Original Article
Middle East Journal of Cancer; April 2015; 6(2): 79-83

Pro-Oxidant - Antioxidant Balance in Patients with High Grade Glioblastoma Multiform

Mohammad Faraji-Rad*, Mehdi Khajavi, Mohammad Hassan Arjmand***, Esmat Shajari****, Daryoush Hamidi Alamdari*****♦**

**Department of Neurosurgery, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
***Department of Neurosurgery, Emdad Academic Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
****Department of Surgery, Ghaem Academic Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*****Department of Neurosurgery, Ghaem Academic Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
******Biochemistry and Nutrition Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

Abstract

Background: Gliomas are the most common primary brain tumors of the central nervous system. Among a number of different bio-molecular events, molecular connections between oxidative stress pathways and their development is prevalent. Oxidative stress is the consequence of an imbalance between pro-oxidant factors and antioxidant defense. This imbalance may lead to DNA damage and changes in growth and function of cells in the brain. Many evidences show that reactive oxygen species in the mammalian brain are directly responsible for cell and tissue function and dysfunction. A brain tumor is correlated with oxidative stress. In this study, we determine the pro-oxidant – antioxidant balance in patients with grade IV brain tumors (glioblastoma multiforme) by the pro-oxidant – antioxidant assay.

Methods: We collected sera from 50 patients with high grade (IV) glioblastoma multiform and 49 healthy subjects. The pro-oxidant - antioxidant assay was measured.

Results: There was a significant increase in pro-oxidant - antioxidant values in patients (158.10±85.71 HK unit) compared to the control group (74.54±33.54 HK; P=0.001.

Conclusion: The pro-oxidant - antioxidant balance assay can show a high level of pro-oxidants in the sera of patients with glioblastoma which indicates the presence of oxidative stress in this group.

Keywords: Glioblastoma multiform, Oxidative stress, PAB assay

♦Corresponding Author:
Daryoush Hamidi Alamdari, PhD
Biochemistry and Nutrition Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: Hamidiad@mums.ac.ir

Introduction

Numerous evidences show that reactive oxygen species (ROS) in the mammalian brain are directly responsible for cell and tissue function and dysfunction. The brain

Received: July 26, 2014; Accepted: November 24, 2014

has a particular predisposition to oxidative stress (OS) which makes it vulnerable to ROS. A brain tumor is correlated with OS. Oxidative stress is defined as an imbalance between pro-oxidants (POX) and antioxidants (AO) in favor of POX. Oxidative stress triggers lipid peroxidation of the cellular membranes, oxidation of proteins and DNA leading to changes in chromosome structure, genetic mutation, and/or modulation of cell growth. Subsequently, a genomic instability occurs which contributes to carcinogenesis. Glioblastoma (or glioblastoma multiforme; GBM) is the most common, aggressive type of glioma. Glioblastoma multiforme is generally located in the main lobes of the brain (otherwise known as the cerebral hemispheres), but can also be found in other brain regions. Most GBMs are advanced when diagnosed. They can invade normal brain tissue and spread from the original tumor location, but rarely to areas beyond the brain.[1]

Antioxidants clean up the excess amount of POX before they damage essential molecules. Antioxidants consist of the water soluble AO (vitamin C, urate, etc.), the lipid soluble AO (vitamin E, A, etc.) and the enzymatic AO (catalase, peroxidase, dismutase, etc.).[2]

The concentration of either POX or AO can be measured by individual POX and AO assays. For example, a diagnostic plot derived from the measurement of 82 assays which characterize both the OS and AO profiles. However, the effect of the POX or the AO molecules in serum is an additive effect which can lead to incorrect measurements. Consequently, various methods have been developed in order to measure total oxidants [such as total oxidative status (TOS) and ferrous ion oxidation xylenol orange (FOX) assays] or total AO [such as the ferric reducing ability of plasma (FRAP) and oxygen radical absorbance capacity (ORAC) assays], separately.[3,4] In this context, the PAB assay has been developed to simultaneously measure the balance of oxidants and AO in one experiment and give a redox index status unlike the above methods.[2,5]

In this study, we have determined the POX–AO balance in patients with high grade glioma brain tumor (IV) by the PAB assay. To the best of our knowledge, this is the first time that the PAB has been determined in patients with high grade glioma brain tumors.

Materials and Methods

Chemicals

In this study the following chemicals were used: 3, 3′, 5, 5′-tetramethylbenzidine powder (TMB, Fluka, Sigma Company, USA), 230 U/mg peroxidase enzyme (Applichem, A3791, 0005, Darmstadt, Germany), chloramine T trihydrate (Applichem, A4331, Darmstadt, Germany), and 30% hydrogen peroxide (Merck Company, Germany). Molecular biology grade reagents were used and preparations performed in double distilled water that was purchased.

Method of pro-oxidant - antioxidant balance (PAB) calculation

The PAB assay is the only available test that can measure the balance of oxidants and AO simultaneously in one experiment.[2,4] It uses two different types of reactions: an enzymatic reaction where the chromogen TMB is oxidized to a color cation (TMB^+) by peroxides and a chemical reaction where the TMB cation is reduced to a colorless compound by AO.[2-4] The photometric absorbance is then compared with the absorbances given by a series of standard solutions that are made by mixing varying proportions (0%–100%) of hydrogen peroxide with uric acid.[2] A low PAB value means that AO are present at greater concentrations than oxidants, while a high PAB value means more oxidants are present than AO. The standard solutions were prepared by mixing varying proportions (0%–100%) of 250 mM hydrogen peroxide with 3 mM uric acid in 10 mM NaOH. The TMB powder (60 mg) was dissolved in 10 ml dimethyl sulfoxide (DMSO). For preparation of the TMB cation solution, 400 ml of the TMB/DMSO solution was added to 20 ml of acetate buffer (0.05 M buffer, pH 4.5), followed by the addition of 70 ml fresh chloramine T (100 mM) solution. The solution was mixed well

and incubated for 2 h at room temperature in a dark place, after which 25 U of peroxidase enzyme solution was added. This mixture was aliquoted into 1 ml aliquots and stored at -20ºC. The TMB solution was prepared by adding 200 µl TMB/DMSO to 10 ml of acetate buffer (0.05 M buffer, pH 5.8) and the working solution was prepared by mixing 1 ml TMB cation solution with 10 ml TMB solution. This working solution was incubated for 2 min at room temperature in a dark place and immediately used. Ten microliters of each sample, standard, or blank (distilled water) were mixed with 200 µl of working solution in each well of a 96-well plate, which was then incubated in a dark place at 37ºC for 12 min. At the end of the incubation period, 100 µl of 2 N HCl was added to each well and the optical density (OD) was measured with an enzyme-linked immunosorbent assay (ELISA) reader at 450 nm, with a reference wavelength of 620 or 570 nm. A standard curve was generated from the values of the standard samples. The values of the PAB assay were expressed in arbitrary units (HK) used by the inventors of the PAB method[2,4] based on the percentage of hydrogen peroxide in the standard solution. The values of the unknown samples were calculated according to the standard curve.

Subjects

There are four grades of brain tumors: grade I (benign and not cancerous), grade II (relatively slow growing), grade III (malignant and cancerous) and grade IV (most malignant). In this study we analyzed sera from 50 patients with GBM, a grade IV brain tumor, who were admitted to Kamyab Hospital. Patients did not require surgical treatment and were under medical treatment. There were 49 healthy participants who comprised the control group that had no clinical problems. Written informed consents were obtained from participants. Samples were collected from April 2013 to June 2013. The PAB assay was measured.

The study protocol was approved in February 20, 2013 by the Ethics Committee for Clinical Research at the Mashhad University of Medical Sciences (MUMS).The approval number is 901139.

Serum collection

Blood samples (1 ml) were collected from each subject during the period of hospitalization where patients were admitted for treatment. Blood samples were collected from patients in the fasted state. Blood were sent to the laboratory for serum separation. Samples were centrifuged at 1500 g for 15 min at room temperature to obtain serum and stored at -80˚C. Hemolytic samples were excluded from analysis.

Statistics

The Statistical Package for the Social Sciences (SPSS) version 16.0 was used for statistical analysis. All parameters were given as mean ± SD and had normal distribution. The group comparisons were assessed by the independent t-test. The significance level was considered less than 0.05 with a confidence interval of 95%.

Results

We observed a significantly higher serum PAB value in the brain tumor patients (158.10±85.71 HK units) compared to the control group (74.54±33.54 HK units, P=0.001). There was a significant increase of OS in patients with GBM (grade IV) compared to the control group (P<0.05).

Discussion

This study showed increased OS in patients with GBM tumor compared to the healthy control group.

The formation of a glioma tumor is a complex process that involves neoplastic changes of cells, resistance to apoptosis, lack of cell cycle control and angiogenesis.[6] Many studies have shown that OS has a substantial role in this process and modulation of OS can have therapeutic potential in treatment.[7-9]

It has been shown that proinflammatory cytokine and OS can interact and provoke one

another, contributing to the development and progression of diseases such as diabetes and cancer.[10]

OS can cause increasing cytokine production by many different mechanisms. The increased POX levels are well known to mediate inflammatory signaling by activating various protein kinases such as c-Jun N-terminal kinase (JNK), phosphatidylinositol 3-kinase (PI3K), and protein kinase C (PKC). These kinases can stimulate redox sensitive transcription factors such as signal transducer and activator of transcription (STAT) and nuclear factor-κ-binding protein (NF-KB). The activation of transcription factors leads to the transcriptional activation of inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin (IL-1), IL-6, IL-8, and IL-18, chemokines, and chemoattractant protein-1 as well as growth factors (transforming growth factor-β, monocyte, and connective tissue growth factor).[11]

Nuclear factor-κ-binding protein has a role in the proliferation and survival of the glioblastoma cell line. A number of studies have shown that activation of NF-KB factor protects some glioblastoma cell lines in vitro from chemotherapeutic agents. Data have confirmed that tumor grade is associated with NF-KB activity; its activity increases progressively with malignancy.[7]

TGF-β2, produced by human GBM cell lines, is involved in the regulation of both suppression of anti-tumor immune surveillance and angiogenesis in malignant gliomas.[12] Malignant gliomas produce IL-10 as a local immunosuppression factor. Levels of expression are correlated with tumor grade.[13]

Different studies have shown that the marker of OS, prostaglandin E2 (PGE2), is produced in malignant glioma tumors and possibly contributes to the ability of the tumor cells to escape immune surveillance.[14]

Cyclooxygenase, as an pro-oxidant enzyme, plays a key role in inflammation through the transformation of arachidonic acid to inflammatory molecules. High cyclooxygenase staining predicts poor prognosis in patients with glioma in general and in patients with GBM in particular.[15]

It has been demonstrated that AO can down-regulate the pro-inflammatory cytokines through two possible mechanisms through their effect on transcription factors that are regulated by redox status and by influencing PGE2 synthesis.[16]

During prolonged OS, changes in brain AO enzyme activities that include superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT). These enzymes normally act to prevent or decrease brain damage caused by excess free radicals. However, they are controlled by polymorphic genes which can be altered by free radicals, leading to dysfunctions in the enzymes' activities. Rao et al. have shown that the red-blood-cell activity levels of SOD were decreased for most types of intracranial neoplasms.[17]

Conclusion

This study has shown the ability of the PAB assay to detect OS in patients with GBM. In further research, this easy elucidation of OS in these patients can be applied to develop effective AO therapies for devising strategies to lessen or delay progression and/or complications. The PAB assay can be used as a quick, one-step method to calculate OS in different sera.

Conflict of interest

No conflict of interest is declared.

Limitations of the study

This study had a limited number of samples due to the lack of patients with high grade gliobastoma at this hospital. In a future study, more samples are necessary for more complete results.

References

1. Ashby LS, Ryken TC. Management of malignant glioma: steady progress with multimodalapproaches. *Neurosurg Focus.* 2006;20(4):E3.
2. Alamdari DH, Paletas K, Pegiou T, Sarigianni M, Befani C, Koliakos G. A novel assay for the evaluation of the prooxidant-antioxidant balance, before and after antioxidant vitamin administration in type II diabetes patients. *Clin Biochem.* 2007;40(3-4):248-54.
3. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem.*

2005;38(12):1103-11.
4. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem.* 2004;37(2):112-9.
5. Alamdari DH, Ghayour-Mobarhan M, Tavallaie S, Parizadeh MR, Moohebati M, Ghafoori F, et al. Prooxidant-antioxidant balance as a new risk factor in patients with angiographically defined coronary artery disease. *Clin Biochem.* 2007; 41:375–80.
6. Conti A, Gulì C, La Torre D, Tomasello C, Angileri FF, Aguennouz M. Role of inflammation and oxidative stress mediators in gliomas. *Cancers (Basel).* 2010;2(2):693-712.
7. Schwartzbaum JA, Oxidant stress and glioblastoma multiforme risk: serum antioxidants, gamma-glutamyl transpeptidase, and ferritin. *Nutr Cancer.* 2000; 38(1):40-9.
8. Bhatti P, Stewart PA, Hutchinson A, Rothman N, Linet MS, Inskip PD, et al. Lead exposure, polymorphisms in genes related to oxidative stress, and risk of adult brain tumors. *Cancer Epidemiol Biomarkers Prev.* 2009; 18(6):1841-8.
9. Tanriverdi T, Hanimoglu H, Kacira T, ZihniSanus G, Kemerdere R, Atukeren P, et al. Glutathione peroxidase, glutathione reductase and protein oxidation in patients with glioblastomamultiforme and transitional meningioma. *J Cancer Res and Clin Oncol.* 2007; 133(9):627-633
10. Virgolici B, Mohora M, Stoian I, Lixandru D, Gaman L, Paveliu F. A comparative oxidative stress study obesity with and without diabetes mellitus. *Rom J Intern Med.* 2005; 43(3-4):261-8.
11. Ramana KV. ALDOSE REDUCTASE: New Insights for an Old Enzyme. *Biomol Concepts.* 2011;2(1-2):103-14.
12. Platten M, Wick W, Weller M. Malignant glioma biology: role for TGF-beta in growth, motility, angiogenesis, and immune escape. *Microsc Res Tech.* 2001;52(4):401-10.
13. Huettner C, Czub S, Kerkau S, Roggendorf W, Tonn JC. Interleukin 10 is expressed in human gliomas *in vivo* and increases glioma cell proliferation and motility *in vitro*. *Anticancer Res.* 1997;17(5A):3217-24.
14. Wojtowicz-Praga S. Reversal of tumor-induced immunosuppression: a new approach to cancer therapy. *J Immunother.* 1997 May;20(3):165-77.
15. Shono T, Tofilon PJ, Bruner JM, Owolabi O, Lang FF. Cyclooxygenase-2 expression in human gliomas: prognostic significance and molecular correlations. *Cancer Res.* 2001;61(11):4375-81.
16. Han SN, Meydani SN. Antioxidants, cytokines, and influenza infection in aged mice and elderly humans. *J Infect Dis.* 2000;182 Suppl 1:S74-80.
17. Rao GM, Rao AV, Raja A, Rao S, Rao A. Role of antioxidant enzymes in brain tumours. *Clin Chim Acta.* 2000;296(1-2):203-12.