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Brain tumor and role of ß-carotene, α- tocopherol, superoxide dismutase and glutathione peroxidase

ABSTRACT

The erythrocyte levels of the antioxidant enzymes SOD and GPx, and serum levels of antioxidants vitamins β-carotene and β-tocopherol were estimated in various types of brain tumors, and were compared with the levels in controls. Statistically significant (P<.001) diminished levels of β- carotene, β-tocopherol, SOD and GPx, were observed in all the brain tumor patients as compared to controls. Malignant tumor also showed a relative decrease in antioxidant levels as compared to benign tumors. Comparison of histopathological sections of brain tumors also suggested a inverse relationship between antioxidant level and grades of malignancy. Marked decrease in antioxidant levels may have a role in genesis of considerable oxidative stress in brain tumors. Further more, the degree of decline in antioxidant levels may indicate severity of malignancy in brain tumors.

Key words: Superoxidase-dismutase, Glutathione peroxidase, ß-carotene, a-tocopherol, Brain tumor

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INTRODUCTION

The role of oxidative stress in the genesis of various types of cancers is well established. Several chemical, cell culture and animal studies also indicate that antioxidants may slow or even prevent the development of cancer.[1] Antioxidant enzymes and vitamins are important group of cellularconstituents responsible for maintaining a balancebetween oxidants and antioxidants within a cell. Among the intracellular antioxidant enzymes, Superoxide-dismutase (SOD)and Glutathione Peroxidase (GPx) are responsiblefor the removal of ROS such as superoxide free radicals andH_2O_2. On the other hand vitamin E, breaks free radical chain reactions as a result of their ability to transfer a phenolic hydrogen to a peroxy free radical of a peroxidized polyunsaturated fatty acid (PUFA). There by preventing peroxidation of PUFA contained in cellular and subcellular membrane phospholipids. Similarly â-carotene acts as an antioxidant by the stabilization of organic peroxide free radicals with in its conjugated alkyl structure. Since â-carotene is effective at low oxygen concentrations, it complements the antioxidant properties of vitamin E which is effective at high oxygen concentration. Brain is considered abnormallysensitive to oxidative damage as brain tissue has high rate of oxygen consumption, high lipid content and relatively low antioxidant defenses, compared to other tissues.[2,3] Relatively few studies have been done to estimate antioxidant enzymes in various brain tumors. Though most of the studies have shown decreased levels of these enzymes and vitamins in various malignancies,[4,5,6] the results are contradictory.[7] Study done in Finland (1994) demonstrated that lung cancer rates of male smokers increased significantly with â-carotene supplementation,[8] yet another study done by Omen GS, Goodman G, Thomquist M, et al. (1994) indicates a possible increase in lung cancer associated with antioxidants supplementation,[9] Although the totality of research supports health benefits from antioxidants, more studies are required before we can include them in our management of cancers.

In order to investigate the oxidative damage which might be involved in the chemical pathology of brain tumors and correlate them to the histological findings and malignancy of tumors, we have examined the activities of various intracellular and extracellular antioxidants in different types of brain tumors and compared them with controls.

MATERIALS AND METHODS

Clinical profile: Thirty clinically diagnosed cases of intracranial neoplasms between age group of

8-70 years were selected for the study. We excluded patients with known clinical conditions associated with altered lipid peroxidation and antioxidant status such as diabetes mellitus, ischemic states, hypertension, renal failure, arthritis, liver disease and pancreatitis by clinical history and relevant lab investigation. The samples were taken preoperative and their diagnosis at the time was based on clinical findings and relevant investigations. The diagnosis was confirmed and staging was done by histopathological findings after the tumors were removed. Thirty age and sex matched healthy controls belonging to the same socio-economic status were also included in the study.

Collection of samples**:** Blood was freshly withdrawn by venepuncture and it was transferred to tubes containing heparin for the estimation of SOD and GPx levels in erythrocytes. Red blood cells were separated by centrifugation and the plasma was discarded along with the buffy coat containing mainly white blood cells. RBC's were washed with 10 volumes of cold saline {for GPx estimation RBC were suspended in 10 volumes of cold buffer (50 mM tris-HCl, pH = 7.5 containing 5 mM EDTA and 1 mM mercaptoethanol)}. Erythrocyte lysate was resuspended in 4 packed cell volumes of ice cold water, samples were stored at -70 C, if not assayed immediately.

Procedure: β-carotene and á-tocopherol assay was done by Bradlay & Hornbeck and Henson & Warwick methods respectively.[10,11] For superoxide-dismutase estimation, BIOXYTECH SOD –525™ kit method[12] was used . The SOD activity in units / ml was divided by the hemoglobin of the given lysate to express the activity as U/mg of hemoglobin. For estimation of glutathione peroxidase, GPx-340™ assay[13] was used, which is an indirect measure of the activity of cytosolic Glutathione Peroxidase (c-GPx). The activity in original sample was expressed as per ml and it was divided with the hemoglobin content of the lysate.

Statistical analysis: Statistical analysis was carried out according to student's paired and unpaired 't' test and correlation was calculated by Pearson's correlation coefficient.

RESULTS

We selected thirty patients of different types of intracranial tumors for our study. The male patients constituted 80 % and the females 20 % of the total number of cases. The maximum number of cancer patients in the study was in the age group of 31-40 years, 20 % of the total cases were in this age group. The range of the age of patients in our study was 8-70 years. We estimated the levels of SOD, GPx, β-carotene and á-tocopherol in intracranial tumor and compared them with controls. The levels of superoxide-dismutase showed a statistically significant decrease in patients with intracranial neoplasms [Table 1], as compared to controls (P<.001). 63 % of the total tumor patients had SOD values lower than the lowest value of controls, similarly the levels of Glutathione Peroxidase in brain tumors was less than that of controls, which was found to be statistically significant (P<.001) [Table 1]. 46 % of the brain tumor cases had GPx value less than the lowest value of controls. The levels of â-carotene and á-tocopherol were also significantly (P<.001) lower in patients of brain tumor as compared to that of controls [Table 1]. 53 % of the total tumor cases had â-carotene value less than the lowest value of controls and 80 % of the total brain tumor cases had β-tocopherol value lower than the lowest value of controls.

When we compared antioxidant levels in benign brain

Table1 : Levels of erythrocyte SOD and GPx and serum beta carotene and alpha tocopherol (Mean ± SD)

Group	β-carotene (mg/l)	α-tocopherol (ìg/ml)	SOD (U/mg of Hb)	GPx (mU/mg of Hb)
Controls	1.6 ± 0.4	7.6 ± 3.1	9.5 ± 1.5	92.5 ± 10.9
	(1.25 – 2.0)	(4.54 – 10.7)	(8.0 – 11)	(81.6 – 103.4)
Patients	0.94 ± 0.4 [a]	4.6 ± 1.9[b]	6.6 ± 2.29[c]	74.6 ± 16.1[d]
	(0.44 – 1.4)	(2.7 – 6.5)	(5.76 – 7.44)	(58.5 – 90.7)

Table 2 : Comparison of the levels of â-carotene, á-tocopherol, SOD, and GPx in malignant and benign brain tumor(mean ± SD)

	Controls	Benign tumor	Malignant tumor
β-Carotene (mg/ml	1.6 ± 0.4	1.07 ± 0.44	0.71 ± 0.46
	(1.25 – 2.0)	(0.63 – 1.51)	(0.25 – 1.17)
a-Tocopherol (µg/ml)	7.6 ± 3.1	5.05 ± 1.11	4.3 ± 1.12
	(4.54 – 10.7)	(3.94 – 6.16)	(3.18 – 5.42)
SOD (U/mg of Hb)	9.5 ± 1.5	6.25 ± 2.07	6.59 ± 3.03
	(8.0 – 11)	(3.18 – 8.32)	(3.56 – 9.62)
GPx(mU/mg of Hb)	92.5 ± 10.9	76.5 ± 14.47	72.54 ± 18.69
	(81.6 – 103.4)	(62 – 91)	(53.84 – 91.24)

β-Carotene: control vs. benign: P<.001, control vs. malignant: P<.001, benign vs. malignant: P<.050

α-Tocopherol: control vs. benign: P<.005, control vs. malignant: P<.001

SOD: control vs. benign: P<.001, control vs. malignant: P<.001

GPx: control vs. benign: P<.001, control vs. malignant: P<.001

tumors, with controls, we found that the levels of these antioxidants in benign tumors were significantly lower than the controls [Table 2]. The β-carotene level was lower in malignant brain tumor than benign tumors, and the difference was found to be statistically significant (P<.050). Although not statistically significant, β-tocopherol and glutathione peroxidase levels in malignant tumors were considerably less than the benign tumor. After histopathological grading [Table 3] we found that Brain tumors of higher grades of malignancy for example astrocytoma, oligodendroglioma, oligodendroglioma G-III, glioblastoma multiforme, medulloblastoma and metastatic adenocarcinoma all showed proportionate decrease levels of β-carotene, β-tocopherol, superoxide-dismutase and glutathione peroxidase, as the tumor became more malignant.

DISCUSSION

In the present study, we found that serum β-carotene and β-tocopherol levels were significantly reduced in brain tumor patients as compared to controls, and the values were even less in malignant tumors, when compared to relatively benign tumors. Our result was in close conformity with similar case studies done by other authors.[14,15,16,17] Few studies have been done to compare the levels of these antioxidants in various histological types of brain tumors. We compared the levels of â-carotene and β-tocopherol in different types of brain tumors, we found that β-carotene and β-tocopherol levels decreases with increasing grades of malignancy and the decrease was statistically significant for oligodendroglioma G-I-II, glioblastoma multiforme and medulloblastoma. The protective effect seen can be because of the antioxidant properties of these two lipid soluble vitamins as various authors have suggested,[19,1] beside their antioxidant action, several carotenoids show enhancement of the immune response, inhibition of mutagenesis, reduction of induced nuclear damage, similarly Vitamin E also has many non-antioxidant functions in the body such as anticoagulant, anti-inflammatory action, effects on gene regulation and immune function.[20] The decrease levels of these nutrient antioxidants in cancer cases may have predisposed the patients to the development of tumor.

We also compared levels of SOD and GPx enzymes in cases of intracranial tumors, and we confirm the results of other authors[4] that levels of these enzymes were significantly less in brain tumor cases than in controls. When we separated the cases on the basis of histopathological type of tumor both enzymes SOD and GPx, showed a notable decrease as the tumor became more malignant, another study has also shown a proportionate decrease of superoxide-dismutase with the increasing grades of malignancy in brain tumor.[21] The low levels of antioxidants in brain tumor patients could be as a result of this increased oxidative damage; or it could be that low values aggravated the free radical damage and increased the chance of developing cancer, indicating antioxidants role in prevention and role of oxidative injury in the causation of cancer.

No global answer can yet be given about the role of individual antioxidant in human tumorigenesis. According to our findings it seems likely that the ability of scavenging oxygen free radicals was impaired in brain tumor patients than controls, because of the lowered levels of antioxidants which predispose the patient to harmful effects of carcinogens. Before we know exactly how it happens, a lot of lacunae need to be filled by further research.

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Table No. 3: Levels of β-Carotene, α-Tocopherol, SOD, and GPx in various type of brain tumors (mean ± SD).

	β–Carotene (mg/ml)	α-Tocopherol (µg/ml)	SOD (U/mg of Hb)	GPx (mU/mg of Hb)
1) Controls	1.6 ± 0.4	76 ± 3.1	9.5 ± 1.5	92.5 ± 10.9
2) Papillary Ependymoma	1.66 ± 0.26	7.7 ± 0.61	7.4 ± 2.14	84.5 ± 12.53
3) Juvenile Pilocytic Astrocytoma	1.25 ± 0.19	5.75 ± 0.78	7.9 ± 2.49	80.5 ± 7.78
4) Astrocytoma G- I – II	1.29 ± 0.45	5.47 ± 0.46	5.76 ± 2.21	84 ± 18.76
5) Oligodendroglioma G- I – II	1.02 ± 0.49	4.76 ± 0.99	5.88 ± 2.16	69.85 ± 17.76
6) Glioblastoma Multiforme	0.51 ± 0.26	4.14 ± 0.93	8.46 ± 2.72	73.6 ± 21.5
7) Medulloblastoma	0.56 ± 0.28	4.05 ± 0.66	6.2 ± 3.54	72.4 ± 15.08
8) Metastatic carcinoma	0.44 ± 0.43	4.5 ± 0.73	5.9 ± 2.51	78.0 ± 9.23

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