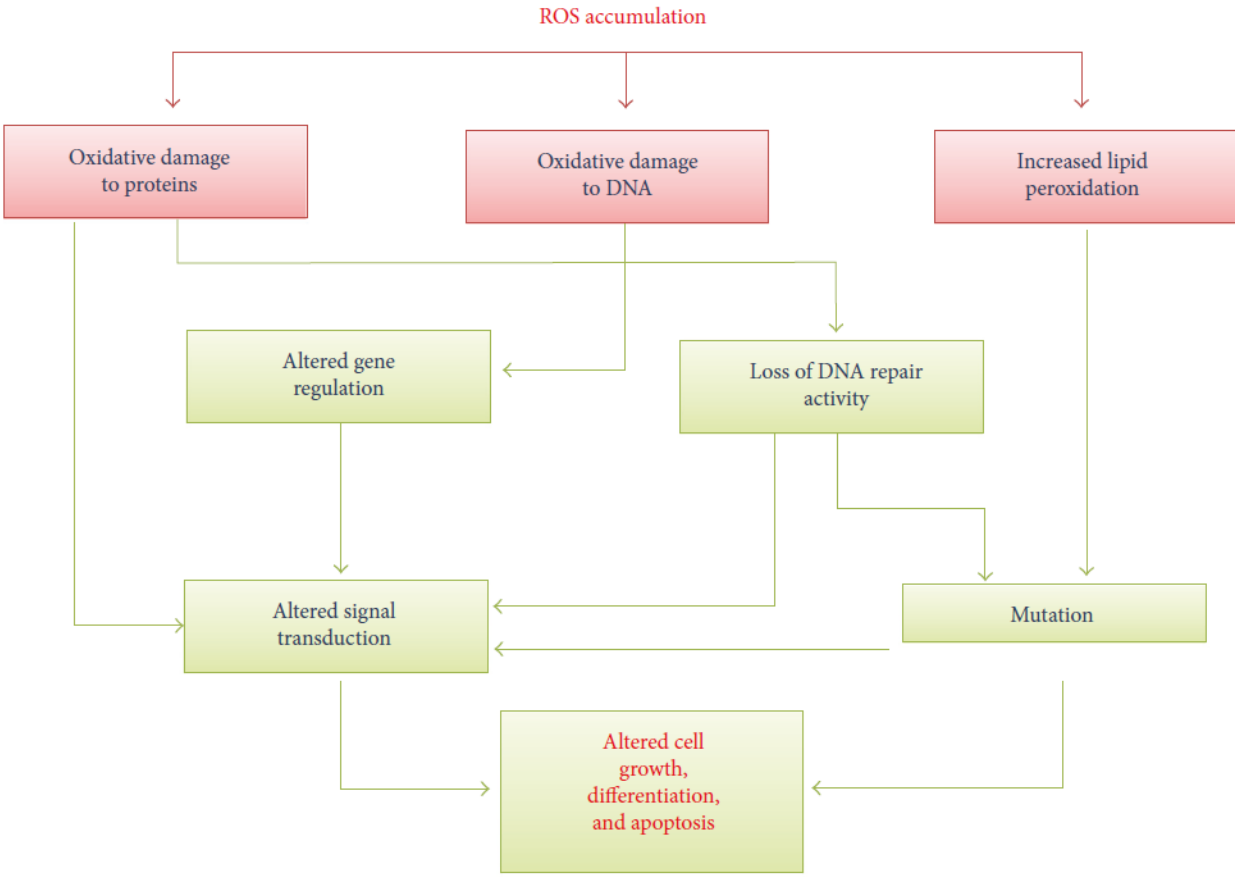


Figure2: The production of abnormally large amounts of ROS and changes in signal transduction and gene expression(Leone et al., 2017).

Tumorogenesis of glioma, the most common primary brain tumors involves multistep process including cellular neoplastic transformation, resistance to apoptosis, loss of control of cell cycle, angiogenesis, and the acquisition of invasive properties. A molecular connection between oxidative stress pathways and the development of glioma has been demonstrated. The Induction, expression and activity of inducible isoform of NO synthase (iNOS or type 2),the enzyme that synthesize NO(one of the reactive nitrogen species)from L arginine,is upregulated creating increased oxidative and nitrosative stress in glial cell significantly contribute to the carcinogenesis of glioma. Increased peroxynitrite levels lead to DNA strand breaks, point mutations and aberrant DNA cross-linking, thereby causing genomic instability which contributes to carcinogenesis of glioma by mutating proto-oncogenes and tumor suppress or genes by causing posttranslational modifications of wild-type p53 protein that are associated with dysregulation of p53 transcriptional activity and downstream signaling pathways, particularly cell cycle checkpoints. Furthermore cell proliferation, growth arrest, and apoptotic pathways of glial cell are affected by elevated peroxynitrite by both activating and inhibitory signaling pathways in glial cells. Level of NOS expression is correlated with degree of malignancy of glioma(Conti *et al*., 2010).

Another review study done by(Salazar-Ramiro *et al*., 2016) on role of Redox Status in different brain cell in the development of Glioblastoma shows that oxidative stress by reactive species of oxygen in brain tissue induces chromosomal and mitochondrial DNA damage and malfunction of DNA repair enzymes which results genetic instability and abnormal metabolic processes, favoring oxidative environment that promote gliomagenesis. The commonest form of modifications by ROS on DNA are -oxo-7,8-dihydroguanine (8-oxoGua) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine, which lead to apurinic/apyrimidinic (abasic) DNA sites, oxidized purines and pyrimidines, single strand DNA breaks (SSBs) and double-strand DNA breaks (DSBs) leading to generation of clustered DNA lesions which finally induce genetic instability and possible development of brain tumors. Alteration of redox homeostasis is involved in the beginning, progression and regression of brain tumor.

Another report by (Ha *et al*., 2014) on Chronic inflammation, states that around 15% of all cancer related deaths are linked with oxidative stress mediated chronic inflammation. Brain damage by different causes like infection results in chronic oxidative stress leading to chronic inflammatory microenvironment which thought to be involved in glioma initiation, promotion, progression and contributes to the neo-vascularization of gliomas by driving malignant conferring genetic mutations. An immunosuppressive microenvironment because of the chronic inflammation shields the tumor mass from clearance by the patient's own immune system.

The biomolecular events initiating and promoting brain tumor development are not yet fully clarified. In brain tumor tissue elevated biomarkers of oxidative stress like Oxidative lesions 8-oxoGua with decreased concentrations of antioxidant enzymes is found to demonstrate the essential mutagenic and carcinogenic potential of oxidative stress. The carcinogenic potential of oxidative stress is related to their capacity to induce genotoxicity and to interfere in crucial cellular processes. ROS can react with or bind bases such as pyrimidines, purines and chromatin proteins, resulting in chromosome modifications, genomic instability, and alterations in gene expression. Accumulation of ROS in cancer cells could damage DNA directly by increasing cellular mutation and/or the enhancement of oncogenic phenotype, or indirectly acting as a secondary messenger in intracellular signaling cascade. DNA damage involves breakage of strand, purine or pyrimidine substitution and DNA-protein cross linkages, are mainly responsible for brain tumor carcinogenesis process(Rinaldi *et al*., 2016).

1.2.4, Creatine kinase

Creatine kinase was discovered by Lohmann in 1934 and two year later the reaction it catalyzes was identified in muscle tissue.CK is a highly conserved enzyme of 40 kDa, and around ~60%its sequence are identical across all species and isoforms (Teixeira and Borges, 2012).CK is an enzyme which involves in the synthesis and use of energy providing molecules to maintain constant levels of ATP and ADP, buffering the cell against rapid depletion of ATP. It's predominantly found in high energy consuming excitable tissues like the heart, skeletal muscles and brain.CK has two cytosolic tissue specific polypeptide chains of M and B which exist as

homo-dimers (MM-CK and BB-CK) and three isomers of CK-MM, CK-BB, and hetero-dimers MB-CK composed of muscle (M) or brain (B) monomers have been identified based on the site they found. CK-BB is commonly found in the brain, gastrointestinal tract and the urinary tract. CK-MB is found in heart, while the skeletal muscles and heart are the main sites for the subtype CK-MM. It catalyzes the reversible transfer of the phosphoryl group from phosphocreatine and ADP, to regenerate ATP and the transfer of high energy phosphate group is important in various cellular processes.CK plays a major role in the production of energy by facilitating the process of energy transduction in muscles, brain and other tissues by catalyzing the formation of ATP(Pulugurtha, 2017).Cells use CK to balance ATP/ADP ratios and buffer temporary demands for ATP(Kuiper *et al*., 2009).

The cytosolic homodimer CKs (CKMM and CKBB) are highly conserved in their sequence and share same tertiary structure. They exist as a homodimer and each subunit are composed of two domains: a smaller N-terminal domain containing a-helices and a larger C-terminal domain with both b-sheets and a-helix secondary structures. The enzyme active site is located at the cleft of the two domains and to facilitate the entry of substrates and inhibitors.GLu232 is a crucial residue responsible for CK catalysis by participating in the positioning of creatine during the reaction(Teixeira and Borges, 2012).

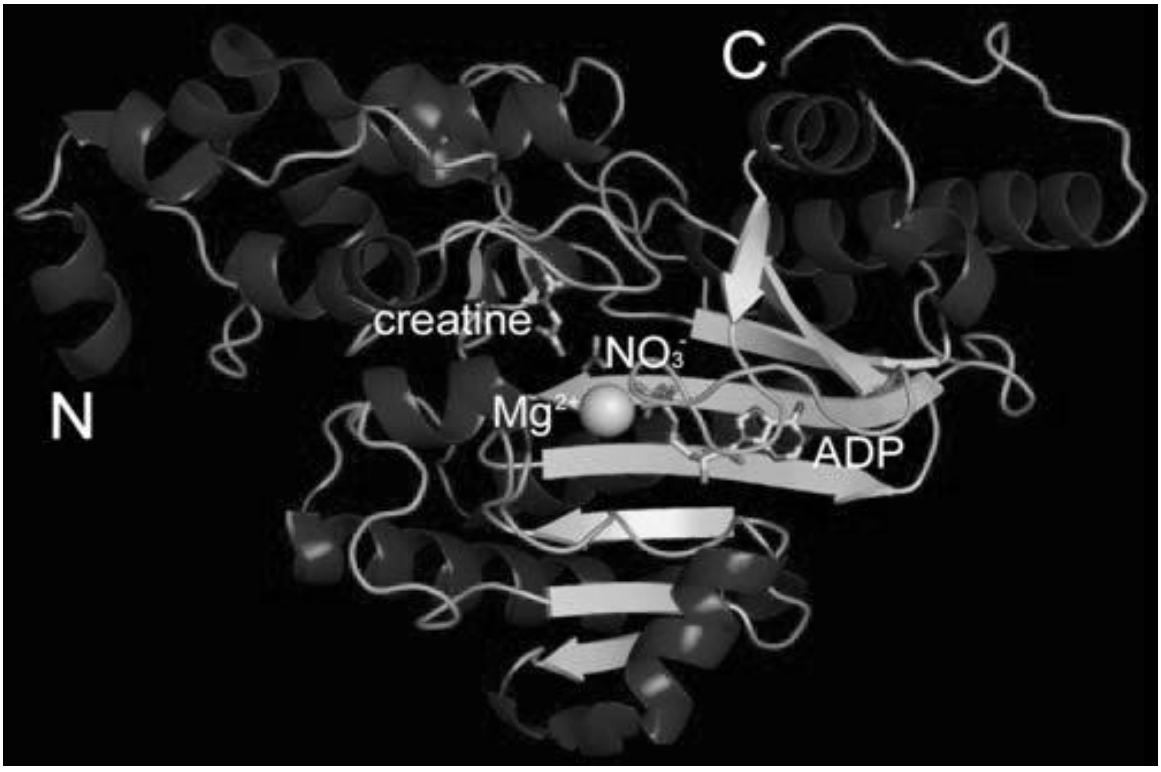


Figure 3, Structure of CK monomer (adopted from Teixeira and Borges 2012).

Creatine kinases catalyze the production of phosphocreatine and ADP from creatine and ATP and during the reaction CK BB forms multi-ligand structures by forming a transition state analog

complex consisting of ADP-Mg2+, NO3-, and creatine. After a phosphoryl group is transferred to creatine from ATP, a phosphocreatine molecule is released and an ADP-Mg2+ complex formed in only one of its two monomers. During the reaction the active monomer (ADP-Mg2+ complex)is in a closed conformation and the other monomer exists in a ligand-free, open conformation resulting in the formation of asymmetric homodimer.$Mg^{2+}$is a metal ion cofactor required in the reaction to align the substrates of the reaction and stabilize the phosphoryl transfer from ATP to creatine. When substrates bind to CK,Mg2+ forms a complex with ATP and stays in the complex during the transition state, and an ADP-Mg2+ complex remains even after the phosphoryl transfer is completed(Paisley, 2012).

Maintenance of energy homeostasis in the brain requires a distinct molecular circuitry which provides tight coupling between energy consumption and production during the performance of sensory, motor and cognitive tasks. It is generally assumed that most energy required in the nervous system is provided through oxidative phosphorylation in the form of adenosine triphosphate (ATP) by mitochondria(Karthick *et al*., 2015).

Brain-type creatine kinase (CK-BB) exists primarily in the brain, retina, heart, smooth muscle, and nervous system and is mainly important to supply energy for ion transport pumps in the brain. High levels of CK flux and activity were found in the cerebellar cortex and grey matter(Teixeira and Borges, 2012).in the bran CK-BB provides ATP mainly for activating the Na+-K+ ATPase ion pumps and ATP-gated K+ channels in neural cells, as they manage the membrane potential and propagate action potentials. In total, these ion transporters account for 50% of the brain's energy usage(Paisley, 2012).

CK-BB is also important for fueling the ATP-dependent cytoskeletal processes in non-muscle cells like oligodendrocytes, astrocytes, macrophages, osteoclasts and tumor cells. Although the exact mechanisms by which how CK-BB activity controls is incompletely understood, CK-B is connected to memory acquisition and behavior, development of the hippocampus, functioning of hair bundle cells in the auditory system, phagocytosis and bone resorption. Astrocytes are cells with highly dynamic surface extensions and CK-BB is highly expressed to enhance migration of astrocytes. Astrocytes use a significant amount of local ATP generation by CK-B for Actin-driven cytoskeletal remodeling and dynamics which requires considerable amounts of ATP to facilitate spreading and motility of astrocytes. The role of astrocytes as active modulators of synaptic transmission, which depends on the intimate physical interactions between astrocytes and neurons, local actin-driven dynamics of astrocytic protrusions play an organizing and maintaining role(Kuiper *et al.*, 2009).

Creatine Kinase has clinical importance and its levels are routinely used in screening, diagnosing, and following the progression of patients of different disease. Sometimes blood level of CK may rise during muscle activity and exercise in a healthy individuals and CK-MM is the main creatine kinase subtype. However, the presence of other CK subtypes in the blood or persistently elevated total CK levels indicate a variety of health conditions such as myocardial

infarction and necrosis, acute skeletal muscle atrophy, muscular dystrophy, burns, epilepsy, streptococcus infection, Streptococcal toxic shock syndrome, brain injury, neurologic disorders like Alzheimer's disease, and cancer of tissues which contain CK. So measurement of total and isoenzymes of CK may be helpful in elucidating the origin of an unexplained or persistently elevation of CK(Tsung, 1981).

1.2.5. Creatine kinase and brain tumor

Proteomics promises the discovery of tumor biomarkers for early detection and diagnosis of cancer. Approximately around 91differentially unique proteins are expressed in human brain cancer. These differentially expressed proteins provided novel information on the differences existing between normal brain and specific brain tumor, and thus might prove useful molecular indicators of diagnostic or prognostic value. Creatine kinase is one of the protein found in brain and shows tumor-specific changes in the proteome of human brain cancer(Alexandria, 2007).

The leakage of intracellular CK from injured tissues results in increase in the level of CK activity in blood which can serve as a biomarker of tissue damage. Due to its prevalent production in the brain, CK-BB may provide useful information about different brain pathologies including physical brain injury, various types of brain cancer and neurodegenerative diseases. Increase in the level of CK-BB activity provides a useful marker for diagnosis and prognosis of brain disease (Coolen et al., 1979). Measurement of serum CK-BB may be useful to confirm suspected CNS disease in cases with a suspicious clinical presentation. However CK-BB activity increase in all pathologic subgroups affecting the brain included leakage from neurons affected by inflammatory or degenerative conditions, or due to the hypoxia associated with brain tumor so it will not allow a diagnostic differentiation so, further diagnostic testing such as diagnostic imaging or CSF analysis is still necessary for a specific diagnosis (Paltrinieri *et al.*, 2017).

In a controlled study of the relationship between neuroblastoma and level of CK-BB level shows, 60% patients with neuroblastoma has elevated serum CK-BB activity and the severity of the disease is associated with an increased incidence of elevated serum CK-BB. Stage IV patients (92%) had elevated serum CK-BB concentrations. The pretreatment elevated serum CK-BB level in patients with neuroblastoma is also correlated with the patient's prognosis. The serum CK-BB levels were elevated more commonly in patients with advanced disease (Stages iii and IV, 77%) than in localized disease (Stages I and 11, 25%) indicating level of CK-BB in the sera of cancer patients was especially high in the presence of metastasis than localized once(Ishiguro *et al*., 2014).

In another comparative animal study conducted on dogs with different central nervous system disorders including space occupying lesions (SOL) of the brain was reported that, dogs with CNS disorders had significantly higher total CK and specifically CK-BB activities compared with healthy dogs, suggesting that CK-BB is released by neuron and other brain cell types damaged due to compressive effects of SOL (brain tumor). However specific neurologic diseases cannot be differentiated based on CK-BB activities and level, unless further confirmatory

diagnostic studies are done. In brain disease the increase in the total CK activity does not depend on the CK-MM release from skeletal muscle and CK-MB from myocardium, they can only contributing to the increased total CK activity. The increased serum CK-BB activity depend either on the release of CK from the cytoplasm of neurons or a damage of the blood brain barrier, as hypoxic conditions because of the compression effect may induce a transient changes of neuronal permeability or alterations in membrane transporters or receptors leakage of intracellular enzymes(Paltrinieri *et al*., 2017).

In experimental study conducted at University of Milan in 2010 by Paltrinieri and his colleague, (2010) to electrophoretically determine the distribution of CK isoenzymes in dogs and cats with different brain tumor and to provide data about the possible use of CK-BB as a biomarker for preliminary of assessment neurologic disorders reported that CK-BB activity increased in all sample cases with brain tumor. Despite the small number of cases, the difference in CK-BB activity between healthy and diseased samples was statistically significant. Even though increases in CK-BB activity provide valuable information, information on histologic type of brain lesions, types of tumors, or degree of invasiveness of the tumors is lacking which precluding about the possible use of CK-BB as a diagnostic or prognostic biomarker for specific CNS diseases(Paltrinieri *et al*., 2010).

1.3. Statement of the problem

Brain tumor is an important heath problem of all age group and the incident is increasing. In infants and young children brain tumor is the second most common form of cancer. In adolescent and young adult brain tumor range from fifth to eighth most frequent cancer. In elderly population the incidence of brain tumor is increasing. Brain tumors differ from other tumors in that both malignant and benign tumors can result in significant morbidity and mortality due to their intracranial location. (Laws and thaper, 1993).

The prevalence of brain tumor is very low, estimated around 4.5 cases per 100,000 individuals. So the use of brain tumor biomarkers for screening brain tumor is undesirable due to low sensitivity and specificity of markers. Very little data are available about the potential use of brain tumor biomarkers for the screening, early diagnosis, classification, and evaluation of the efficacy of therapy and also many of the studied markers has very low specificity and biopsy of the brain is not feasible(Feld and marton, 1978).

The worldwide annual incidence rate for primary brain tumors was estimated to be 3.7/100,000 in males and 2.6/100,000 in females, with a higher incidence in developed countries (males = 5.8/100,000 and females = 4.1/100,000) than in less developed countries (males = 3.0/100,000 and females 2.1/100,000). According to a report done in 2002 the estimated numbers of new malignant brain tumor cases diagnosed is around108,221 males and 81,264 females in the world(Robles et al., 2014).In 2012, 256134 new cases of brain cancer have been reported around the world. The highest numbers of brain cancer occurs mostly in 5countries, included China (65627 cases), America (21611 cases), India (18831 cases), Brazil (11737 cases), and the Russian Federation (7377 cases).Survival rate of brain tumor is low and it estimated that the 2 and 5-year survival rates is 36.2 and 27.6% respectively for patients with malignant brain tumors indicating the higher mortality rate of this disease(Khodamoradi *et al*., 2012).

Generally, there is far less research about tumors of the brain in Africa. About fifty-three percent of the estimated 12.7 million new cancer cases in 2008 were from developing countries, and the yearly incidence is expected to rise to 27 million by the year 2050. In a hospital-based cancer registry conducted in West African countries in 1990, brain tumor was ranked third in incidence among all neoplasms. Another report from Southwest Nigeria indicated that brain cancer represented 3.9% of total cancers and is the sixth most common neoplasm of all cancer.

In Ethiopia data on the incidence and prevalence of cancer are lacking. According to a population-based cancer registry conducted at Addis Ababa since 2011, indicates that noncommunicable diseases, especially cancer is becoming the second leading cause of death in the adult population of Ethiopia. In Ethiopia in 2015, an estimated 21,563 and 42,722 cancer cases were registered in males and females respectively. Out of this 362 men and 380 female new cases of brain tumor were estimated (Tessema *et al*., 2018).

1.4. Significance of the study

Now a day's brain tumor is becoming a major global health problem of both developed and developing countries. It has long been recognized that the interactions of tumor cell with their microenvironment may affect and contribute to tumor cell growth and metastasis. The ability of oxidative stress to affect tumor cell microenvironment and their interaction will be illustrated providing evidence for the activity of oxidative stress in brain tumor development and progression. So this study will suggest if the initiation, progression and metastasis of brain tumor can be prevented or reduced by preventing oxidative stress development in our body. Measuring creatine kinase isoenzyme level is used as a tumor marker for the diagnosis of many diseases and in this study we will try to elucidate if CK-BB can be used as plausible marker for screening and early diagnosis of brain tumor.

Furthermore, this study will serve as a baseline for other broad and nationwide research that shall be undertaken on brain tumor and related topics.

2. OBJECTIVE

2.1. General Objective:

- To evaluate the total oxidative stress and Creatine kinase (BB) level for screening and diagnosis of brain tumor among brain tumor patients attending Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia.

2.2. Specific Objective:

- To evaluate the total oxidative stress status of brain tumor patients and compare it with apparently healthy control groups.
- To assess the Creatine kinase BB level of brain tumor patients and compare it with apparently healthy control groups.
- To evaluate serum level of total oxidative stress and creatine kinase BB correlation with brain tumor size.
- To assess the association of Oxidative stress with the initiation and growth of brain tumor.
- To evaluate the correlation of serum creatine kinase BB level with brain tumor.

3. METHOD AND MATERIALS

3.1. Study Area

This study was conducted at Tikur Anbessa Specialized Teaching Hospital Addis Ababa, Ethiopia. TASH is a large referral teaching hospital, under the administration of Addis Ababa University, located in Lideta Sub City of Addis Ababa. This referral hospital has about 700 beds and is the main teaching hospital for both clinical and preclinical training of most disciplines. The hospital has separate neurology ward which provide specialized service for patients with different types of brain tumor and brain injury.

3.2. Study Design and period

Hospital based comparative cross-sectional study design was used to determine serum level of total oxidative stress and CK-BB among brain tumor patients attending Tikur Anbessa Specialized Hospital (TASH) between the period April, 2018 to October, 2018.

3.3. Population

3.3.1. Source Population

All newly diagnosed cancer patients who visit Tikur Anbessa Specialized Hospital of Addis Ababa, Ethiopia.

3.3.2. Study Population

The study population was all newly diagnosed brain tumor patients visiting the neurology and oncology department of Tikur Anbessa Specialized Hospital during the study period.

3.4. Sampling Technique

To recruit study subjects purposive sampling technique was used to include all brain tumor patients during the study period and by using structured questionnaire(annexure) all necessary socio-demographic data were collected. Accordingly, 50 brain tumor patients and 40 control groups were participated.

3.5. Sample Size Determination

Many international reports regarding oxidative stress and creatine kinase measurement are based on relatively small sample size due to practical constraints. When calculating the sample size requirements for this study, a number of factors were taken into consideration including cooperation and attrition, practical constrains such as time, subject availability and finance, subgroup analysis and sensitivity of the measurement used. Therefore by examining the sample size of other comparative study carried out internationally on assessing oxidative stress and creatine kinase BB level in brain tumor patient sample size varies from 30 to 80. However power analysis and sample size calculation was not reported in any of these studies. Therefore based on

logistical feasibility and uncertainties in sample size consideration, 50 brain tumor patients were included in this study.

3.6. Study variables

3.6.1. Dependent variables

- Serum creatine kinase BB
- Serum Total oxidant stress

3.6.2. Independent variables

- Socio demographic characteristics
- Age
- Sex
- Brain Tumor
- Family history, BMI

3.7. Inclusion and exclusion criteria

3.7.1 Inclusion criteria

All volunteer brain tumor patients of both sexes who meet the required information at the study area during data collection were included in the study.

3.7.2 Exclusion criteria

Patients who have the following finding were excluded from the study

- Spinal cord tumors
- Metastatic brain tumors, whose origins are external to the brain
- Primary brain lymphomas, which are essentially hematological malignancies.
- Brain tumor patient who presented with additional conditions, such as other malignancies, advanced organ failure or active infection
- Other diseases that are capable of raising the serum level of total oxidative status

3.8. Data and Specimen collection handling and storage

A total of 90 blood samples (50 from brain tumor patients and 40 from apparently healthy controls) were collected after a brief explanation and based on the participants consent and willingness for participation.

Blood sample was collected by professional experienced laboratory technologists, nurses working at TASH neurosurgery department. Interviewer administered structured questionnaire

data collection tool was used. The data collection was mainly focused on the objective of the study. All necessary information was collected from patients using clinical data and patient's medical records (charts) using structured formats (see Annex) consisting of collection of data on socio- demographic characteristics, medical history and laboratory investigations and recorded using data abstract format. Under preceding instructions brain tumor patients and healthy controls were checked regarding fasting by interview on the morning of the examination by the physician or Nurse. Brain tumor patients were selected who confirmed to have brain tumor by radiologic (CT- scan and MRI) examination and before any type of treatment. Appropriate information on demography and health status was obtained from standardized clinical files designed for the program. 5ml of fasting venous blood samples were obtained by vein puncture from vein of the arm of each participant using sterile syringes then transferred to serum separator container and allowed to stand for 30 min to coagulate and centrifuge at 3000 rpm for 10 minutes in order to separate the serum. Serum was separated by sterile pipette and transferred to 3ml Eppendorf test tubes and incubated at -80 oC until analyzed.

At the time of diagnosis each brain tumor patient's tumor size was measured from radiographic (CT-scan or MRI) images of brain tumor patients and the largest diameter of tumor lesion was measured. Tumors size were grouped in to, < 2 cm, 2-4 cm and > 4 cm.

3.8.1 Biochemical assays and Laboratory analysis

Measurement of total oxidative stress was assessed using ELISA microplate reader LT 4000 in Microbiology lab of Addis Ababa University. Measurement of Creatine kinase BB were done spectrophotometer using calibrated fully automated cobas c clinical analyzer (china) according to the reagent manufacture's instruction.

A, Determination of Total Oxidant Stress (TOS)

Plasma concentrations of different oxidant species can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive, and costly and require complicated techniques. Since the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the total oxidant stress status (TOS) of a sample is measured. TOS was determined based on the principle of Erel.

Principle: Total oxidant status of plasma samples were measured using novel robotized colorimetric estimation way for Total oxidant stress (TOS). In this process, oxidants present in the sample oxidize the ferrous particle o-dianisidine complex to the ferric complexes. The oxidation reaction was upgraded by glycerol molecules, which are richly present in the reaction medium. A colored compound was formed when the ferric ion reacts with xylenol orange in an acidic medium. The color strength, which can be measured spectrophotometrically at 560 nm wave length (ELISA microplate reader LT 4000), is correlated to the total quantity of oxidant molecules present in the plasma. The assay was calibrated with hydrogen peroxide and the

results were expressed as µmolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Eq /l) (Erel 2005). Standard curve was used to calculate the concentration of the study sample (figure3).

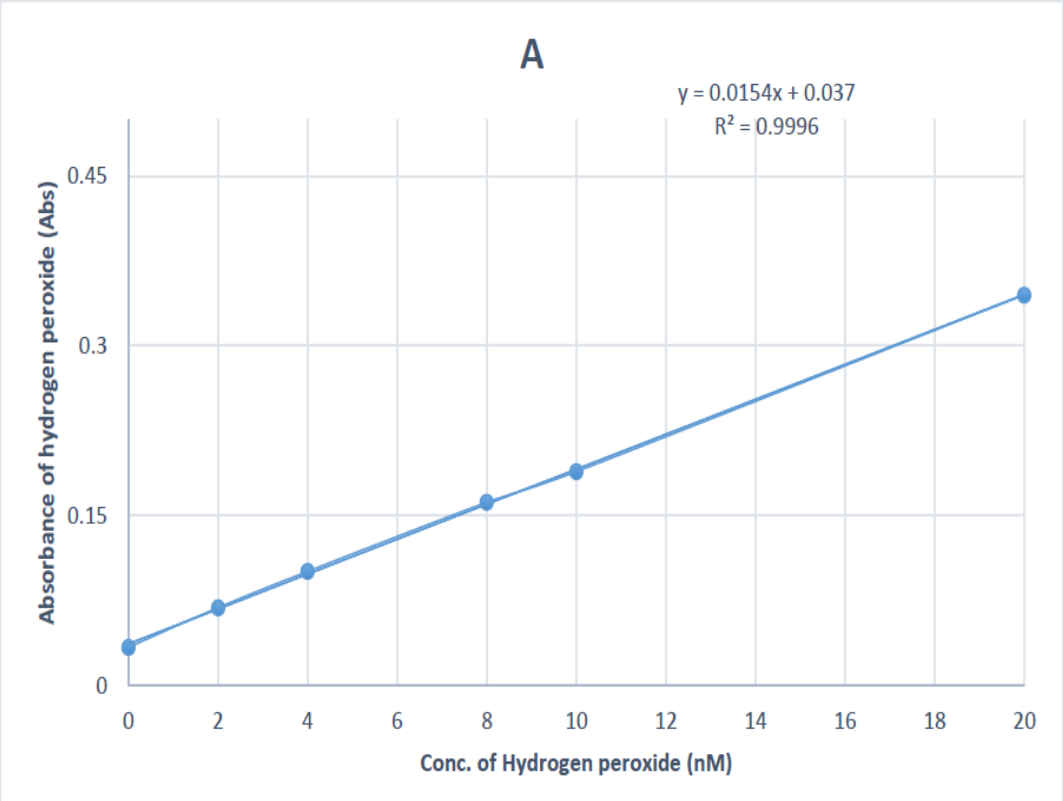
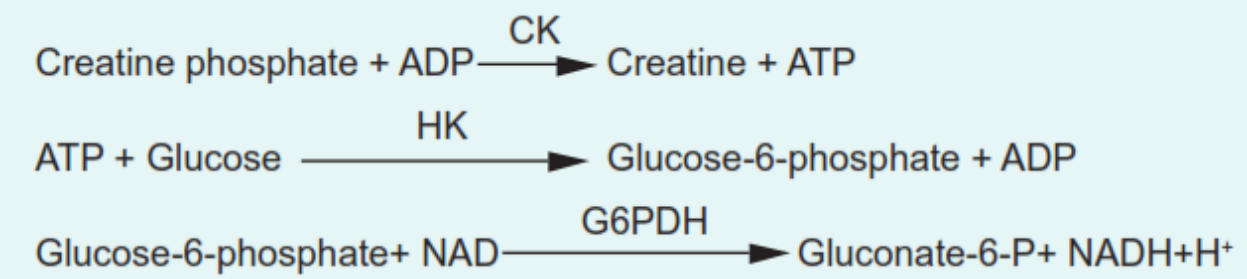


Figure 4: Standard curve for estimation of Total Oxidant Stress (TOS)

B, Creatine kinase (CK) assay methods

Creatine kinase (CK) appears as three isoenzymes, which are dimers composed of two types of monomeric subunits. These isoenzymes comprise combinations of the two type of monomers, M (for skeletal muscle derived) and B (for brain derived), as represented by the notations MM, MB and BB. Creatine Kinase BB (CK-BB) is a dimeric enzyme that catalyzes the reversible reaction of creatine and adenosine triphosphate (ATP) to form phosphocreatine and adenosine diphosphate(ADP). In healthy person CK-BB appears in the serum rarely.

Standardized methods for the determination of CK-BB using the “reverse reaction” and activation by N-acetyl cysteine (NAC) were recommended by the German Society for Clinical chemistry (DGKC) and the International Federation of Clinical Chemistry (IFCC), in 1977 and 1990 respectively. CK was activated by N-acetylcysteine (NAC). First the activated CK catalyzes the dephosphorylation of creatine phosphate to form creatine and ATP. In a coupled reaction catalyzed by hexokinase (HK) glucose was phosphorylated by ATP to form D-glucose -6-phosphate (G6P). Finally, glucose -6-phosphate dehydrogenase (G6PDH) catalyzed the oxidation of G6P by NADP+ to form 6- phosphogluconate and NADPH.



The rate of NADPH formation was directly proportional to the catalytic activity of CK-BB. It was determined by measuring the increase in absorbance photometer at 340 nm.

Reference intervals strongly depend on the patient group regarded and the specific clinical situation. For healthy people with intact blood brain barrier, serum level is almost always negligible. CK-BB activity of children is significantly (almost twice) higher than adult. However the CK-BB activity of adults of both sexes is approximately equal and serum CK-BB level above 10µg/l is considered abnormally high for neurologic patients(Ishiguro et al., 2014).

3.9. Data processing and analysis

All data were checked, cleared and feed into Epi-data (version 3.5.1, 2008) and then exported to SPSS (version 22.0, 2012, America) software for statistical analysis. Descriptive statistics like mean, standard deviation, and percentage were carried out for socio-demographic characteristics. Continuous variables were expressed as the mean ± standard deviation (SD) while categorical variables were expressed as frequencies and percentages. Student independent sample T-test was performed to analyze between-group difference and comparison of means of continuous variables. ANOVA was run to see the difference in the mean values of total oxidative stress and creatine kinase BB among deferent tumor sizes. Chi-square test was performed to see the association between Creatine kinase BB and brain tumor. Correlation analysis was used to see the relationship between, brain tumor size and level of creatine kinase BB and total oxidative stress. Binary logistic regression was used to assess the association between total oxidative stress status and brain tumor. Odds ratios (OR) and 95% confidence intervals (95% CI) for the association between oxidative stress and brain tumor were expressed. P-value was considered significant at $p < 0.05$.

3.10. Ethical Consideration

Ethical approval for the study was granted by the respective research ethics committees from Biochemistry Department College of Health Sciences Addis Ababa University with protocol number M.sc 06/18 and meeting number DRERC 08/18. Furthermore, A formal collaboration letter from Department of Biochemistry was provided to Tikur Anbesa specialized hospital, neurosurgery department. The obtained data was used only for the purpose of this research and not linked with patient personal information and before blood sample collection and filling of questionnaire, signed informed consent was taken from each participant.

3.11. Data Quality Assurance and management

The data quality assessment was started with socio-demographic data collection and goes to blood sample collection, laboratory test and final data entry and statistical analysis. Blood sample was taken in aseptic techniques with standard operational procedure. The kit was made free from contamination and check for consistency. All the laboratory procedures were handled by professional laboratory technologists and results were checked for completeness on daily basis by the immediate supervisor. Attention in data insertion to software on computer was sought. The complete result was rechecked repeatedly to maintain the overall quality of data.

4. RESULT

4.1. Socio-demographic characteristics

A total of ninety study participants (50 brain tumor cases and 40 health controls) were recruited in this study. The mean age of brain tumor patients and healthy controls was 35.25 and 36.2 years with a minimum age of 18 and 23 years and a maximum age of 62 and 60 years respectively. The socio-demographic characteristics are displayed in table 2 below. Most of the participants of the study were males 27/50(63.3 %) in the brain tumor patients and 24/40 (60 %) in the healthy controls. Equal number of brain tumor patients in the study were living in rural and urban areas with each 25/50(50%) but most of the healthy control lives in urban36/40(90). 18/50 (36%), 29/50(58%) 3/50 (6%), brain tumor patients and 27/40(67.5%), 13/40 (32.5%) , 0/40 (0.0%) healthy controls were single, married and widowed respectively. Out of fifty brain tumor cases 21 (42%) and out of the forty controls 5(12.5%) were illiterate. None of brain tumor cases 0/50 and 3/40(7.5%) healthy controls smokes and 26/50(52%) brain tumor patients and 11/40(27.5%) healthy controls drink alcohol.

Table 2. Socio-demographic profile of study participants of brain tumor patients and control groups at Tikur Anbesa hospital, Addis Ababa, Ethiopia, 2018

Variables		Brain tumor cases N=50	Healthy control N=40
Age (mean ± SD) in years.		35.25±11.8	36.2±9.9
Gender [n (%)]	Male	31(62)	24(60)
	Female	19(38)	16(40)
Residence [n (%)]	Urban	25(50)	36(90)
	rural	25(50)	4(10)
Marital status [N (%)]	Single	18 (36)	27 (67.5)
	Married	29(58)	13 (32.5)
	widowed	3 (6)	0 (0.0)
Educational level [N (%)]	Illiterate	21 (42)	5 (12.5)
	Primary school	13 (26)	7 (17.5)
	Secondary school	4 (8)	6(15)
	College/University	12(24)	22(55)
Alcohol [N (%)]	Yes	26(52)	11(27.5)
	no	24(48)	29(72.5)
Smoking [N (%)]	Yes	0(0)	3(7.5)
	no	50(100)	37(92.5)

SD= Standard Deviation, %= percentage, N= number, NA= Non-Applicable. Categorical variables are presented in frequency and percentage while continuous variables are presented as mean ± (SD)

Table 3 depicts the Family history of brain tumor, BMI group and physical exercise habits of the study participants. The majority of participants had no family history of brain tumor (98% cases and 0% controls).There was no statistically significant difference between cases and control groups in terms of BMI grouping , physical exercise habits, and family history of brain tumor ($p > 0.05$).

Table 3. General socio-demographic data of study participants of cases and controls at Tikur Anbesa hospital, Addis Ababa, Ethiopia, 2018

Characteristics		**Controls (N=40)** **Frequency (%)**	**Cases (N=50)** **Frequency (%)**
Family history of brain tumor	**Yes**	**0(0)**	**1(2)**
	no	**40(100)**	**49(98)**
Physical exercise habits	**Never**	**25 (62.5)**	**31 (62.2)**
	Once a week	**0 (0.00)**	**4 (8.4)**
	2-3 times a week	**10 (25)**	**10 (20.2)**
	4-6 times a week	**5 (12.5)**	**5 (11.6)**
BMI grouping	**≤ 18 (underweight)**	**2 (4.2)**	**3 (6.3)**
	18.5-24.9 (normal weight)	**30 (75)**	**37 (73.7)**
	25-29.9(over weight)	**8 (20.9)**	**10 (20.0)**

%= percentage, N= number, BMI=Body Mass Index, Categorical variables are presented in frequency and percentage while continuous variables are presented as mean (SD)

4.2. Clinical and radiologic characteristics of brain tumor patients

Clinical and radiological results of all brain tumor patients were studied, tabulated in table 4. At the time of diagnosis, clinical and radiologic feature of tumors of each brain tumor patients (CT scan and MRI imaging) were examined, out of the 50 brain tumor patients most 31/50(62%) were benign and the rest 19/50(38%) were malignant tumor cases. In brain tumor patients the mean tumor size was 3.6 ± 1.12cm (range, 1.8-6.5 cm) and most 33(66) of them had tumor size between 2-4 cm (table 4).

Table4. Clinical and Radiologic characteristics of tumors of brain tumor patients at Tikur Anbesa hospital, Addis Ababa, Ethiopia, 2018

Clinic-Radiologic characteristics		**Frequency [n (%)]**	**mean ±SD**
Tumor size	**Less than 2 cm**	**3(6)**	**1.9± 01 cm**
	2-4cm	**33(66)**	**3.2±0.55 cm**
	> 4cm	**14(28)**	**5.1±0.76 cm**
Tumor type [n (%)]	**Benign**	**31(62)**	**NA**
	malignant	**19(38)**	

Values are expressed as Means+ SE. %= percentage, n= number, NA= Non-Applicable.

4.3. Estimated levels of biochemical parameters

4.3.1. Serum levels of TOS in brain tumor patients and healthy control groups

The serum concentration of total oxidative stress status (TOS) in brain tumor patients and healthy control groups were examined. The mean total oxidative stress of brain tumor patients was 10.34±3.23 μmol H2O2 Equiv/l and 6.9467±2.91μmol H2O2 Equiv/l (Table 5) in healthy controls.

Independent sample t-test showed that, compared to healthy control group the levels of TOS in brain tumor patients were highly significantly ($p < 0.001$) elevated (Table 5).

Table 5: Serum TOS levels among brain tumor patients and healthy control samples, Tikur Anbessa teaching hospital, July, 2018.

Serum parameter	**Control group (N=40, mean ±SD)**	**Brain tumor patients (N=50, mean ±SD)**	**p-value**
TOS (µmole H2O2 eqv. /l])	**6.9467±2.91**	**10.34±3.23**	**0.001**

Values are expressed as Means+ SD. TOS= total oxidative stress

4.3.2. Serum CK-BB level in brain tumor patients and healthy control groups

CK-BB concentration in blood sample was considered abnormal when it exceeded 10µg/L (Ishiguro et al., 2014).

At the time of diagnosis the serum CK-BB concentration of brain tumor patients and healthy control groups were examined (Table 6). 8/40(20%) healthy controls has no detectable serum CK-BB level and in controls who have measurable CK-BB (3.87±2.62 µg/L, range 1.25-9.7 µg/L) none of them has elevated (> 10 µg/L) serum CK.BB level. Out of the 50 brain tumor patients 32/50(64%) has elevated (range 11.2- 123 µg/L) serum CK-BB level.

Table 6: Serum creatine kinase BB levels among brain tumor patients and healthy control samples, Tikur Anbessa teaching hospital, July, 2018.

	CK-BB (mean ±SE) In µg/L	CK-BB< 10 µg/L No (%)	CK-BB>10 µg/L No (%)
Healthy control(n=40)	3.87±2.62 µg/L	40(100)	0(0)
Brain tumor patients(n=50)	35.12±31.25 µg/L	18(36)	32(64)

Values are expressed as Means+ SE, CB.BB =creatine kinase BB

Independent sample t-test showed that there was a statistically significant difference in creatine kinase BB levels between case and control groups (p =0.001) (table 5) and that the brain tumor patient have significantly higher levels of CK-BB level than healthy controls.

Table 7: Independent sample t-test

Variable	Healthy control (n=40, mean ±SE)	Brain tumor patients (n=50,mean ±SE)	p- value
CK-BB	3.87±2.62 µg/L	35.12±31.25 µg/L	0.001

Values are expressed as Means+ SE, CK.BB – creatine kinase BB

4.3.3. Comparison of total oxidative stress level between benign and malignant tumors within brain tumor patient

As shown in the table below the total oxidative stress level among brain tumor patients varies between benign and malignant tumor types. The TOS in malignant brain tumor patients (12.76 ± 3.38, range 8.50-21.06) was highly significantly elevated (P=0.001) than the benign brain tumor patients (8.86±2.03, range 6.19-15.40).

Table 8: Serum TOS levels of benign and malignant forms among brain tumor patients

Serum parameter	Benign brain tumor (n=31, mean ±SE)	Malignant Brain tumor patients (n=19, mean ±SE)	p-value
TOS (µmole H2O2 eqv. /l])	8.86±2.03	12.76 ± 3.38	0.001

Values are expressed as Means+ SE, TOS= total oxidative stress

4.3.4. Comparison of CK-BB level between benign and malignant tumors within the brain tumor patient

Even though it was not statistically significant (P > 0.05), the serum concentration of creatine kinase BB of malignant tumors (25.98 ±25.98 µg/L) was lower than the benign tumor types (38±34.33 µg/L).

Table 9: Serum CK-BB level of benign and malignant forms among brain tumor patients

Serum parameter	Benign brain tumor (n=31, mean ±SE)	Malignant Brain tumor patient (n=19, mean ±SE)	p-value
CK.BB In µg/L	38±34.33	25.98 ±25.98	0.357

Values are expressed as Means+ SE, CK-BB creatine kinase BB

4.3.5. Comparison of total oxidative stress and creatine kinase BB level between different tumor sizes

Tumor size was measured from brain radiographic (CT-scan or MRI) images of brain tumor patients and the largest diameter of tumor lesion was measured. Tumors were grouped in to, < 2 cm, 2-4 cm and > 4 cm based on their size. The Total oxidative stress (TOS) and creatine kinase BB level in blood sample were compared between less than 2 cm, 2-4 cm and greater than 4 cm tumor sizes of brain tumor patients.

4.3.5.1 Comparison of total oxidative stress level between different tumor sizes

The mean total oxidative stress level of 4cm and larger tumor sizes (11.43 ± 3.52 μmole H2O2 eqv. /l) were higher compared to both 2-4 cm(9.97 ± 3.17 μmole H2O2) and < 2 cm (9.35± 0.37 μmole H2O2) tumors sizes and 2-4 cm tumor sizes have higher TOS level than < 2 cm. But the difference in TOS level among different brain tumor sizes was not statistically significant (p=0.323).

Table 10: Analysis of Variance in TOS level between different tumor sizes

Serum parameter in brain tumor patients	Tumor size			ANOVA
	< 2cm N= 3 Mean± SD	2-4 cm N= 33 Mean ±SD	>4cm N= 14 Mean ±SD	P-value
TOS (μmole H2O2 eqv. /l)	9.35± 0.37	9.97 ± 3.17	11.43 ± 3.52	0.323

Values are expressed as Means+ SE, TOS= total oxidative stress

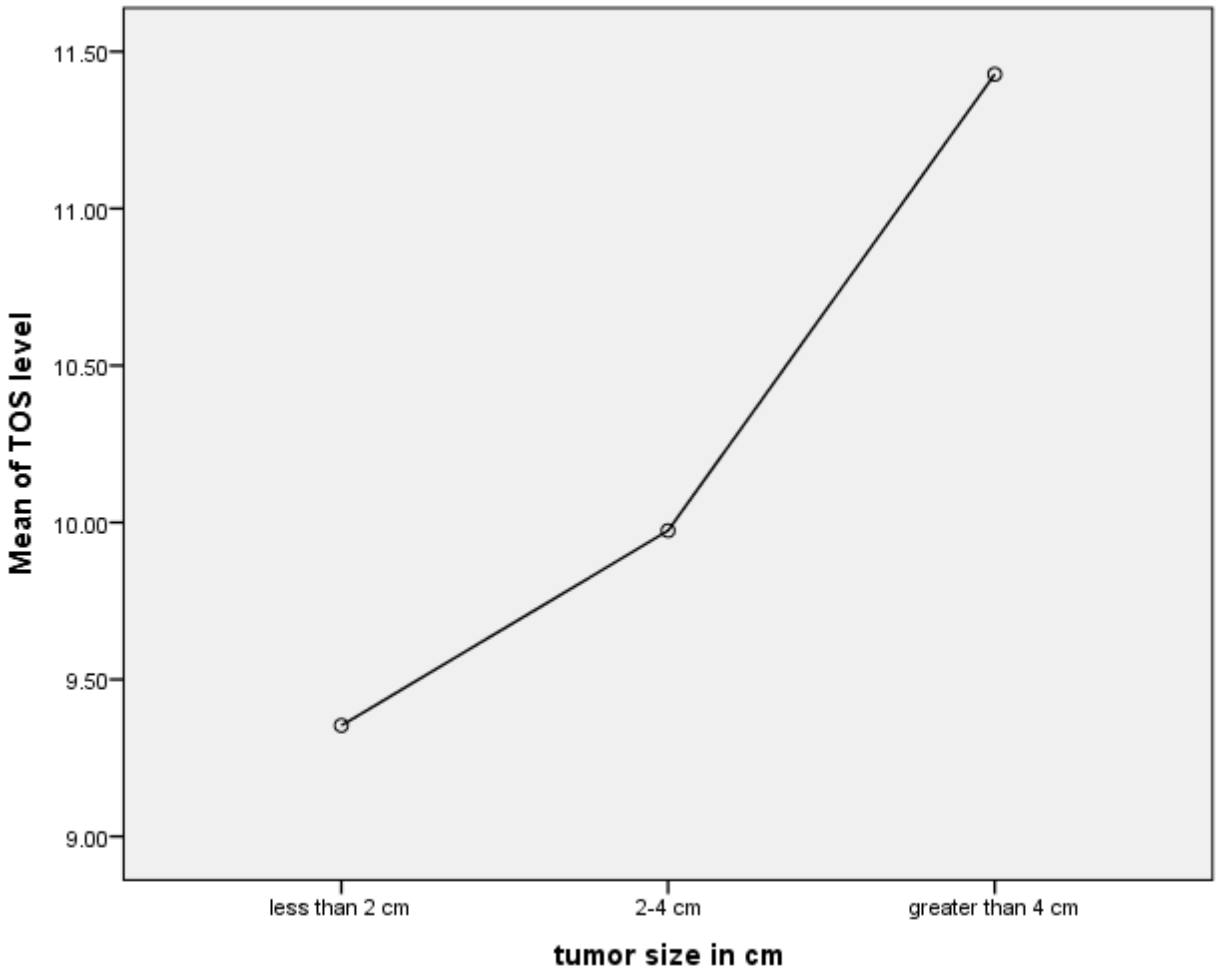


Figure 5: Mean plots of TOS level in different tumor sizes of brain tumor patients

4.3.5.2 Comparison of CK-BB level between different tumor sizes

Analysis of variance also showed that the serum CK-BB level was also higher in larger(> 4cm) tumor sizes (40.06±34.76 µg/l) than both 2-4 cm (34.95±30.04 µg/l) and 2 cm and lower sized (8.53±3.6 µg/l) tumors and level of CK-BB of 2-4 cm was also higher as compared to < 2 cm tumors but again the difference in CK-BB level among different tumor size was not statistically significant(p=0.293).

Table 11: Analysis of Variance in CK- BB level between different tumor sizes

Serum parameter in brain tumor patients	Tumor size			ANOVA
	< 2cm N= 3 Mean± SD	2-4 cm N= 33 Mean ±SD	>4cm N= 14 Mean ±SD	P-value
CK-BB(µg/l)	8.53±3.6	34.95±30.04	40.06±34.76	0.293

Values are expressed as Means+ SE, CK-BB creatine kinase BB

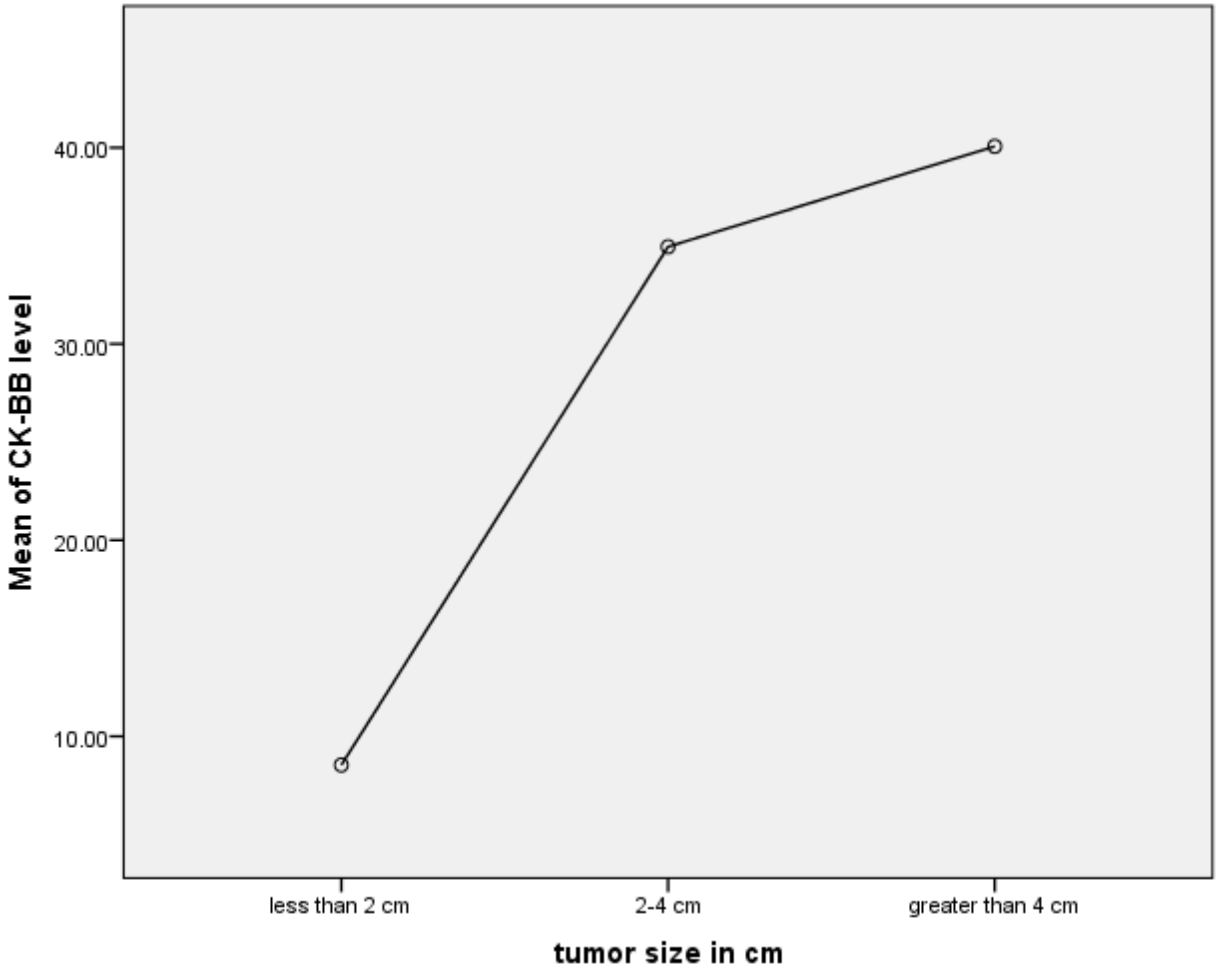


Figure 6: Mean plots of serum CK-BB level in different tumor sizes of brain tumor patients

4.3.6. Correlation between tumor size and serum level of TOS and CK-BB in brain tumor patients

The TOS and CK-BB level of brain tumor patients was analyzed and correlated with size of brain tumor.

4.3.6.1 Correlation between tumor size and serum level of TOS in brain tumor patients

Correlation analysis showed that the level of TOS was positively but non-significantly correlated with size of tumor among brain tumor patients (r= 0.15, p = 0.28).

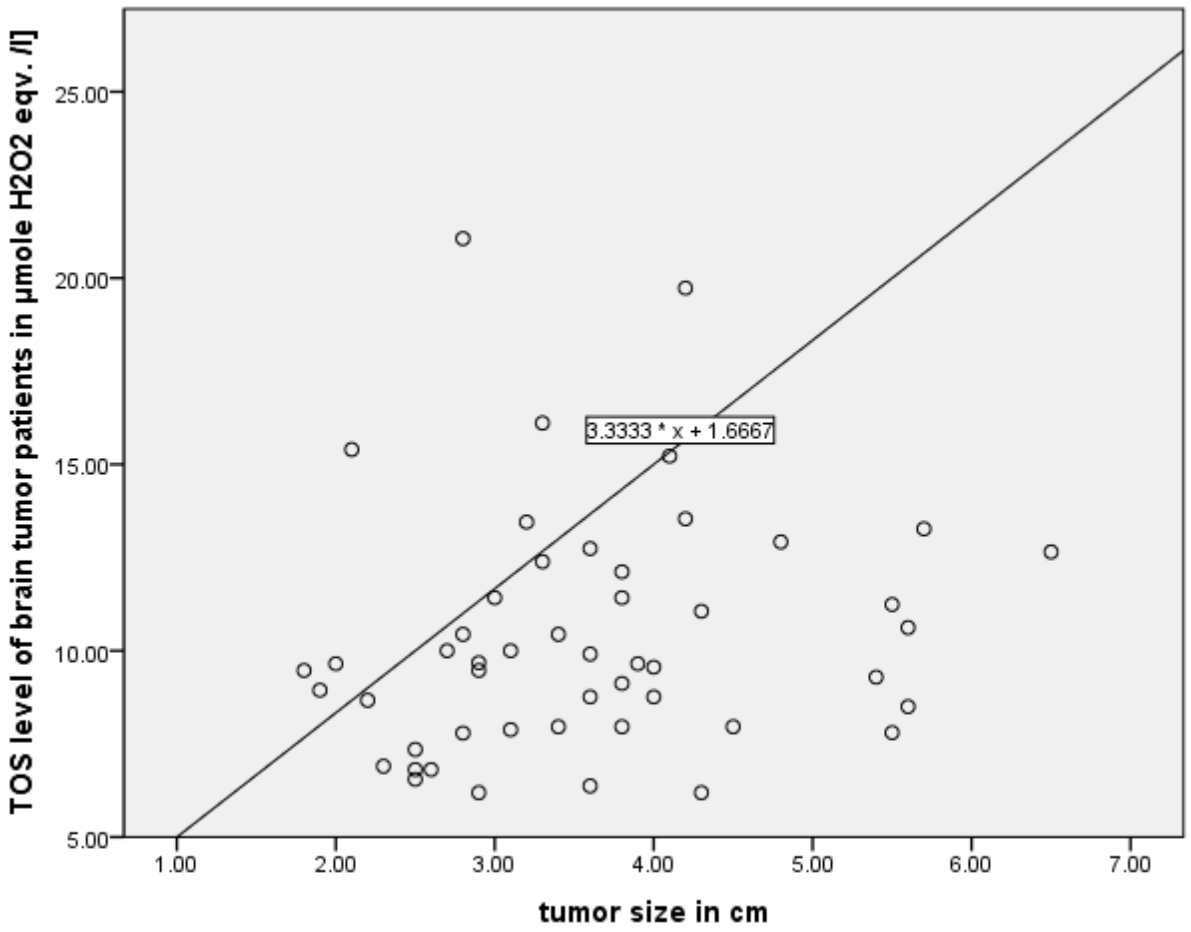


Figure 7: Scatter plot correlation between TOS level and tumor size in brain tumor patients

4.3.6.2 Correlation between tumor size and serum level CK-BB in brain tumor patients

The serum level of CK-BB also has a non-significant most nearly linear, negative (inverse) correlation with the size of tumor in brain tumor patients (r= -0.013, p=0.813).

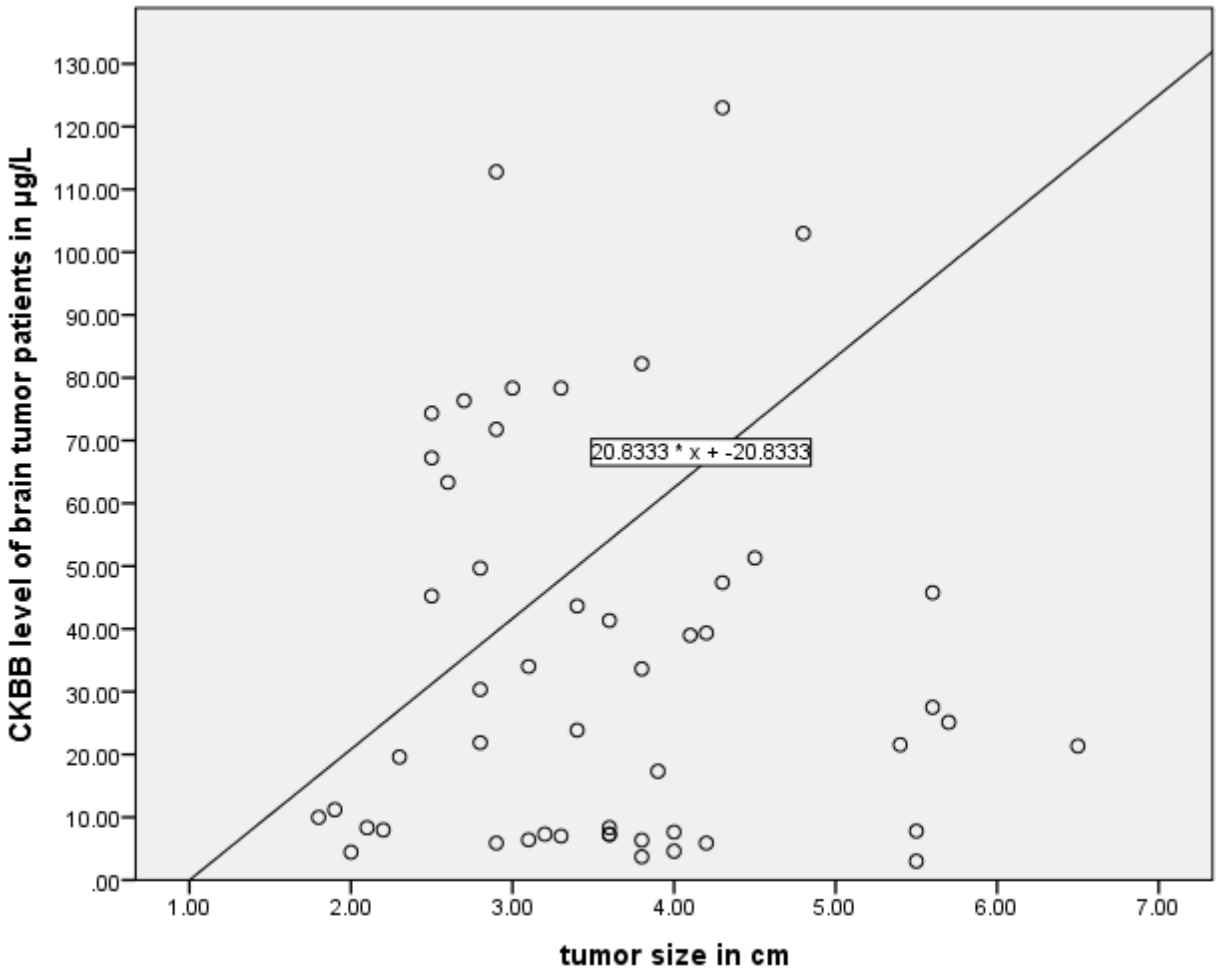


Figure 8: Scatter plot correlation between CK- BB level and tumor size in brain tumor patients

4.3.7. Association between total oxidant stress level and brain tumor

Table 11 showed the estimated odd ratio of brain tumor risk according to the serum TOS levels using bivariate logistic regression analysis. The risk of brain tumor was significantly increased by 1.535 times in the highest group for serum TOS compared to lowest TOS group (participants who had increased TOS level had the possibility of having brain tumor by a factor of 1.535) (p= 0.001, OR= 1.535, CI=1.247-1.889). Table 5, independent sample t-test showed that compared to

healthy control group the levels of TOS in brain tumor patients was significantly ($p < 0.001$) elevated.

Table 12 association of TOS and brain tumor, TASH, Addis Ababa, Ethiopia, 2017/18

Parameter	OR	p-value	95% Confidence interval
TOS	1.535	0.001	1.247-1.889

TOS total oxidative stress, OR odds ratio

4.3.8. Association of serum level of CK-BB and brain tumor

High serum concentration of CK-BB was found in brain tumor patients than healthy controls and it was significantly (p=0.001) associated with brain tumor as observed from result of chi-square test.

Table 13: Distribution of CK- BB in brain tumor patients and healthy controls at TASH, Addis Ababa, Ethiopia, 2018

		Brain tumor	Healthy control	*p*-value
CK-BB >10 µg/L	Yes	32(64%)	0(%)	0.001
	NO	18(36%)	40(100%)	

>10 µg/L abnormal high creatine kinase level, CK-BB creatine kinase BB

4. DISCUSSION

In this study, serum level of total oxidative stress (TOS) and creatine kinase BB (CK-BB) were determined in search of a potential biomarker for the early diagnosis, treatment follow up, prognosis and treatment target for brain tumor disease. Those serum parameters were studied on 90 (50 brain tumor patients and 40 apparently healthy control groups) participants. The result of the present study demonstrated that there were significantly higher serum level of total oxidative stress and CK-BB in brain tumor patients compared to healthy control groups ($p < 0.05$).

Altered redox status is implicated in mutagenesis and tumorogenesis, tumor cell proliferation, development of metastases, and resistance to chemotherapy and radiotherapy of brain tumor (Bogosavljević *et al*., 2015). Reactive free radical species may participate in carcinogenesis through induction of gene mutation which results in cell damage and the consequences of signal transduction and transcription factor, and the redox status of cancer cell usually differs from that of normal cell (Olinski *et al*., 2007). Olinski and his colleagues, (2007) reported that brain tumor is a disease of genes. Any agent capable of reacting with DNA and chemically modifying and damage DNA is tumorigenic. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have mutagenic and tumorigenic potential by causing somatic mutations and are the main factor responsible for brain tumor development. Levels of oxidative stress markers like MDA and 4-HNE are often elevated in serum, urine and tumor tissue of brain tumor patients as compared to healthy individuals (Olinski *et al*., 2007; Tafani *et al.,* 2015).

In the present study the TOS level in the serum of brain tumor patients were significantly ($p = 0.001$) higher than apparently healthy controls (Table 5). These finding are in agreement with numerous previous studies conducted by Hardiany *et al*., (2012); Olinski *et al*., (2007); Zukiel *et al.*, (2004); Zhao *et al*., 2012; Tafani *et al*., (2015). The possible reason for high level of total oxidative stress in brain tumor patients may be due to high free radical load (which leads to loss of polyunsaturated fatty acid in membranes and disturbing membrane integrity which in turn may result in the accumulation and release of lipid peroxidation products to the circulation of brain tumor patients) and insufficient antioxidants. Because of metabolic and signal aberration, tumor cells usually exhibit elevated oxidative stress levels (Gorrini *et al*., 2013). Brain tumor cell may also produce a significant amount of reactive oxygen species specially H_2O_2 without exogenous stimulation which consequently results in the development of a high oxidative stress condition leading to oxidative insult on DNA and reduced DNA repair which may promote tumor cell proliferation, metastasis and progression(Olinski *et al*., 2007).

Previous work suggest that although exact mechanisms of release remain unclear, newly synthesized macromolecules by both benign and malignant brain tumor cell are often released into the circulation. Because of alterations of tissue architecture in tumors microenvironment including alteration of vascular supply and changes related to abnormal growth and necrosis, damage and break down of blood-brain barrier, released tumor products may get access to the

circulation. CK-BB is found in high amount in the cytoplasm of both benign and malignant brain tumor, and often released to circulation and may appear in abnormal high concentrations (Silverman *et al*., 1979).

Our study showed that, 32(64%) of the 50 brain tumor patients had elevated serum CK- BB (>10µg/L) and the rest 18/50 (36 %) had normal serum CK-BB level. The study also shows that none of the healthy control (0/40) participants had above normal serum CK-BB and 8/40(20%) of them has no detectable serum CK-BB level. This indicates CK-BB activity is higher in brain tumor patients than healthy individuals. Our study result also showed that the mean catalytic activities of CK-BB were significantly ($p < 0.001$) increased in brain tumor patients when compared to healthy controls. This is in agreement with previous studies done by Herschkowitz and cumings, (1964); Schwartz *et al*., (1989); Ishiguro *et al*., (2014); Rubery *et al.,* (1982). It is believed that the source of the raised serum CK-BB is the tumor tissue itself(Rubery *et al*., 1982).

The possible reason for high activity of CK-BB in tumor cell may be due to the process of high cell proliferation, migration or invasion than normal cells. This is to say that large tumor cell population requires a higher and rapid energy source as compared to normal cell population. Under aerobic condition, tumor tissues can metabolize approximately ten –fold more glucose in a given time than normal tissue. In order to meet this large and rapid energy demand, tumor cells use CK-BB activity as one option which is helpful for metabolic requirement of tumor cells. The possible mechanism of high CK-BB activities may be due to higher expression of CK-BB gene in tumor cells as compare to normal cells(Coolen *et al*., 1979). Brain tumor cells with high tendency to growth and proliferation requires high and continuous energy supply in the form of ATP from high carbohydrate metabolism to maintain their cellular activities and elevation of CK-BB may serve these requirements. This increased CK-BB activity in brain tumor cell is often released into the circulation by mechanism of local cell destruction, exertion of compression effect on the surrounding compression sensitive normal brain cells and structures containing CK-BB activity and even may induce CSF pressure changes which may also release CK-BB and appear in an abnormally high concentration in the circulation of brain tumor patients (Rubery *et al*., 1982; Matias *et al*., 1986).

Comparison of redox conditions between malignant and benign tumors is essential for understanding the role of reactive free radicals in the pathophysiology of aggressive or malignant brain tumor (Bogosavljević *et al*., 2015). In the present study we compare oxidative stress level of malignant brain tumor (like glioblastoma) and benign brain tumor (like pituitary macroadenoma) and our result showed that the mean serum values of total oxidative stress in patient with malignant form of brain tumor was significantly($p<0.001$) higher than benign once. This finding is in accordance with the studies of Bogosavljević *et al*., (2015); Olinski *et al*., (2007) reported that level of total oxidative stress in glioblastoma were higher than meningioma. In discrepancy to our findings, Zhao *et al*., (2012) have found higher oxidative stress activity in meningioma compared to high-grade astrocytoma. This discrepancy might be related to

potentially lower number of study participant patients recruited in the previous study (10 meningioma and 10 high-grade astrocytoma).

Some of the mechanisms responsible for the higher oxidative stress level characteristic of cancerous(malignant) brain tumors as compared to benign once may be due to cancerous tumors may exhibit extensive granulocyte activation with a release of reactive oxygen species, tumors cells may stimulate the defense systems of the body so that they react against the tumor to produce cytokines that can produce large amounts of ROS(may be accountable for the elevated plasma level of TNF), release of a large number of cancer cells into the blood stream and their penetration into other target tissues, are generally responsible for increased oxidative DNA damage and decreased concentration of antioxidants in cancerous brain tumor (Olinski et al., 2007; Zhao et al., 2012; Bogosavljević et al., 2015).

In the same way, we also examined the serum CK-BB level of malignant and benign brain tumor patients. The result showed that CK-BB level of cancerous or malignant brain tumor patients were not significantly ($P>0.05$) different from benign brain tumor cases. Even though the different was not statistically significant, the serum concentration of CK- BB of malignant tumors was lower (25.98 ±25.98 µg/L) than the benign types (38±34.33 µg/L). In contrary to our finding pan and his colleagues, (2013) reported a statistically significant lower CK-BB level in malignant tumor patients than benign tumor patients(Pan et al., 2013).

The low serum CK-BB level activity in malignant cells may be due to the host immune response. Immune response is an important factor in determining tumor development, progression and metastasis. CK-BB was demonstrated to be an important regulator of T cell development and activation. The intracellular ATP concentration and the ATP regeneration capacity of T cells were considered to have strong impact on T cell response signal strength. CK-BB helps keep the ATP pool constant and takes an important part in immune response affecting cancer development and progression, and decreased serum CK-BB levels may reflect the status of immunity of the host against the cancer(lower the CK-BB levels, the poorer the host immunity to malignancy was, contributing to tumor progression(Pan *et al*., 2013).

Tumor size is considered a highly valuable prognostic factor for brain tumor next to the location and the type of brain cell that makes the tumor. Tumor size was of prognostic importance independent of other known prognostic variables like: age and type of tumor, as brain tumor patients with larger tumor size lesions are often associated with significantly poor clinical outcome(Chang *et al*., 2017). In our study tumor size was; 3/50 cases (6 %) ≤ 2 cm, 33/50 cases (66 %) 2-4 cm, and 14/50 cases (28%) > 4 cm. The result indicates a low incidence of smaller tumors (≤ 2 cm) and high incidence of larger brain tumors (2-4 cm and> 4 cm). This could be attributed to a later stage of detection and presence of certain genetic or environmental carcinogens which lead to development of large tumor size in the locality(Benhawy and Sheredy, 2014).

In this study, the mean TOS level of brain tumor patients with tumor sizes of > 4cm was higher compared to tumor sizes of both between 2-4 cm and ≤ 2 cm and TOS level of tumor sizes of between 4-2 cm was again higher than tumor sizes of ≤ 2 cm indicating the TOS level gradually increase as the tumor size increase. This result may suggest the involvement of oxidative stress in tumor growth and progression. However the difference in TOS level across different tumor sizes of brain tumor patients was not statistically significant (p=0.323). These reports disagree with the reports of Mahlke et al., (2011); Hardiany et al., (2012). Mahlke and his colleagues, (2011) reported that a persistent increase in oxidative stress level in brain tissue is important not only for brain tumor initiation but also for tumor promotion and progression. Oxidative stress appeared to promote and regulate brain tumor growth by promoting brain tumor angiogenesis, tumor cell proliferation, metastasis and limiting tumor cell apoptosis, which are all essential for tumor growth in size, progression to more aggressive and metastasis of existing tumors (Mahlke et al., 2011).

Furthermore, our study result showed that TOS and size of brain tumor were positively but non-significantly correlated (r= 0.15, p=0.28). In contrary to our findings many studies have reported a statistically significant and positive correlation between TOS level and brain tumor size Conti et al., (2010); Hardiany et al., (2012); Zhao et al., (2012); Chang et al., (2017); Leone et al., (2017). Oxidative stress and deregulation of redox signaling are strongly involved in any steps of brain tumor development(Hardiany et al., 2012). Brain tumor progression and growth is a complex, multistep process that includes resistance to apoptosis, loss of control of the cell cycle, angiogenesis, and the acquisition of invasive properties. Within a growing tumor microenvironment a number of different biomolecular event occur but it is largely orchestrated by inflammatory molecules which play an indispensable role in the growth process by promoting proliferation, survival and migration of tumor. High oxidative stress level in brain tumor cell induces tumor cell growth and self-renewal by targeting protein kinase A and Notch signaling pathways(Conti *et al*., 2010). The possible reason for these differences may be due to different study design that is cross sectional or sample size of study participant in different laboratories.

Oxidative stress may affect proliferation of brain tumor cells by a ligand-independent transactivation of receptor tyrosine kinase and may induce tissue invasion and metastatic dissemination by activation of metalloproteinases. Oxidative stress can also induce release of vascular endothelial growth factor and angiopoietin which may promote brain tumor angiogenesis and anoikis(Leone *et al*., 2017).

Surprisingly, even within the different sizes of brain tumor CK-BB value was different. In the results section(Table 10), Analysis of variance showed that the mean serum CK-BB level in patients with>4 cm tumor was higher than in patients with 4-2cm and ≤ 2 cm tumors and CK-BB level of tumor sizes of between 4-2 cm was again higher than tumor sizes of ≤ 2 cm. But the difference in CK-BB level among different tumor size was not significant (p>0.05). Even though it was not statistically significant CK-BB activity gradually increases with increasing tumor size which may suggest that high CK-BB activity may be important for brain tumor progression in

size. This finding was in contrary with a study by pan and his colleagues, (2013). They demonstrated that the mean serum CK-BB level in patients with larger tumor size was significantly lower than in patients with lower tumor size(Pan et al., 2013). The possible reason for these differences may be due to different study design that is cross sectional or sample size of study participants in different laboratories.

Furthermore, the present investigation also demonstrated that in brain tumor patients a negative or inverse and statistically non-significant ($r= -0.013$, $p>0.05$) correlation were obtained between serum level of CK-BB and size of brain tumor lesion which is the indicators of the volume of damaged brain tissue (as the amount of damaged brain tissue increased, the value for CK-BB decreased). This lack of significant negative correlation between tumor size and serum level of CK-BB may be due to an intact blood-brain barrier, the rapid elimination or inactivation of CK-BB, or some combination of these factors (Schwartz *et al*., 1989). These findings are in contrary with the study of Pan and his colleagues, (2013) reported a statistically significant negative correlation between CK-BB level and tumor size. The lower CK-BB activity the poorer the host immunity to tumor cells and this may contribute to tumor progression or development to large size. CK-BB activity progressively decreased with progression of tumor size and may be totally absent in full-grown tumors largely due to reduced gene expression, rather than increased protein degradation(Pan et al., 2013). Furthermore, CK-BB activity in other types of cancer patient such as breast cancer, lung cancer, and prostatic cancer patients reported with significant positive correlation with tumor size (Carney *et al*., 1984; Forman, 1979).

Generally, tissues have different rates of metabolic activity and oxygen consumption. When cells have a high production of reactive species than cellular antioxidant defenses attempts by the cells to remove these toxic specious induces oxidative stress. Oxidative stress has long been implicated in cancer development and progression(Pacheco and Gonsebatte, 2009). Free radicals (reactive oxygen and nitrogen species) are powerful DNA damaging agents. They can induce base modification (alteration in guanine and thymine bases), free base sites (apurinic and apyrimidic), strand breakage, sister chromatid exchanges and DNA-protein cross linking. This may inactivate additional tumor suppressor genes within tumor cells or further increase expression of proto- oncogenes. Genetic instability due to persistent carcinoma cell oxidative stress will therefore, increase the malignant potential of the tumor (Hardiany et al., 2012; Brown and Bicknell, 2001). Damage due to free radicals and oxidative stress is one of the most important factors in brain tumor initiation, development and progression (Olinski et al., 2007; Sotgia et al., 2011).

The brain is one of the most metabolically active tissues in the body and consumes about 15–20% of the total energy generated in our body(Cobleya *et al*., 2018). Because of increased mitochondrial energy metabolism from high glucose and PUFA metabolism high concentration of reactive free radical is produced. To combat the high ROS and eradicate oxidative stress damaged macromolecules, brain cells utilize highly effective antioxidant defenses, efficient repair and removal mechanisms. However, the brain is more susceptible to oxidative stress than

other organs when compensatory antioxidant defenses fail to counteract excess oxidative stress which may result in impairment in neuronal function and oxidative induced DNA mutations (Garbarino *et al*., 2015; Wagner *et al*., 2013).

Brain tumor cells show accelerated metabolism than normal cells which results in increased production of reactive oxygen species specially H_2O_2. Furthermore altered activity of other sources of ROS, inadequate oxygen supply, may also promote ROS production and dysfunctional redox signaling in brain tumor cell (many critical mutations in oncogene proteins affect redox-sensitive regulatory moieties) which all contribute to increased level of oxidative stress and inefficient removal by antioxidative system (Bogosavljević et al., 2015). Regarding serum TOS and brain tumor risk, our study result revealed that oxidative stress was statistically significantly associated with brain tumor development and the risk of brain tumor development was increased by 1.535 times in the highest TOS level group (brain tumor patients) compared to low TOS level groups (healthy control)(OR= 1.535, 95% CI(1.247-1.889), P= 0.001). Overall, results of our study confirm that markers of OS are increased in brain tumor patients compared with healthy controls. These results are consistent with recent studies examining the risk of oxidative stress in brain tumor development Jorgenson et al., (2013); Yasueda et al., (2016); Conti et al., (2010); Tews, (1999); Ramiro et al., (2016); Chang et al., (2017). Oxidative stress can promote pro-tumorigenic signaling, facilitate tumor cell proliferation, survival, and adaptation to hypoxia(Reczek and Chandel, 2016).

Supporting the key involvement of oxidative stress in carcinogenesis, patients with different cancers type and taking anti-oxidants (N-acetyl-cysteine, metformin) either alone or as adjuvant with chemotherapy and radiotherapy showed anti-tumor properties like regression of tumor size, less risk of mortality as well as reduced rates of recurrence by reducing reactive oxygen and nitrogen species production from mitochondrial(Sotgia et al., 2011). A review article by Yasueda and his colleagues, (2016) also showed that minerals and vitamins with antioxidant capacity like ubiquinone, carotenoids, retinol, tocopherol, ascorbic acid, and folate significantly increased survival rate and improve clinical response of cancer patients during chemotherapy and/or radiotherapy(Yasueda et al., 2016).

Neuroglia or nerve cells of brain contain high CK-BB isoenzyme activity to maintain the ATP level from carbohydrate metabolism mainly for the movement of ions across the nerve cell membrane. Increased glycolytic metabolism in most brain tumor cells confers a selective advantage for survival and proliferation in the unique hypoxic tumor microenvironment and is accompanied with tumorogenesis termed the Warburg effect. Compared to other CK isoenzymes CK-BB is rapidly eliminated or inactivate. The half-life of CK-BB in human serum was estimated to be around 5 h .Therefore, measurable CK-BB activity is rarely seen, and in the serum of normal adults it is very low and below the detection limit of most methods(Schwartz et al., 1989). In brain tumor patient destruction of cerebral tissues and alteration of cellular permeability may occur resulting in increased CK-BB activity in the circulation. Brain tumor demonstrated dysregulation of CK-BB activity (Pan et al., 2013). Brain tumor has increased CK-

BB enzymes activity often released it into the circulation upon breakdown of tumor and surrounding cerebral tissue and found abnormal high amounts of this isoenzyme in the serum of brain tumor patients. An intact blood-brain barrier seems to block the penetration of CK-BB activity into the serum effectively but brain tumor patients exhibited some damage and break down of their blood-brain barrier (Herschkowitz and cumings, 1964).

In this study we found significant percentage of brain tumor patients with abnormally high concentrations of CK-BB in their serum. Chi-square test showed that the high serum concentration of CK-BB found in brain tumor patients was significantly ($p=0.001$) associated with brain tumor. This finding is in agreement with the findings of several previous studies where people with brain tumor have been shown to have a higher serum CK-BB level, suggesting that CK-BB isoenzymes may be used as biologic marker and may have some predictive value in early diagnosing, staging, identify early recurrence of the tumor and monitor the efficacy of various therapeutic modalities of brain tumor Ishiguro et al., (2014); Silverman et al., (1979); Rubery et al., (1982); Durany et al., (1997); Matias et al., (1986). Since CK-BB is produced in high concentration by a wide variety of tumors the potential use of serum CK-BB as a screening test for a particular tumor is limited(Rubery et al., 1982).

Serum CK-BB is produced and increased in the serum of patient with various malignant and non-malignant conditions. Patients with several different adenocarcinoma types, prostatic carcinoma, breast, colon, ovary, and colorectal tumors may have increased CK-BB in their serum suggesting that CK-BB may be a marker of more general applicability rather than a specific marker associated with specific untreated brain tumors, so this must be substantiated by further investigation. Strong evidence associating CK-BB with malignant cells has been lacking. The presence of increased CK-BB in most malignant and benign brain tumor patients alone may not be a confirmatory diagnostic biological marker but can be used as an adjunct to the histologic (cytologic) and radiologic diagnosis of brain tumor. Individuals with typical clinical symptoms suggestive of brain tumor and with abnormal high concentration of CK-BB might increase the sensitivity of tumor detection(Silverman et al., 1979).

6. CONCLUSION

The present study demonstrated that in brain tumor patients the serum total oxidative stress and creatine kinase BB level were significantly elevated in comparison to healthy controls. Patients with malignant brain tumor suffer a high degree of oxidative stress than benign brain tumors but CK-BB level was not significantly different between benign and malignant cases. The study also found a non-significant difference and correlation in the total oxidative stress and CK-BB level among different brain tumor size suggesting oxidative stress may not play key role in the progression and growth of brain tumor and level of CK-BB may not depend on the clinical characteristics of tumor. The total oxidative stress and CK-BB level was significantly associated with brain tumor. Therefore, it is possible to conclude that increased oxidative stress may have significant influence in the initiation of brain tumor and individuals with high oxidative stress level may have high risk of developing brain tumor. Measuring of serum TOS and CK-BB level may be simple non-invasive method for brain tumor diagnosis among suspected cases of brain tumor and reduction of oxidative stress is thought to be a very important measure for primary prevention of brain tumor.

7. RECOMMENDATION

- It is recommended that supplementation of diet and an antitumor therapy with antioxidant is a part of treatment plan for the management of brain tumor patients.
- Brain tumor patients have to modify their feeding habit with high intake of fruit and vegetables, legumes, grains and cereals, fish and antioxidant levels with their therapy.
- Monitoring or evaluation of free radical and CK-BB level in brain tumor patients will be important in diagnosis of brain tumor and can indicate disease progression and clinical outcome of treatments.
- This study is short, cross sectional and more studies need to be done in larger cohort of participants using additional oxidative stress markers including oxidatively damaged DNA metabolites and important enzymatic antioxidants.
- Chemotherapy and radiotherapy induce the production of free radicals; and thus, assessment of oxidant /antioxidant status of brain tumor patients on or after treatment is recommended.
- Controlled studies are required to be conducted to see the impact of antioxidant supplementation in brain tumor patients in an attempt to decrease the effect of oxidative stress.
- It is expected that more studies will be undertaken in Ethiopia because data on biological biomarkers for screening of brain tumor and role of oxidative stress in brain tumor development are very meager in developing countries.

8. STRENGTH AND LIMITATION OF THE STUDY

8.1. Strength of the Study

Measurement of total oxidative stress and CK-BB level in brain patients and correlations with its development is attempted for the first time in Ethiopia. Therefore, this study is expected to offer the baseline information for further studies of brain tumor patient in Ethiopian population, in relation to initiation and growth of brain tumor.

The study involved healthy control samples to clearly show the contrasting features in oxidative stress and CK-BB observed in brain tumor patients.

8.2. Limitation of Study

Although this study revealed pertinent information with respect to oxidative stress and CK-BB in brain tumor patients, the sample size screened was small and cross-sectional and due to this the result of this study may not reflect total population of brain tumor patients in Ethiopia. The cost of reagents and supplies were high so that we have limited ourselves to 90 study participants and the different assays that would have been measured were also proportionately limited. In addition we did not investigate the impact of treatment, surgery, chemo and radio therapy, on oxidative stress and CK-BB level. Influences of other factors like diet, medication were also not taken into consideration during this study. Furthermore the total antioxidant capacity was not assessed.

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10. ANNEXES

10.1. Information sheet (English Version)

Research Project: The evaluation of total oxidative stress and Creatine kinase (BB) level for screening and diagnosis of brain tumor among brain tumor patients attending Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia.

Sponsoring organization: Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University

Principal Investigator: Fitalew Tadele (BSC, MSc in biochemistry candidate)

Advisors: Dr. Solomon Genet, Dr. Menakath Menon, Dr. Abat Sahlu

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, three advisors; from Addis Ababa University biochemistry department and neurosurgery clinic. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to evaluate the total oxidative stress and creatine kinase BB level among brain tumor patients so that we will suggest if oxidative stress involves in the pathogenesis of brain tumor and if creatine kinase can be used as a biological marker for the diagnosis of brain tumor.

Procedures and what will be expected from you for participation

In order to perform the indicated study at Tikur Anbessa hospital you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected by a principal investigator and nurses. Then, you are requested to give your consent to the sample collector. After consent, 5ml blood specimen will be collected from you by specimen collector and face to face interview for additional questions.

Potential risks and Discomforts

There will be minor discomfort during blood specimen collection. During collection of specimen from you, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. But based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the early detection of brain tumor. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is completely voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result for free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Name, Fitalew Tadele Phone: 0918290166 E-mail: fitalewtadele@gmail.com

10.2. Informed consent (English version)

Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University, Consent form for the participation of the study participants in the research project.

Name of the study participant ……

Code number…..…….

I have clearly been informed about the research project that it aims to evaluation of total oxidative stress and Creatine kinase (BB) level for screening and diagnosis of brain tumor among brain tumor patients attending Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. The objectives of the research project have clearly been explained to me and I have been told that the results obtained from me will help me as well as the community for better management of the brain tumor disease. I had been also informed about the confidentiality of this research project. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study.

Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and blood sample as the doctors find best for me.

Signature: _____ Date_____

10.3. Questionnaire (English version)

Thank you for your willingness to participate; your cooperation is very important to the success of the study. This is a questionnaire you are asked to fill out. Please answer the questions as frankly and accurately as possible. All information obtained in the study will be kept confidential.

1. Personal identification:

a) Full name of the subject: _____

b) Subject code number: _____

c) Card number._____

2. Demographic detail

a) Age: _____

b) Gender:

Male □ Female □

c) Region: _____

d) Marital status:

Single □ Married □ Divorce □

e) Educational level: Illiterate □ primary school High School □ College and above □

f) Occupation _____

3. Residential area: Rural □ Urban□

4. Group: study □ Control□

5. Alcohol consumption

Do you consume alcohol? □ Yes □ No

6. Smoking 1. Do you smoke? □ Yes □ No

7. Body Mass Index:

a) Weight (in Kg) _____

b) Height (m)_____

c) BMI_ (Kg/m2) _____

8, family history of brain tumor yes □ no □

9, Exercise activities

How often do you do any (can be strenuous) exercise?

Rarely/never □ Less than once a week □

Once a week □ 2-3 times a week □

4-6 times a week □ every day □

For physician or clinician use only

A) Brain tumor type, benign □ malignant □

B) Brain tumor size, < 2 cm □ between 2-4 cm □ > 4 cm □

THANK YOU FOR YOUR PARTICIPATION

10.4. Information sheet (Amharic version).

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል።እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ።

የጥናቱ ርዕስ. Evaluation of Total oxidative stress and Creatine kinase BB level among brain tumor patients for screen and diagnosis of brain tumor at Tikur Anbessa specialized hospital Addis Ababa Ethiopia.

እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቀሚና ምቹ ሆነው ተመርጠዋል። የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ። በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም።በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል።

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደም ናሙና እንደሚወሰድና ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል።ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል።ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም። ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል አርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል።በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም። ነገር ግን ደም ሲወሰድ መጠነኛ የህመም ስሜት ሊያስከትል ይችላል።ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም።

ህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው።ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት። የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን brain tumor ለማወቅና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል።

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ።ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት።የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ። ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ።

አድራሻ፡- FitalewTadele ሞባይል 0918290166

ኢ-ሜይል: fitalewtadel@gmail.com

10.5. Informed consent (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

ለዚህ ጥናት መረጃና የስምምነት ቃሌን የምሰጠዉ በአጠቃላይ የጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፈቃደኝነት ነው። በመጠይቁ ላይ የምሰጠዉ የእኔ መረጃ እንደማይባክን እንደሚያዝም ተነግሮኛል። በተጨማሪም ጥናቱ ዉስጥ ላለመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት ጭምር መብቴ መሆኑንና ከጥናቱ በመዉጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ ተገልጾልኛል። ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ፈቃደኝነቴን ሰጥቻለሁ።

በተጨማሪም የምሰጠዉ የደም ናሙና ለ Total Oxidative Stress እና creatine kinase BB ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ። ማንኛዉንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ። በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች በጊዜዉ ለሀኪሜ እንደሚሰጥልኝ እና ዉጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል። በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤአለሁ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

እኔ_____የተባልኩት ግለሰብ ይህን ሁሉ በማገናዘብ በምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ።

ፊርማ _____ ቀን_____

ተሳታፊ _____ _____

10.6. Amharic questionnaire

የቤተሰብ መረጃ መሰብሰቢያ ቅጽ ቁጥር

መጠይቅ ክፍሌ አንድ፡ የቤተሰብ ሁኔታ

A. የቤት ቁጥር…….

B. እድሜ……

C. ጾታ…….

D. ክልል…….

2. የጋብቻ ሁኔታ ሀ. ያላገባ ለ.ያገባ ሐ. የፈታ/ች መ. ባለቤቷ/ቱ በህይወት የለሌ

3. የቤተሰብ ኃላፊ የትምህርት ደረጃ ሀ. ያልተማረ ለ. መጻፍና ማንበብ የሚችሌ ሐ. የመጀመሪያ ደረጃ መ. ሁለተኛ ደረጃ እና ከዛ በላይ

4. የቤተሰብ የስራ ሁኔታ ሀ. የመንግስት ሰራተኛ ለ. አርሶ አደር ሐ. ነጋዴ መ.ሌላ (ይግለጹ)

5. የመኖሪያ ቦታ ሀ. ከተማ ለ. ገጠር

6. የሰውነት ክብደት ልኬት (ኪ.ግ./ሜ2)……

7. ክብደት በኪሎ ግራም…….

8. ቁመት.....

9. የአልኮል መጠጥ አዉ የለም

10. ሲጋራ ማቸሰ ትዉ የለም

11. የሰዉነት አካላዊ እንቅስቃሴ

የሰዉነት አካላዊ እንቅስቃሴ ምን ያህል ጊዜ ያዘወትራሉ

ምንም እንቅስቃሴ አላደርግም ከ 1 ያነሰ ሳምንት በሳምንት 1 ቀን

2-3 ጊዜ በሳምንት 4-6 ጊዜ በሳምንት

12. በቤተሰብ ዉስጥ የቸንቅላት ካንሰር ታሪክ አለ አለ የለም

10.7. Declaration

I declare that this research paper entitled Evaluation of total oxidative stress and Creatine kinase (BB) level for screening and diagnosis of brain tumor among brain tumor patients attending Tikur Anbessa specialized hospital Addis Ababa, Ethiopia is my original work and has not been presented for any degree in any other university. All sources of materials used for the research have duly been acknowledged.

Fitalew Tadele

Signature_____

Date_____

10.8. General procedures and reagents for measuring total oxidative stress

Procedure: Reagent 1 and 2 were prepared.

Reagent 1: [114mg of xylenol orange and 8.18gm of NaCl] were dissolved in 900mL of H_2SO_4 solution 25mM. The final reagent was composed of 150mM xylenol orange, 140mM NaCl, and 1.35M glycerol, pH 1.75.

Reagent 2: [1.96 gm of ferrous ammonium sulfate and 3.17 gm of o-dianisidine dihydrochloride] were dissolved in 1000mL of H_2SO_4 solution 25mM. TOC of serum was measured by adding 225µL reagent 1 into 35µL serum sample and then 11µL of reagent 2 was added. Finally, absorbance was measured by ELISA microplate reader LT 4000 at wavelength 560 nm.

Blood samples were obtained following an overnight fasting state, and were collected on ice at 4°C. The serum samples were separated from the cells by centrifugation at 3000 rpm for 15 min and were stored at -80°C and used for the analysis of the Total Antioxidant Status (TAS) and Total Oxidant Status (TOS).