Study of Some Antioxidants in Brain Tumor Patients

Kusay A. Al-Chalabi[1] , Saba Z. Al-Abachi[2] , Layla A. Mustafa[2]

[1]*Biology Dept. , College of Science , University of Mosul , Mosul , Iraq*

[2]*Chemistry Dept. , College of Science , University of Mosul , Mosul , Iraq*

(Received / / 2007, Accepted / / 2008)

Abstract

Oxidative stress may be a key feature, and hence important determinant of blood serum of brain tumor patients. To investigate this, we determined some antioxidants status [albumin, uric acid, glutathione, ceruloplasmin, nitric oxide, and superoxide dismutase activity (SOD) and lipid peroxidation status {malondialdehyde (MDA}] in a blood serum of (31) patients with different types of brain tumor. A follow-up study was done with (25) post operative patients. On comparison with blood serum from normal individuals, albumin was significantly decrease while uric acid, nitric oxide and malondialdehyde were significantly increase. Glutathione and superoxide dismutase were decreased, but the decrease was statistically insignificant. Ceruloplasmin levels remained in the normal range. In a comparative study of pre operative and post operative cases, serum albumin and malondialdehyde levels in post operative brain tumor patients were significantly increase, while the uric acid was significantly decrease than those in the pre operative state. There was no significantly difference in the serum level of glutathione, ceruloplasmin, nitric oxide and in the activity of (SOD). The conclusion of the present study showed that the brain tumor patients which have a compromised antioxidant status before surgery and it remains poor for one month following the operation.

Introduction

Oxygen metabolism in aerobic organisms has obvious beneficial effects, but adverse effects of oxygen also occur because of the generation of reactive oxygen species (ROS). Most macromolecules can undergo oxidative reactions that are mediated by ROS. The adverse effects of ROS on biological systems have become a major focus of current biomedical research [1].

Oxygen free radicals and lipid peroxides have been implicated in the pathogenesis of many diseases, including diabetes mellitus, cancer, rheumatoid arthritis, infections diseases, atherosclerosis, and aging [2]. Free radicals are atomic or molecular species containing one or more unpaired electrons. They are generally highly reactive species and tend either to lose an electron, thereby acting as reducing agents, or to gain an electron, acting as oxidizing agents. In aerobic cells, the most important free radicals reactants are oxygen derivates (hydroxyl radical, $OH^{\cdot}$, superoxide anion, $O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and certain transition metals. Cells possess an array of antioxidant defenses that help to prevent the formation of free radicals and to limit their damaging effects [3]. This defense system includes antioxidant molecules, such as albumin, uric acid, glutathione, and various antioxidant enzymes. Superoxide dismutase, the first line of defense against oxygen-derived free radicals, catalyses dismutation of O_2 to H_2O_2. Glutathione peroxidase, a selenoprotein, reduces both lipid and nonlipid hydroperoxides, as well as H_2O_2, and oxidizes glutathione. Oxidized glutathione is reduced back to glutathione by glutathione reductase [4].

Reactive free radicals that are formed within cells can oxidize biomolecules, leading to cell death and tissue injury. Free radicals can attack almost any component of the cell, but lipids, proteins and nucleic acids are particularly important targets. Lipids of cells membranes and organelles are frequently damaged, resulting in lipid peroxidation [5]. The process of lipid peroxidation involves oxidative degradation of polyunsaturated fatty acids to malondialdehyde, which is commonly measured by the chromogenic thiobarbituric acid reaction and expressed as total thiobarbituric acid reactive substrates [6].

The aim of this study is to determine some oxidative stress and antioxidant status in serum of patients with brain tumor before and after one month following the operation.

Tools & Methods

Patients and Control Subjects:

Patients were enrolled in the present study to the neurosurgery unit in Ibn-Sina Hospital in Nineveh Governorate.

Blood serum of (31) patients before surgery and blood serum of (25) patients postsurgery for one month were collected. These samples were diagnosed clinically and radiologically as having brain tumor ranging in their age between (15-70) years. Blood samples from (35) control subjects were obtained for comparison.

Samples:

Blood was freshly withdrawn by vene-puncture of each patient immediately before and after one month of operation.

Serum then were separated by centrifugation at (3000 xg) for (10) minutes, and then it was divided in aliquot and kept frozen at (-20 °C) for the different assays [7].

Method:

Albumin was determined by colorimetric method using manufactured kit by Syrbio [8].

Uric acid level was estimated by using the Uricase Enzymatic method of [9] using manufactured kit by Biomerieux.

Glutathione was measured according to the modified method of [7].

Ceruloplasmin concentration was estimated using the modified method of [10].

Peroxynitrite level was estimated following the modified method of [11].

Malondialdehyde was determined by the method of [6].

Superoxide dismutase activity was measured by using a modified photochemical nitroblue tetrazolum method [12].

Results and Discussions

The results in table (1) showed that there is a significant decrease ($P<0.001$) in albumin concentration in serum of

patients with brain tumors which was (3.80 ± 0.13 and 4.21 ± 0.11 gm/dl) in a pre and post operative brain tumor patients respectively, in comparison with (5.05 ± 0.05 gm/dl) in control. The percent decrement for pre and post operative brain tumor patients was about (25% and 17%) respectively compared with control.

Table (1): Concentration of some oxidative stress and antioxidant status in serum of brain tumors and control subjects

Parameters	Mean ± SE		
	Control n = 35	Pre operation n = 31	Post operation n = 25
Albumin (gm/dl)	5.05 ± 0.05	3.80 ± 0.13***	4.21 ± 0.11***
Uric acid (µmole/L)	389.4 ± 16.8	667.8 ± 33.49***	519.0 ± 21.31***
Glutathione (µmole/L)	6.01 ± 0.13	5.85 ± 0.13	5.55 ± 0.1**
Ceruloplasmin (µmole/L)	297.05 ± 13.85	297.92 ± 17.52	293.63 ± 20.56
Peroxynitrite (mmole/L)	38.35 ± 1.24	156.26 ± 11.49***	161.03 ± 9.4***
Malondialdehyde (µmole/L)	0.14 ± 0.008	0.45 ± 0.04***	0.58 ± 0.03***
Superoxide Dismutase	0.005 ± 0.0008	0.006 ± 0.0004	0.006 ± 0.0005

*** Significant difference between control at (P< 0.001)
** Significant difference between control at (P< 0.01).

In the follow- up study (i.e. after one month of operation) albumin concentration in post operative brain tumor patients were significantly higher(P<0.05) than those in the pre operative state as shown in figure (1).

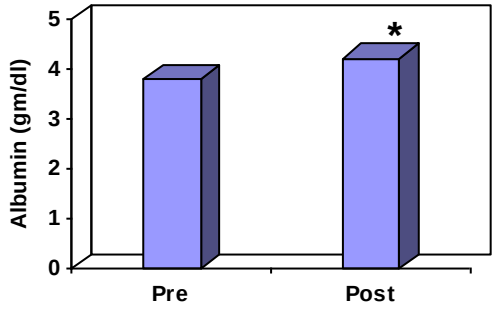

Fig. (1) Comparison of albumin concentration between pre and post operation

The results of the present study was agree with those obtained by Alta'ee in patients with different types of cancer [13] and from lung cancer patients [14].

These results of albumin concentration may be due to that serum proteins play several roles in the human body, some of them, such as albumin may be considered an important component of plasma antioxidant activity, primarily, binding free radicals, free fatty acid, divalent cations, hypochloride (HOCl) and bilirubin [15].

Thus, albumin concentration may be changed under oxidative stress associated with cancer. Low albumin levels (hypoalbuminaemia) are found in many conditions, where there are: 1. impaired synthesis as a result of liver disease [16]. 2. increased breakdown of protein due to tissue damage or inflammation, 3. reduced absorption of amino acids [17].

The concentration of serum albumin remains low after one month post operatively in carcinoid tumor patients. However the results showed that serum albumin in the post operative was still lower than the controls, but higher than the pre operative stage. It has been well documented that serum albumin, in particular, are very much affected by all types of trauma including the surgical operation. The degree of hypoalbuminemia after surgical operation depends on the severity of injury and the pre, and post operative nutritional state [14].

The results in table (1) showed a significant increase (P<0.001) in unic acid concentration which was (667.8 ± 33.49 and 519.0 ± 21.31 µmol/L) in serum of pre and post operation brain tumor patients respectively in comparison with (389.4 ± 16.8 µmol/L) in control group. The percent increment was about (71% and 33%) in the two groups of patients respectively in comparison with control group.

There were several results which were conformable to our results of uric acid concentration in patients with lymphomas and leukemia [18], and in patients with glioma cells [19].

Uric acid is the final break down product of nucleic acid and purine catabolism in human, its formation occurs only in tissues that contain the enzyme xanthin oxidase. The increased body burden of uric acid is a result of increased *de novo* purine synthesis, increased purine nucleoide degradation diminished renal excretion of urate, or a combination of these defects. The causes of the increment could be due to the increased nucleoprotein production and catabolism which are important in the hyperuricemia that occurs with brain tumors [18].

On the other hand, the results of comparison between pre and post operative patients showed that serum uric acid in the post operative patient was significantly (P<0.001) lower than those in the pre operative cases as shown in figure (2). During one month post surgery, little improvement in uric acid concentration was seen in brain tumor patients. These results are due to that the uric acid is one of the antioxidant which present in plasma in high concentration. It efficiently scavenges radicals. Urate might be particularly important in providing protection against certain oxidizing agents. It has been suggested that the increase in life span that has occurred during human evolution might be attributable to the formation of stable non-reactive complexes with iron, but it is also a direct free radical scavenger [2].

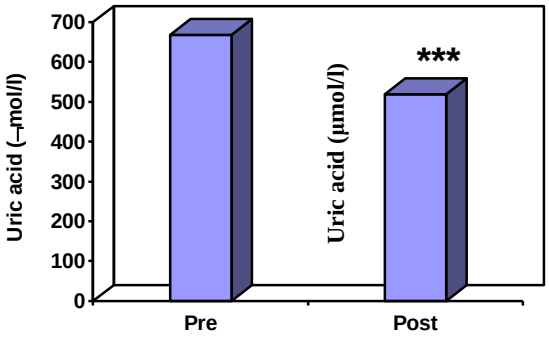

Fig. (2) Comparison of uric acid concentration between pre and post operation

Compared with control group, glutathione concentration was found to be decreased in serum of pre operative patients, but the decrease was not significant, while an obvious significant decrease (P<0.01) was found in post operative brain tumor patients as shown in table (1).

Also the results indicated that there was no significant difference in serum glutathione concentration in pre and post operative patients as shown in figure (3). Pervious studies have shown low levels of glutathione in patients with brain tumors [20] and in patients with breast, bladder, liver cancers [13].

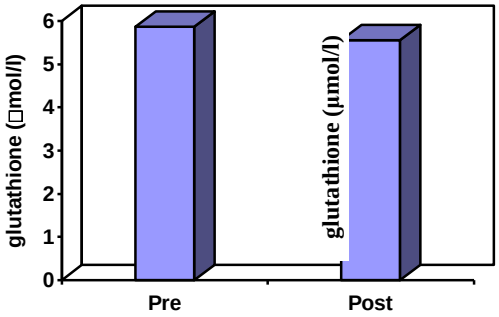

Fig. (3) Comparison of glutathione concentration between pre and post operation

Glutathione plays an important role in the protection of cells against damage from free radicals and also influences cytotoxicity to some kinds of chemotherapeutic agents [20]. Another investigation suggested that the glutathione depletion could be attributed to the fact that the intracellular level of glutathione is varied with growth, nutritional status, hormonal balance and the hepatic resource of glutathione [12]. Or the low level of glutathione is due to that the glutathione is one of the most abundant reductant and acts as a thiol donor converting disulfides to thiols and as a cofactor for glutathione peroxidase and glutathione S-transferase [21].

In this study, pre and post operative serum concentration of ceruloplasmin was measured in patients with brain tumor. The results in table (1) and figure (4) showed that there were no significant differences in ceruloplasmin concentration in pre and post operative brain tumor patients.

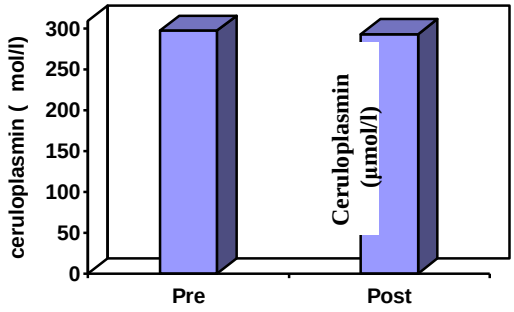

Fig. (4) Comparison of ceruloplasmin concentration between pre and post operation

Serum ceruloplasmin had variable levels in different states studied ; being high in the gynaecological carcinoma [22], low in the nephrotics and almost unchanged in the primary hyper triglyceridemia [23]. In spite of the ceruloplasmin may contribute to the oxidative damage produced by ROS which affect major classes of biomolecules, nucleic acid, proteins and lipid [23].

Patients with brain tumor showed a significant overproduction (P<0.001) in peroxy nitrite concentration in pre and post operative brain tumor patients, which was (156.26 ± 11.49 and 161.03 ± 9.4 mmol/L) respectively in comparison with (38.35 ± 1.24 mmol/L) in control. The percent increment was about (307% and 320%) respectively.

Also, the results in figure (5) showed that there was no significant difference in peroxy nitrite concentration before and after operation of brain tumor patients.

These results were in accordant with other studies which they show a significant higher of peroxy nitrite concentration in patients with prostate cancer [24].

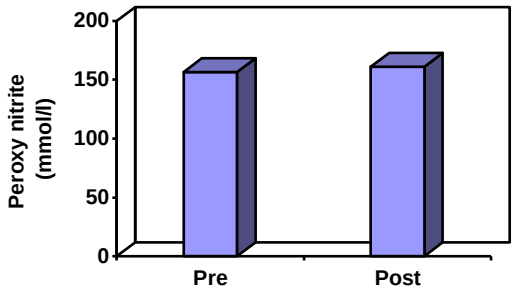

Fig. (5) Comparison of Peroxy nitrite concentration between pre and post operation

The role of nitric oxide (NO) in cancer is ubiquitous. (NO) was reported to inhibit cell proliferation, to induce differentiation, and to decrease the metastatic spread of different tumor cell lines [25]. In addition, (NO) is a reactive compound and can react with other free radicals such as superoxide ($O_2^{\cdot-}$) and may cause the production of the more destructive compound (Peroxy nitrite) [26]. Accordingly, it may be suggested that oxidant stress and (NO) may have multiple effects on the intiation and progression of cancer [27]. In addition, (NO) can be a very effective antioxidant to the ROS. The antioxidant

mechanisms is through the versatile chemistry of (NO) with ligand-metal and radical-radical. Because of the extremely complexity in nitric oxide function and mechanism [27].

Results in table (1) showed a significant increase ($P<0.001$) in malondialdehyde (MDA) concentration which was (0.45 ± 0.04 and 0.58 ± 0.03 µmol/L) in serum of pre and post operation brain tumor patients respectively in comparison with (0.14 ± 0.008 µmol/L) in controls. The percent increment was about (221% and 314%) respectively which is in agreement with other published data [28], [3].

Free radical attack essentially effects polyunsaturated fatty acid in cell membranes, producing lipid peroxidation which generates hydro peroxides and long lived aldehydes.

The end products of these reactions are MDA. It is also known that oxidative stress has a role in the process of cancer and inflammation [29]. Lipid peroxidation products and ROS have been found to be very active in binding to DNA to cause mutation and initiate cancer [28]. Also lipid peroxidation products formed or increased in various organs are released into serum. Several aldehydes including MDA have been shown to react with nucleic acids, thus possibly contributing to mutagenesis and carcinogenesis. Oxidative damage to membrane lipids initiated lipid peroxidation [29].

In the follow-up study and after one month of operation, MDA level in post operative brain tumor patients was significantly higher ($P<0.01$) than those in the pre operative cases as shown in figure (6). During one month post surgery, no improvement in extent of lipid peroxidation was seen in these patients. Higher level of MDA was associated with depletion of antioxidant and several forms of scavergers, which may suggest that there is an increase in oxidative stress in brain tumor patients [30].

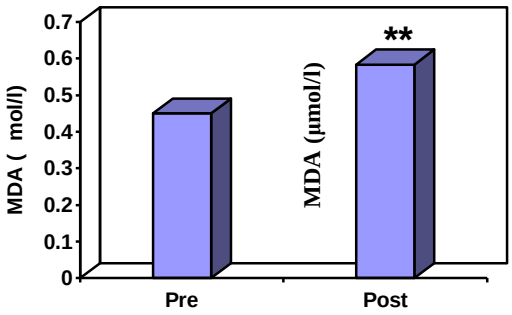

Fig. (6) Comparison of MDA concentration between pre and post operation

In this study, pre and post operative serum activity of superoxide dismutase (SOD) enzyme was measured in patients with brain tumor. The results in table (1) and in figure (7) indicated that there were no significant differences in SOD activity in patients compared to the controls, nor between pre and post operative value for this enzyme in the patient group.

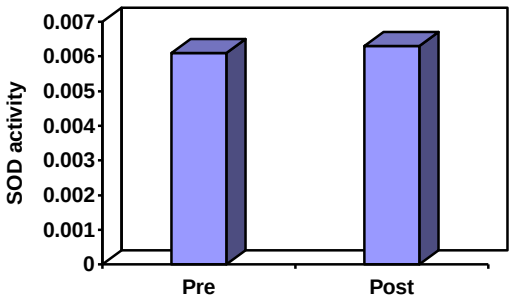

Fig. (7) Comparison of SOD activity between pre and post operation

There were several results which were observed a significant diminished level of SOD activity in brain tumor patients [31] and in patients with gastric cancer [32]. No explanation can be given for this at present except, may be a non accurate test due to a lack in equipment accuracy.

References

1. Kamat J. P. and Devasagayam T. P. (2000). "oxidative damage to mitochondria in normal and cancer tissues, and its modulation". Toxicology, 155: 73-82.
2. Young I. S. and Woodside J. V. (2001). "Antioxidants in health and disease". J. Clin. Pathol., 54: 176-186.
3. Celil U., Seyithan T. and Nuri B. (2003). "Lipid peroxidation and antioxidant enzyme activites in experimental maxillary sinusitis". Annals of Clin & Laboratory Sci. 33: 18-22.
4. Polat M., Taysi S., Gul M., Cikmon O., Yilmaz I., Bakan E. and Erdogan F. (2002). "oxidant/antioxidant status in blood of patients with malignant breast tumor and benign breast disease". Cell Biochem. Funct, 20: 327-331.
5. Gupta S., and Deshmuk U. (1994). "Formation and function of free radicals in human body". Ann Nat. Acod. Med. Sci. (India), 30 (1): 45-54.
6. Benge J. A. and Aust S. D. 1978. "Estimation of serum malondialdehyde level". Methods, in Enzymology, Academic press, London, 51: 302.
7. Tietz N. W. (1999). "Text Book of Clinical Chemistry". 3rd ed., Burtis C. A. and Ashwood E. R. (eds) W. B. Saunders Company, London. pp. 192-250.
8. Webester D. 1977. "The lmmediate reaction between bromocresol green and serum as a measure of albumin content". Clin. Chem., 23 (4): 663-665.
9. Newman D. J. and Price C. P. (1999). "Renal function and nitrogen metabolism". In Tietz Textbook of Clinical Chemistry, 3rd ed., Burtis C. A. and Ashwood E. R. (eds). W. B. Saunders Company, London. pp. 1239-1250.
10. Menden E. E., Boiano H. M., Murthy L. A. and Petering H. G. 1977. "Modification of phenylene diamine oxidase method to permit non-antomated ceruloplasmin determination in batches of rat serum or plasma micro samples". Analtical, 10: 197-204.
11. Vanuffelen B. E., Van Derzee J. and Dekoster B. M. (1998). Biochem J., 330: 719. Cited by Al-Zamely etal., (2001).
12. Al-Zamely O. Y., Al-Nimer M. S. and Muslih R. K. (2001). "Detection the level of peroxy nitrite, and related with antioxidant status in the serum of patients with acute myocardial infarction". Nation J. of Chem. 4: 625-637.
13. Alta'ee A. H. H. (2003). "A new relationship between cytidine deaminase activity and cancer via oxidative hypothsesis". M. Sc. Thesis, College of Science, University of Babylon, Iraq.
14. Al-Ani A. T., El-Hassani N. B., and Al-Hadithi A. H. (1997). "A study of serum total protein and its electrophoretic patterns in lung cancer patients". Iraqi J. pharm. Sci., 8 (1): 1-15.
15. Halliwell B. (1997). "Antioxidants and human disease: a general introduction", Nutr. Rev., 55: 44-52.
16. D'Hases P. C., Couttenye M. M., Lamberts L. V., Elseviers M. M., Goodman W. G. and Schrooten I., (1999). "Aluminum, iron, lead, cadmium, copper, zinc, chromium, magnesium, strontium and calicium content in bone of end-stage renal failure patients". Clin. Chem., 45 (9): 1548-1556.
17. Mayne P. D. (1998). "Clinical Chemistry in Dignosis and Treatment". 6th ed., Arnold a member of the Hodder Headline Group plc., London. pp. 157-159.
18. Henry J. B. (2001). "Clinical Dignosis and Management by Laboratory Methods". 20th ed., W. B. Saundres company. USA. pp: 184-187.
19. Griguer C., Oliva C., Kelly E., Giles G., Lancaster J. and Gillespi G. (2006). "Xanthine oxidase-dependant regulation of hypoxia-inducible factor in cancer cells". Cancer Res., 66 (4): 2257-2263.
20. Matsumoto Y., Saoka N., Tsuchida T., Fujiwara T., and Nagao S., (1992). "Quantitative analysis of glutathione and glutathione S-transferase in human brain tumors, C_6 rat glioma cells and drug resistant C_6 cells", No Shinkei Geka. 20 (10): 1069-1074.
21. Jianfang Hu (2001). "Oxidative stress and aging". Medical Laboratories, free Radicals in Biology and Medicine 77: 222.
22. Anita Ch., Felix W. and Mano A. (1993). "Serum ultrafiltrable copper, total copper and ceruloplasmin concentrations in gynaecological carcinomas". Ann. Clin. Biochem. 30: 545-549.
23. Al-Kaisey H., Hammodi N., Al-Shamma Gh. And Al-Shamma I. (2002). "Oxidative stress in hyperlipidemia of various etiologies". J. Fac. Med. (Baghdad), 44 (2): 253-258.
24. Marilenna K., Anastassia H. and George N. (2000). "Wine antioxidant polyphenols inhibit the proliferation of human prostate cancer cell lines". Nutrit and cancer, 37 (2): 223-233.
25. Bani D., Masini E., Bello M., Bigazzi M. and Sacchi T. (1995). "Relaxin activates the L-arginine-nitric oxide pathway in human breast cancer cells". Cancer Res. 55: 5272-5275.
26. Jonsen E. G. (1994). "Nitric oxide reactivity". Chem. Eng. News, 14: 4-6.
27. Zhen G. (2003). "The anti-oxidant effects of nitric oxide". Free radicals in biology and medicine: 77: 222.
28. Uzun K., Vural H., Ozturk T., Ozer F., and lmecik I. (2000). "Diagnostic value of lipid peroxidation in lung cancer". Eastern J. of Medicine, 5 (2): 48-51.
29. Singal p., petkau A., Hrushovetz S. and Foetster J., 1988. "Free radicals in health and disease, Molecular and Cellular Biochemistry". 84: 121.
30. Murray R., Granner D., Mayes P. and Rodwell V. (2003). "Harper's Illustrated Biochemistry". 26th . ed., lnge Medical Books/Mc Graw-Hill Companies. pp. 118-121.
31. Aggarwal S., Subberwal M., Kumar S. and Sharma M. (2006). "Brain tumor and role of β-carotene, α-tocopherol, superoxide dismutase

patients with gastric cancer pretiminary study". Pol. Merkur lekarshi. 19 (112): 521-525.

and glutathione peroxidase". J. of Cancer Research and Therapeutics. 2(1): 24-27.

32. Czeczot H., Scibior, D., Skrzycki M. and podsiad M. (2005). "Antioxidant barrier in

دراسة بعض مضادات الاكسدة في مرضى اورام الدماغ

قصي عبد القادر الجلبي[1] و صبا زكي العباچي[2] و ليلى عبد الله مصطفى[2]

[1] *قسم علوم الحياة ، كلية العلوم ، جامعة الموصل ، الموصل ، العراق*

[2] *قسم الكيمياء ، كلية العلوم ، جامعة الموصل ، الموصل ، العراق*

(تاريخ الاستلام: / /٢٠٠٧ ، تاريخ القبول: / /٢٠٠٧)

الملخص

يتضمن البحث قياس بعض مضادات الاكسدة في مصل دم مرضى أورام الدماغ والتي تشمل (الألبومين – حامض اليوريك – الكلوتاثايون – السيرو بلازمين – اوكسيد النتريك وفعالية أنزيم السوبر اوكسيد دسميوتيز (SOD) وقياس مستوى المالونداياليديهايد كدالة لدرجة تأكسد الشحوم في مصل دم (٣١) مريض مصابين بأورام الدماغ. ثم متابعة (٢٥) مريض بعد إجراء العملية الجراحية. إضافة إلى مجموعة من (٣٥) شخصاً طبيعياً كمجموعة سيطرة.

تشير النتائج إلى وجود نقصان معنوي في تركيز الألبومين بينما تظهر زيادة معنوية في حامض اليوريك، اوكسيد النتريك والمالونداياليديهايد في مصل دم المرضى المصابين بأورام الدماغ مقارنة مع مجموعة السيطرة، بينما لوحظ أن تركيز الكلوتاثايون وفعالية أنزيم SOD قد أظهرت انخفاضاً غير معنوياً (حددت فعالية أنزيم SOD في مرضى أورام الدماغ بطريقة غير مباشرة من خلال ظهور دلالة في انخفاض الكثافة الضوئية للفورمازين المتكون من اختزال $O_2^{\cdot-}$ لصيغة نايتروبلوتترازوليوم مع NBT والذي بدوره يتولد مع تشعيع مصل الدم). أما فيما يتعلق بمستوى السيريلوبلازمين فقد وجد انه يبقى ضمن مستواه الطبيعي.

عند دراسة المقارنة بين المرضى قبل وبعد العملية فان النتائج تشير إلى أن مستوى الألبومين والمالونداياليديهايد في مرضى أورام الدماغ بعد إجراء العملية قد ازداد معنوياً بينما يظهر حامض اليوريك انخفاضا معنوياً مقارنة بالحالة قبل إجراء العملية. كما تبين النتائج عدم وجود تغيراً معنوياً في مستوى كل من الكلوتاثايون–السيريلوبلازمين–اوكسيد النتريك وفعالية أنزيم SOD.

نلاحظ في البحث الحالي أن مرضى أورام الدماغ الذين يملكون مضادات أكسدة قليلة قبل إجراء العملية أنها تبقى ضعيفة بعد شهر واحد من متابعة المرضى.