ROS

RESEARCH ARTICLE

Oxidative Stress and Serum Creatine Kinase BB Levels can Help Mark Severity and Stage of Brain Tumor

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ABSTRACT | The exact cause of brain tumor is still unknown, and it is a multi-factorial disease. Brain tumor can originate from brain cells or secondary from other organs via metastasis. Generally, tumor cells show aberrant metabolism of free radicals. The current study aims at evaluating creatine kinase BB (CK-BB) and total oxidative stress (TOS) levels among brain tumor patients for diagnosis, prognosis, and treatment strategy. Hospital-based comparative cross-sectional study was conducted on a total of 90 participants (50 brain tumor patients and 40 healthy controls) from April 2018 to October 2018. Venous blood samples were collected for TOS and CK-BB activity measurement. Purposive sampling technique was implemented to select study participants in the hospital. Catalytic activity of CK-BB and TOS were significantly increased in the serum of brain tumor patients as compared to controls. TOS levels in patients with malignant brain tumor were significantly higher than those with benign tumor, but CK-BB levels were not significantly different between benign and malignant types of brain tumor. Both serum levels of TOS and CK-BB were significantly associated with brain tumor. The CK-BB activity was significantly associated with brain tumor and can be used as markers for diagnosis. Participants with increased TOS had significantly higher risk of brain tumor development compared to healthy controls. In conclusion, oxidative stress status and CK-BB level may be used as possible brain tumor markers.

KEYWORDS | Brain tumor; Cancer stage; Creatine kinase BB; Oxidative Stress; Tumor marker

ABBREVIATIONS | BMI, body mass index; CI, confidence interval; CK-BB, creatine kinase BB; MRI, magnetic resonance imaging; OR, odds ratio; ROS, reactive oxygen species; TOS, total oxidative stress

CONTENTS

1. INTRODUCTION

Brain tumor is a multi-factorial and debilitating disease. It is characterized by uncontrolled proliferation and growth of cells originating from intracranial cells and the meninges. The incidence and prevalence of brain tumor is poorly understood, as there is scarcity of data. Globally the prevalence of brain tumor is very low, estimated around 4.3 to 18.6 per 100,000 per year [1]. In infants and young children, brain tumor is the second most common form of cancer and in adolescents and young adults brain tumor ranges from 5th to 8th most frequent cancer [2]. In 2012, 256,134 new cases of brain cancer were reported around the world [3]. Despite the increased incidence and prevalence, brain tumor continues to receive a relatively low public health priority in Africa, because of limited economic resources and neglection [4]. In a hospital-based cancer registry conducted in West African countries in 1990, brain tumor was ranked third in incidence among all cancer. In Ethiopia in 2015, 362 male and 380 female new cases of brain tumor were estimated [5].

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 the adenosine triphosphate (ATP)-intensive neuronal and glial activity. The ubiquitous isoform of creatine kinase BB (CK-BB) is primarily found in the brain cells and associated with ion pumps [6, 7]. The high CK-BB activity in neuroglia and nerve cells of the brain provides energy to activate Na^+-K^+ ATPase and ATP-gated K^+ channels, which are essential for neural cell membrane potential and propagation of action potentials, activities that account for 50% of the brain's energy usage [8]. Brain tumor cells require large and rapid supply of energy for proliferation, and they use CK-BB activity as one option for energy delivery. A possible reason of high CK-BB activity may be the higher expression of CK-BB gene in tumor cells compared to normal cells [9].

In organisms, free radicals are produced continuously and spontaneously inside all metabolically active cells [10, 11]. These reactive species are produced endogenously from enzymatic reactions, phagocytosis, prostaglandin synthesis, and non-enzymatically in the lysosomes, mitochondria, peroxisomes, and other organelles. Apart from natural oxidants produced in the body, exogenous agents like solar and ionizing radiation, air pollutants, organic solvents, and heavy metals in food and water can also produce free radicals [12, 13]. Oxidative stress occurs as a result of an imbalance between oxidant generation and the capacity of the antioxidant systems of the body. Antioxidants in the body control the status of over oxidation, and a continuing imbalance in support of oxidation causes different problems when it beats the limit of such control. Free radicals and antioxidant can reinforce differing impacts on cells according to their concentrations [10, 14]. Reactive oxygen and nitrogen species (ROS/RNS) may participate in carcinogenesis via induction of gene mutations that result in cell damage and aberrated signal transduction and transcription factors, and the redox status of cancer cells usually differs from that of normal cells. Because of metabolic and signaling aberrations, cancer cells exhibit elevated oxidative stress levels [15, 16].

The human brain is a highly metabolically active organ which consumes much of the basal oxygen budget of the body with increased production of free radicals [17]. Because of this 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 oxidatively induced DNA mutations [18, 19]. The present study aimed at identifying early metabolic biomarkers for diagnosis, prognosis, and therapeutic targets for brain tumors. This might be possible through evaluation of CK-BB and total oxidative stress (TOS) levels in the serum of brain tumor patients.

2. MATERIALS AND METHODS

2.1. Subjects

A hospital-based comparative cross-sectional study was conducted at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. The study was conducted from April 2018 to October 2018. Fifty brain tumor patients and forty apparently healthy individuals as control group were included in the study. The study was ethically approved by the Ethical Review Committee of Biochemistry Department College of Health Sciences, Addis Ababa University, Ethiopia.

2.2. Blood Sample Collection Procedure

Socio-demographic data were recorded using structured questionnaire and before sample collection patients were well informed about the aim of the study and requested to donate venous blood sample (~5 ml). Then, venous blood samples and the subjects' responses to questionnaires were collected by professional nurses.

2.3. Blood Sample Preparation

Venous blood samples (50 brain tumor patients and 40 apparently healthy individuals) of ~5 ml were collected using sterile syringes and emptied to serum separator tubes. Serum samples were transferred into Eppendorf tubes after the blood samples were centrifuged at 2,600 *g* for 5 min and stored at −80°C until the day of analysis.

2.4. TOS Assay

TOS was measured based on the method of Erel [20]. The color intensity, which can be measured spectrophotometrically at 560 nm, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide (H_2O_2) and the results are expressed in terms of micromolar H_2O_2 equivalent per liter (μmol H_2O_2 Eq/L).

2.5. CK-BB Assay

CK-BB activity was measured spectrophotometrically following the method of Szasz et al. [21]. The change in absorbance was followed at 340 nm. Standardized procedures for the determination of CK-BB using the reverse reaction and activation by *N*-acetyl cysteine (NAC) were employed.

2.6. Tumor Size Determination and Tumor Stage Classification

Tumor size was measured from brain radiographic (CT-scan or MRI) images of brain tumor patients. Tumors were grouped into < 2 cm, = 2–4 cm, and > 4 cm based on their size. Classification of tumor tissues as benign and malignant was based on radiologic characteristics of the tumors from the CT-scan and MRI images.

2.7. Data Processing and Statistical Analysis

All data were checked, cleared, and fed into EpiData (version 3.5.1, Odense, Denmark), and then exported to SPSS (version 22.0, Chicago, IL, USA) software for statistical analysis. Descriptive analysis, Pearson correlation, Chi-square test, Binary logistic regression, independent sample t-test, and one-way ANOVA were used for this study. Continuous variables were expressed in mean ± SD, and $p \leq 0.05$ was considered as statistically significant.

3. RESULTS

The sociodemographic characteristics are displayed in **Table 1**. 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. Most of the study participants were male. Equal numbers of brain tumor patients in the study were living in rural and urban areas. Majority of participants had no family history of brain tumor. There was no statistically significant difference between patients and control groups in body mass index (BMI), physical exercise habits, and family history of brain tumor ($p > 0.05$). At the time of diagnosis, clinical and radiologic features of tumors of each brain tumor patient (CT scan or MRI) were examined. Among the 50 brain tumor patients, 31 were benign and 19 malignant tumor cases. In brain tumor patients, the mean tumor size was 3.6 ± 1.1 cm and most of them had tumor size between 2 cm and 4 cm (**Table 2**).

The serum levels of TOS in brain tumor patients and healthy control groups were measured and tabu-

Table 1. Sociodemographic results of brain tumor patients and controls

Variable		Brain tumor cases (n = 50)	Healthy controls (n = 40)
Age (year)		35.3 ± 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	Married	18(36)	27(67.5)
	Single	29(58)	13(32.5)
	Widowed	3(6)	0(0)
Education	Illiterate	21(42)	5(12.5)
	Primary	13(26)	7(17.5)
	Secondary	4(8)	6(15)
	College	12(24)	22(55)
Alchhol	Yes	26(52)	11(27.5)
	No	24(48)	29(72.5)
Smoking	Yes	0(0)	3(7.5)
	No	50(100)	37(92.5)
Family history	Yes	1(2)	0(0)
	No	49(98)	40(100)
Sport	No	31(62.2)	25(62.5)
	Once a week	4(8.4)	0(0)
	Twice a week	10(20.2)	15(12.5)
	4-6 times a week	5(11.6)	5(12.5)
BMI	< 18	3(6.3)	2(4.2)
	18–24.9	37(73.7)	30(75)
	25–29.9	10(20)	8(20.9)

Note: Categorical variables are presented in frequency and percentage (%) while continuous variables are presented as mean ± SD. BMI denotes body mass index.

Table 2. Clinical and radiologic characteristics of tumors of brain tumor patients

Tumor fcharacterustic		Frequency, n(%)	Mean ± SD (cm)
Tumor size (cm)	< 2	3(6)	1.9 ± 0.1
	2–4	33(66)	3.2 ± 0.55
	> 4	14(28)	5.1 ± 0.76
Tumor type	Benign	31(62)	
	Malignant	19(38)	

Note: Categorical variables are presented in frequency and percentage (%) while continuous variables are presented as mean ± SD.

lated in **Table 3**. Compared to the control group, the levels of TOS in brain tumor patients were highly significantly elevated ($p < 0.001$) (95% CI: 2.09–4.69). At the time of diagnosis, serum CK-BB levels of brain tumor patients and healthy controls were examined. Eight healthy controls had no detectable serum CK-BB level and the rest had no elevated enzyme level. Most brain tumor patients had elevated serum CK-BB levels. Brain tumor patients had higher levels of CK-BB than healthy controls (**Table 3**).

Table 3. Serum TOS and CK-BB levels among brain tumor patients and controls

Serum parameter	Brain tumor cases	Health controls	p Value
TOS (μmol H_2O_2 eq/L)	10.34 ± 3.23	6.95 ± 2.91	< 0.001
CK-BB (μg/L)	35.12 ± 31.25	3.87 ± 2.62	< 0.001

Note: TOS, total oxidative stress; CK-BB, brain type creatnine kinase.

Table 4. Serum TOS and CK-BB levels among brain tumor patients with benign and malignant forms

Serum parameter	Benign cases (n = 31)	Malignant cases (n =19)	p Value
TOS (μmol H_2O_2 eq/L)	8.86 ± 2.03	12.76 ± 3.38	< 0.001
CK-BB (μg/L)	25.98 ± 12.98	38.00 ± 34.33	0.357

Note: TOS, total oxidative stress; CK-BB, brain type creatnine kinase.

Table 5. Analysis of variance in TOS and CK-BB levels of brain tumor patients with different tumor sizes

Serum parameter	Tumor size (cm)			p Value
	< 2 (n = 3)	2–4 (n = 33)	> 4 (n = 14)	
TOS (μmol H_2O_2 eq/L)	9.35 ± 0.37	9.97 ± 3.17	11.43 ± 3.52	0.323
CK-BB (μg/L)	8.53 ± 3.60	34.95 ± 30.04	40.06 ± 3 4.76	0.293

Note: TOS, total oxidative stress; CK-BB, brain type creatnine kinase.

Our finding showed that TOS levels varied between benign and malignant brain tumor types. The TOS levels in malignant brain tumor patients were highly significantly elevated ($p < 0.001$) than the benign brain tumor patients (**Table 4**). Although it was not statistically significant, the serum levels of CK-BB of malignant tumor patients were higher than those of the benign tumor patients (**Table 4**). Tumor size was measured from brain radiographic (CT-scan or MRI) images of all brain tumor patients and the TOS levels in blood samples were compared between patients with tumor sizes of < 2 cm, = 2–4 cm, and > 4 cm. The mean TOS levels of patients with tumor sizes of > 4 cm were higher compared to patients with both 2–4 cm and < 2 cm tumors sizes, and patients with 2–4 cm tumor sizes have higher TOS level than those with < 2 cm tumor sizes. But the difference in TOS levels among patients with different brain tumor sizes were not statistically significant as shown in **Table 5**. Analysis of variance also showed that the serum CK-BB level was also higher in patients with larger (> 4cm) tumor sizes than those with both 2–4 cm and < 2 cm tumor sizes, and the level of CK-BB of patients with 2–4 cm tumor sizes was also higher compared to those with < 2 cm tumors sizes, but again the difference in CK-BB levels among patients with different tumor sizes was not statistically significant (**Table 5**).

The TOS levels of brain tumor patients were analyzed and correlated with the sizes of brain tumor. Correlation analysis showed that the levels of TOS were positively, but non-significantly, correlated with the sizes of tumor among brain tumor patients ($r= 0.15$, $p = 0.28$). The CK-BB levels of brain tumor patients were analyzed and correlated with the sizes of brain tumor. The serum levels of CK-BB also had a non-significant negative (inverse) correlation with the sizes of tumor in brain tumor patients ($r= –0.013$, $p = 0.813$). The risk of brain tumor was significantly increased by 1.535 times in the highest group of serum TOS compared to the lowest TOS group (participants who had an 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). Independent sample t-test showed that compared to the control group, the levels of TOS in brain tumor pa-

tients were significantly ($p < 0.001$) elevated, and higher serum concentrations of CK-BB were found in brain tumor patients than healthy controls ($p < 0.001$) associated with brain tumor from result of chi-square test.

4. DISCUSSION

Brain tumor is a health burden of both high and low economic levels and is associated with increased risk of permanent disability, morbidity, mortality, and/or decreased quality of life [22, 23]. Hence, early specific and sensitive biomarkers for diagnosis, prognosis, and potential treatment targets for brain tumor are required to improve outcomes and delay the progression to higher grade tumor [24]. This study showed that the serum TOS and CK-BB levels were significantly increased in brain tumor patients compared to controls, and this finding was consistent with those reported in previous studies [25–29]. A possible reason for the high levels of TOS in brain tumor patients may be the high free radical load that leads to oxidation of membrane lipids and disturbance of membrane integrity which in turn may result in the accumulation and release of lipid peroxidation products to the circulation. It is believed that due to metabolic and signal derangements, tumor cells usually exhibit elevated oxidative stress [19]. Brain tumor cells produce a significant amount of ROS, especially H_2O_2, without exogenous stimulation, which eventually results in the development of a high oxidative stress condition. This leads to oxidative insult on DNA and reduced DNA repair which may promote tumor cell proliferation, metastasis, and progression [26, 30].

Brain tumor tends to increase CK-BB levels in the serum, which could be due to damage and leaking out of the enzyme to the circulation. We found that the mean catalytic activity of CK-BB was significantly increased in brain tumor patients compared to healthy controls. This is in agreement with previous studies [31–34]. The CK-BB level in the serum samples was considered abnormal when it exceeded 10 µg/L [33]. The source of the raised serum CK-BB is the tumor tissue itself due to cell lysis. A high CK-BB activity in tumor cells may be due to the high cell proliferation, migration, or invasion compared with normal cells. The large tumor cell population requires a higher and more rapid energy source compared to normal cells. Under aerobic conditions, tumor tissues can metabolize more glucose than the normal tissue. In order to meet this large and rapid energy demand, tumor cells use CK-BB activity as one option for their energy supply. Another possible reason for the high CK-BB activity could be the high expression of CK-BB gene in tumor cells compared to normal cells [35].

The mean serum levels of TOS in patients with malignant brain tumor were significantly higher than those with benign ones. This finding agrees with researchers who reported that the levels of TOS in glioblastoma were higher than meningioma [26, 36]. Others found higher oxidative stress in meningioma compared to high-grade astrocytoma [28]. Reasons for the higher oxidative stress levels of malignant compared to benign brain tumors may be that cancerous tumors exhibit extensive granulocyte activation with release of free radicals. Tumor cells may stimulate the defense systems of the body so that they react against the tumor by producing cytokines and large amounts of ROS. Release of cancer cells into the blood stream and other target tissues can increase free radicals and decrease the levels of antioxidants in brain tumor [26, 28, 36].

The CK-BB levels of malignant brain tumor patients were higher than those of benign tumor cases. Similar to our finding, Pan et al. reported a significantly lower CK-BB level in malignant tumor patients than benign ones [37]. Tumor size is considered a highly valuable prognostic factor for brain tumor next to the location and the type of brain cells. Brain tumor patients with larger tumor size lesions are often associated with significantly poor clinical outcomes [38]. 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 indicating that the TOS level gradually increases as the tumor size increases. This result may suggest the involvement of oxidative stress in tumor growth and progression. oxidative stress appeared to promote and regulate brain tumor growth by promoting brain tumor angiogenesis, tumor cell proliferation, and metastasis and by limiting tumor cell apoptosis, which are all essential for tumor growth in size and progression to more aggressive forms and metastasis of existing tumors [39]. Furthermore, our result showed that TOS and size of brain tumor were positively but non-significantly correlated ($r = 0.15$, $p = 0.28$). Other studies reported a statistically signif-

icant positive correlation between the TOS levels and brain tumor sizes [25, 38, 40]. Oxidative stress and deregulation of redox signaling are strongly involved in many steps of brain tumor development. It was reported that a high oxidative stress level in brain tumor cells induced tumor cell proliferation, growth, survival, and self-renewal by targeting protein kinase A and Notch signaling pathways [15].

The serum CK-BB levels in patients with tumor sizes > 4 cm were the highest and those with sizes < 2 cm were the lowest. The finding that CK-BB activity gradually increased with increasing tumor size suggested that a high CK-BB activity may be important for brain tumor size progression. Furthermore, CK-BB activity in other types of cancer patients such as breast cancer, lung cancer, and prostatic cancer patients were reported having significant positive correlation with tumor sizes [41, 42]. We found that a high serum concentration of CK-BB was significantly ($p < 0.001$) associated with brain tumor. This agrees with findings of previous studies suggesting that CK-BB may be used as a marker and may have some predictive value in early diagnosing, grading, and identifying early recurrence of the tumor and in monitoring the efficacy of various therapeutic modalities of brain tumor [31, 33, 34]. The presence of increased CK-BB in brain tumor patients may not be a lone confirmatory biological marker but can be used as an adjunct to the histological and radiological diagnosis of brain tumor. Free radicals are powerful DNA-damaging agents. They can induce base modifications, strand breakage, sister chromatid exchanges, and DNA-protein cross linking. This may inactivate tumor suppressor genes of tumor cells or further increase expression of proto-oncogenes, thereby increasing the malignant potential of the tumor [25, 43]. The brain is more susceptible to oxidative stress than other organs when compensatory antioxidant defenses fail to counteract excess oxidative stress causing impairment in neuronal function [18, 19]. One of the most important factors in brain tumor initiation, development, and progression is oxidative stress [44]. Our study revealed that oxidative stress is significantly associated with brain tumor development and the risk of brain tumor development was increased by 1.535 times in the brain tumor group compared to controls [OR = 1.535; 95% CI, 1.247–1.889; $p = 0.001$]. These results are consistent with findings in recent studies [15, 38, 45–48]. Hence oxidative stress can promote pro-tumorigenic signaling and facilitate tumor cell proliferation, survival, and adaptation to hypoxia.

5. CONCLUSION

Serum TOS and CK-BB levels were significantly elevated in brain tumor patients. Patients with malignant brain tumor suffered a higher degree of oxidative stress than benign ones. The TOS and CK-BB levels were significantly associated with brain tumor. Increased oxidative stress significantly influences the initiation and aggravation of brain tumor. Measuring serum TOS and CK-BB levels may be a simple non-invasive method for brain tumor diagnosis and prognosis among suspected cases of brain tumor and may guide the treatment strategy of brain tumor. Measuring oxidative stress level and CK-BB may be used as additional markers for brain tumor diagnosis and prognosis.

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