RESEARCH ARTICLE

Trace Elements, Heavy Metals and Other Biochemical Parameters in Malignant Glioma Patients

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Abstract

Aim: We aimed to assess relevance of mineral, trace element and heavy metal levels in patients with malignant gliomas. Methods: In this study, erythrocyte catalase (CAT), and carbonic anhydrase (CA), serum copper (Cu.), zinc (Zn), lead (Pb), iron (Fe), cadmium (Cd), cobalt (Co), manganese (Mn), and magnesium (Mg) levels were measured in 22 healthy humans and 22 malignant glioma patients. Metal concentrations were assessed by atomic adsorption spectrophotometry, while biochemical methods were used for CAT and CA. Results: The Cd, Fe, Mg, Mn, Pb and Zn levels were significantly elevated in the patients as a whole compared to controls($P<0.05$), while Cu was decreased and Co demonstrated no change. Although mean CAT activity were significantly lowered, CA exhibited significant increase. Conclusions: The results of the current study indicate that antioxidant enzymes may have a role in the genesis of considerable oxidative stress in patients with malignant glioma. CAT and CA seem to play particular roles in the pathophysiology of this disease.

Keywords: Malignant glioma - catalase - carbonic anhydrase - trace elements - heavy metals

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Introduction

Gliomas are the most common intra-axial tumors arising from the central nervous system. They are graded from low to high grade tumors by their histologic appearance and grade 3 and 4 are considered malignant glioma. The malignant gliomas tend to be faster growing, more aggressive and more invasive into the surrounding brain tissue. Anaplastic astrocytoma and glioblastoma multiforme are the most malignant and aggressive form of gliomas which have very poor prognosis.

Lipid peroxidation which is mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues (Tas et al., 2005). Antioxidants (CAT, Gpx) are compounds that dispose, scavenge, and suppress the formation of free radicals or oppose their actions and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated (Cotgreave et al., 1988; Sies, 1991). During the past few years, superoxide radical activity in tumor cells has received increasing attention. For this reason, many researchers have turned towards the research for SOD and CAT activity, which are the basic antioxidants in damaged tumor cells (Gonzales et al., 1984; Guner et al., 1996; Cobanoglu et al., 2010).

Carbonic anhydrase is a tumor-associated metalloenzyme that belongs to the physiologically important family of at least 13 different mammalian carbonic anhydrases, CAs (Jarvela et al., 2008). Numerous studies in the last decade have demonstrated fundamental roles of carbonic anhydrase (CA) in tumor progression and shown a negative correlation in CA levels in cancer cases (Gramlich et al., 1990; Venta, 1991; Yokoyama et al., 1997; Pastorekova et al., 2008). Relatively a few studies have been done to estimate antioxidant enzymes in malignant glioma.

Living organisms have developed mechanisms of utilising vital trace elements such as zinc and copper, and reducing to the minimum the toxic influence of heavy metals like cadmium, mercury and lead (Solioz et al., 1994). Pb has been shown to pass the blood– brain barrier, which may result in elevated lead levels in brain tissue. Pb is thought to play a facilitative role in carcinogenesis, involving inhibition of DNA synthesis and repair, oxidative damage and interaction with DNA-binding proteins and tumor suppressor proteins (Inskip et al., 1995; Wijngaarden and Dosemeci, 2006). Cd is a ubiquitous toxic heavy metal and, unlike organic compounds, it is not biodegradable and has a very long biological half- life (Messner et al., 2009). Zn is an important trace element in biology. An important pool of Zn in the brain is the one present in synaptic vesicles in a subgroup of glutamatergic neurons. In this form it can be released by

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Table 1. Descriptive Statistics and Comparison Results for Studied Chacteristics

Parameters	Control Group				Patient Group				p
	Mean	St. Dev.	Max.	Min.	Mean	St. Dev.	Max.	Min.	
Cd (µg/dI)	0.003	0.003	0.01	0.001	0.04	0.03	0.1	0.007	0.001
Co (µg/dI)	0.03	0.03	0.09	0.002	0.04	0.03	0.11	0.002	0.44
Cu (µg/dI)	1.28	0.23	1.78	1.00	0.94	0.06	1.03	0.84	0.001
Fe (µg/dI)	0.71	0.21	0.96	0.4	1.25	0.33	1.76	0.5	0.001
Mg (µg/dI)	14.0	1.42	16.3	10.6	16.7	1.76	19.2	13	0.001
Mn (µg/dI)	0.02	0.008	0.04	0.01	0.06	0.01	0.1	0.05	0.001
Pb (µg/dI)	0.16	0.05	0.25	0.09	0.53	0.17	0.79	0.31	0.001
Zn (µg/dI)	0.42	0.12	0.58	0.23	1.00	0.42	2.34	0.49	0.001
CA EU/(gHb)-1	0.28	0.12	0.46	0.04	0.85	0.38	1.81	0.15	0.001
CAT EU/(gHb)-1	32.4	6.42	47.1	20.4	8.34	2.12	11.2	3.76	0.001

electrical stimulation and may serve to modulate responses at receptors for a number of different neurotransmitters (Cuajungco and Lees, 1997). This study is the first one to show the relationships of erythrocyte CAT and CA activities, and the serum levels of Cu, Zn, Co, Fe, Mn, Mg, Cd and Pb in malignant glioma.

The aim of this study was to investigate CAT, CA, some mineral, trace element and heavy metal concentrations in malignant glioma.

Materials and Methods

Biochemical Analysis

22 patients with malignant glial tumors were operated in Neurosurgery Clinic of Yuzuncu Yil University between january 2007 to july 2010. The age of patients ranged between 35-75 (mean, 55). 16 of these patients (74%) were diagnosed as glioblastome multiforme and 6 (26%) were diagnosed as anaplastic astrocytoma in histopathologic examination. Venous blood samples of the patients with malignant glioma radiologically (MRI) were obtained before operation and diagnosis was confirmed histopathologically after surgery. The study included a total of 44 subjects (22 brain tumor and 22 healthy human) and venous blood samples of malignant glioma were obtained from the antecubital fossa vein in patient with malignant glioma in accordance with the guidelines set out in the Declaration of Helsinki. Consent was given by family members of all the patients included in this work. The study was approved by the local ethics committee. Serum was separated by centrifugation and the samples were processed immediately. The serum samples were placed in deionised polyethylene tubes and kept at -80 centigrade in a deep-freeze (without thawing) until the day of study.

Determination of serum concentrations of Cu, Zn, Mg, Mn, Pb, Co, Cd, and Fe was performed by Atomic Absorption Spectrophotometer measurements, in which a UNICAM-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK) was used.

Biochemical analysis of CAT activity in erythrocytes was performed with a method described by Aebi (Aebi, 1984) in the Biochemistry Laboratory of Chemistry Department, Faculty of Science, Yuzuncu Yil University. Briefly, the supernatant (0.1 ml) was added to a quartz cuvette containing 2.95 ml of 19 mmol l-1 H2O2 solution prepared in potassium phosphate buffer (0.05 M, pH 7.00). The change in absorbance was monitored at 240 nm for

Table 2. Area Under the Curve Test Result Variable(s): Catalase (CAT)

Area	Std. Error(a)	Asymptotic Sig. (b)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	0.00	0.00	1.00	1.00

a, Under the nonparametric assumption; b,Null hypothesis; true area, 0.5

Table 3. Coordinates of the Curve Catalase (CAT)

Positive if Greater Than or Equal To(a)	Sensitivity	1 - Specificity
2.76	1.00	1.00
4.90	1.00	0.91
6.44	1.00	0.82
6.99	1.00	0.77
7.37	1.00	0.73
7.65	1.00	0.68
8.07	1.00	0.59
8.59	1.00	0.55
8.76	1.00	0.50
8.82	1.00	0.46
8.93	1.00	0.41
9.26	1.00	0.36
9.77	1.00	0.32
10.1	1.00	0.27
10.3	1.00	0.23
10.3	1.00	0.18
10.5	1.00	0.14
10.7	1.00	0.09
11.0	1.00	0.05
15.8	1.00	0.00
22	0.96	0.00
24.5	0.91	0.00
25.5	0.86	0.00
26.7	0.82	0.00
27.7	0.77	0.00
28.2	0.73	0.00
29.0	0.68	0.00
30.2	0.64	0.00
31.2	0.55	0.00
31.8	0.50	0.00
32.2	0.46	0.00
33.7	0.36	0.00
35.6	0.32	0.00
36.7	0.23	0.00
38.8	0.18	0.00
40.8	0.09	0.00
44.3	0.05	0.00
48.1	0.00	0.00

a: The smallest cut off value is the minimum observed test value minus 1, and the largest cut off value is the maximum observed test value plus 1. All the other cut off values are the averages of two consecutive ordered observed test values.

Table 4. Area Under the Curve Test Result Variable(s): Carbonic Anhydrase (CA)

Area	Std. Error(a)	Asymptotic Sig. (b)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.96	0.04	0.00	0.89	1.03

The test result variable(s): Carbonic anhydrase has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased; a, Under the nonparametric assumption; b, Null hypothesis; true area = 0.5

Table 5. Coordinates of the Curve Carbonic Anhydrase (CA)

Positive if Greater Than or Equal To(a)	Sensitivity	1 - Specificity
-0.97	1.00	1.00
0.08	1.00	0.96
0.12	1.00	0.91
0.13	1.00	0.86
0.14	1.00	0.82
0.16	0.96	0.77
0.19	0.96	0.73
0.22	0.96	0.68
0.25	0.96	0.64
0.28	0.96	0.59
0.29	0.96	0.55
0.30	0.96	0.5
0.31	0.96	0.46
0.32	0.96	0.41
0.32	0.96	0.36
0.32	0.96	0.32
0.33	0.96	0.27
0.36	0.96	0.23
0.4	0.96	0.18
0.41	0.96	0.14
0.42	0.96	0.09
0.45	0.96	0.05
0.48	0.96	0.00
0.55	0.91	0.00
0.61	0.86	0.00
0.63	0.82	0.00
0.66	0.73	0.00
0.71	0.68	0.00
0.72	0.64	0.00
0.75	0.59	0.00
0.78	0.55	0.00
0.79	0.46	0.00
0.80	0.41	0.00
0.81	0.36	0.00
0.82	0.32	0.00
0.86	0.27	0.00
0.90	0.23	0.00

5 min using a spectrophotometer (Shimadzu UV-1201, Japan).

CA activity was assayed by hydration of carbondioxide. Hydration of carbondioxide was measured by the method of Rickli and Wilbur-Anderson with bromothymol blue as an indicator (Rickli et al., 1964).

Statistical Analysis

Descriptive statistics for studied variables (characteristics) were presented as mean, standard deviation, minimum and maximum values. Student t test was used to compare Control and Patient group means for the studied variables. Cut off value of CAT and CA were determined by ROC analysis. Statistical significance levels were considered as 5%. The SPSS (ver. 13) statistical program was used for all statistical computations.

Results

Cu level were significantly lower (P<0.05) in level of serum of patients with malignant glioma as a whole compared to controls. Serum Cd, Fe, Mg, Mn, Pb and Zn levels were found to be increase in patients group compared to control group (P<0.05). There is no statistically significant difference between healthy human and patient groups in Co serum level (P>0.05). Although mean CAT activity was significantly lower, CA significantly increased in the patients group than in the control group (P<0.05) (Table 1). The cut off value for CAT was 15.80 and its specificity and sensitivity was 100% (Table 2 and 3). The cut off value for CA was 0.481 and its specificity and sensitivity was 95% (Tables 4 and 5).

Discussion

Glioblastome multiforme and Anaplastic astrocytoma consist of at least 35% of primary brain tumors and their 50.0 incidence are 3/100.000 (Libermann et al., 1979; Cobb and Youmans, 1982). Survival time of patients with intracranial malignant glioma varies from 8 months to 2 25.0 years although they are applied multimodality therapy, such as surgery, chemotheraphy and radyotheraphy (Levin et al., 1979).

Free radical-mediated damages may play an important role in cancerogenesis (Popov et al., 2003). Several studies have indicated that oxidative stress plays a significant role in cancer development and outcome of treatment. Evidence has accumulated that exposure to reactive oxygen species (ROS) can lead to direct DNA damage, inducing cancer (Halliwell, 2007). Catalase resides in the peroxisomes and catalyzes the dismutation of H2O2 into oxygen and water. In various brain tumor cells, antioxidant enzyemes have been found to be decreased (Popov et al., 2003). A decrease in catalase activity has been seen in most of the brain tumor and lung cancer patients (Rao et al., 2000; Cobanoglu et al., 2010). The present study showed decreases in CAT activitys in patients as compared with control subjects. Catalase deficiency may lead to accumulation of reactive oxygen metabolites and this may cause the initiation of carcinogenesis. The reason for this change in CAT activity may indicate a possible link between decreased antioxidants and increased free radical levels, supporting the idea that there is a persistence of oxidative stress in malignant glioma.

CA is an extremely attractive molecule in cancer research because it has several important properties that make it completely unique. Carbonic anhydrase is a hypoxia-induced enzyme that has many biologically important functions, including its role in cell adhesion and invasion (Supuran et al., 2003; Jarvela et al., 2008). It has also been suggested that CA has a direct role in tumor progression and the regulation of pH balance during

tumorigenesis. In addition, the effect of CA on tumor cell invasion is still under debate as some recent results have suggested that there is no evidence of such a correlation (Svastova et al., 2003; Robertson et al., 2004). The relevance of the relationship between carbonic anhydrase and brain tumors is not shown, because despite these studies, there are few data on the activity of CA in brain tumors. Therefore, determination of CA enzyme activity is important in brain tumors. Therefore, we decided to measure the CA activity. The present study showed that CA activity in malignant glioma group increases as compared with control groups. In the current study, the cut off value for carbonic anhydrase (CA) was 0.481 and its specificity and sensitivity was 95% for malignant glioma. If value of CA is above 0.481, it may be possible that pearson is at risk for malignant glioma. We found that serum level of catalase and carbonic anhydrase have a important role in forming of malignant glioma.

Carcinogenic elements may act as either genotoxic or epigenetic carcinogens. In the current study, in group with malignant glioma, level of Zn increased. Zn plays an important role in brain metabolism. It's part of an antioxidant "chain reaction" that destroys many of the free-radical molecules in the brain. The levels of four trace elements (Fe, Cu, Zn, and Co) have been investigated in 52 patients suffering from larynx cancer at different stages of the disease (Zakrzewski et al., 1972). Metal-containing dust from either polluted environments or cigarette smoke is a well-acknowledged risk factor for the development of cancer (De Palma et al., 2008). Decreased gastrointestinal absorption and tissue-specific absorption of Zn may have contributory effects.

Mg could also be a important factor. Some research has shown that magnesium levels in the blood of people with active schizophrenia are lower than normal and that the levels are higher in persons whose schizophrenia is in remission. Optimal Mg intake may be prophylactic against initiation of some neoplasms. Since cancer cells have high metabolic requirements, it is not indicated (alone) in the treatment of cancer (Collery et al., 1981; Cobanoglu et al., 2010).

Serum Mn level was found to be higher in malignant glioma compared to controls in this study.

Mn seems to be very important microelement during development. This microelement participates in the reduction-oxidation reactions due to its chemical properties (Floriańczyk et al., 2007). Information regarding the carcinogenicity of Mn in humans or animals is not available (Schwartz, 1975). It is thought that manganese and Mn superoxide dismutase play an important role in the differentiating cells (St.Clair et al., 1994).

The present study showed that Fe levels increased in patients with malignant glioma as compared with control subjects. Although Fe is an essential nutritional element for all life forms, it is known that excess Fe and Fe deficiency also lead to oxidative DNA damage (Ames, 2001). Defective Fe homeostasis may be involved in the development of some diseases within the central nervous system. Although the expression of genes involved in normal Fe balance has been intensively studied in other tissues, little is known about their expression in the brain tumors (Hanninen et al., 2009).

Serum Cu levels are known to increase in several malignancies such as osteosarcomas, some gastrointestinal tumors, and lung cancer (Turecky et al., 1984). Some studies have shown that serum Cu in tumour cells or lung epithelial lining fluid of individuals with lung cancer increases (Diez et al., 1989; Mahabir et al., 2006), but in a study, it has been found that the level of serum Cu decreased in lung cancer patients (Cobanoglu et al., 2010). In this study serum copper levels in 22 patients with malignant gliomas were studied and Cu level decreased in patients with malignant glioma in comparison with healthy subjects.

In the scientific literature there are only five other cases of severe adverse effects on nervous system associated with Co released from prosthesis. Moreover, Co neurotoxicity for acoustic and optic nerves has been reported following occupational exposures to the metal or, few decades ago, after oral intake during therapy of refractory anaemia (Schirrmacher, 1967). In the present study, serum Co level was no found a statistical significant difference when compared to control group (Table 1) ($P>0.05$). It may thus be important to be specific about the chemical structure of the Co and confounding exposures.

A recent report in the literature suggested a link between occupational exposure to Pb and brain tumor (Cocco et al., 1988). In our study, Pb levels increased in malignant glioma compared to controls. Although the etiology of primary malignant brain tumor remains largely unknown, there are several clues that exposure to Pb may impact brain tumor risk (Wijngaarden and Dosemeci, 2006). Pb increase oxidative stress and it are established toxic and carcinogenic metal (Stohs and Bagchi, 1995). In addition, the epidemiological literature for an association between lead exposure and brain tumor is inconclusive (Wijngaarden and Dosemeci, 2006).

In our study, serum Cd levels were higher in malignant glioma groups than those of controls. Cd is known to be one of the most toxic environmental and industrial pollutants. Its industrial applications were developed based on its unique chemical and physical properties. Most studies have not given information on an number of relevant confounders by Cd. Cd are established toxic and carcinogenic metals (Nawrot et al., 2002; Messner et al., 2009). In the current study, we identified a relationship between biochemical elements studied, except for cobalt, and forming of malignant glioma, however, there was a more meaning relationship between healthy group and patient group for catalase and carbonic anhydrase. Therefore, we theorize that persons with low catalase level and high carbonic anhydrase level may be more likely affected from intracranial malignant glioma. That is, it is possible that high catalase level and low carbonic anhydrase level have a protective role in healthy people for malignant glioma.

As a result, the antioxidant enzymes may have a role in the genesis of considerable oxidative stress in patients with malignant glioma.

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