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Oxidant and Antioxidant Activity Alteration in Anaplastic Astrocytomas Patients Following Radiation Therapy

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Abstract

Radiation therapy has been used in cancer treatment for many decades, although effective in killing tumor cells, ROS produced in radiotherapy threaten the integrity and survival of surrounding normal cells. Radiation damages cell by direct ionization of DNA and and other cellular targets and by indirect effect through ROS. A linkage between an increase in cellular ROS and the pathogenesis of several chronic diseases including cancer has been established. When the antioxidants control

mechanisms are exhausted or overrun, the control redox potential shifts towards oxidative stress, in turn increasing the potential for damage to nucleic acids, lipids and proteins. Human cells normally function in a reduced state, but oxidative stress results in imbalance towards a more oxidised state resulting in lower levels of antioxidants. In the present study the damage caused by ROS and the effect of radiation in anaplastic astrocytomas (WHO grade III) patients were assessed by analyzing MDA levels, GPX and GR activities. Blood samples were collected before and after radiation treatment. The MDA levels showed a highly significant elevation both before and after radiotherapy which reflects increased lipid peroxidation. The activity of antioxidant enzymes GPx and GR were found to be decreased highly significantly in same patients before and after radiotherapy when compared to healthy controls which showed the lack of antioxidant defense. Radiation induces lipid peroxidation by inactivating the antioxidant enzymes, thereby rendering the system in management of the free radical attack. Hence the degree of radiation affects the extent of the depression of the antioxidant enzyme activities and increased lipid peroxidation.

Key words: Anaplastic astrocytomas, Glutathione peroxidase, Glutathione reductase, Lipid peroxidation, Radiotherapy.

INTRODUCTION

Cancer is the second leading cause of death worldwide. It is estimated that there are approximately 2-2.5 million cases of cancer in India at any given point of time, with around 700,000 new cases being detected every year [1]. Radiation therapy has been used in cancer treatment for many decades; it is used to eradicate cancer and as a palliative to relieve pain associated with metastases. In the course of treatment, radiation produces numerous biological perturbations in cells; because normal cell toxicity limits the doses used in effective treatment, approaches are designed to strike a balance between eliminating cancer cells and protecting normal tissues [2]. The biological effects they produce are thought mainly to be caused by the production of free radicals from interaction with the cell constituents, especially water [3]. Oxygen, normally present in most biological systems, aggravates the damage done by radiation. Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions. The shift in the balance between oxidants and antioxidants in favor of oxidants is termed “oxidative stress” [4]. Ionizing radiation, in the presence of O_2, converts hydroxyl radical, superoxide, and organic radicals to hydrogen peroxide and organic hydroperoxides.

These hydroperoxide species react with redox active metal ions, such as Fe and Cu, via Fenton reactions and thus induce oxidative stress [5].

The brain has a particular predisposition to oxidative stress which makes it vulnerable to free radicals. They trigger 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. It was shown that oxygen-derived free radicals play an important role in brain tumor development due to DNA strand backing, appearance of point mutations and aberrant DNA cross-linking [6]. Brain tumor development involves not only oxidative aggression but also a reduced response of antioxidant defense. During prolonged oxidative stress, changes in brain antioxidant enzymes activities, including superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase(GR) , catalase (CAT), glutathione transferases (GTSs) appear. These enzymes normally act to prevent or decrease brain damages caused by free radicals in excess [7]. Free radicals can trigger lipid peroxidation chain reactions by abstracting a hydrogen atom from a side chain methylene carbon. The lipid radical then reacts with oxygen to produce peroxyl radical. Peroxyl radical initiates a chain reaction and transforms polyunsaturated fatty acids into lipid hydroperoxides. Lipid hydroperoxides are very unstable and easily decompose to secondary products, such as aldehydes (such as 4-hydroxy-2, 3-nonenal) and malondialdehydes (MDAs) [8]. Glutathione is a ubiquitous thiol containing tripeptide which is important defense against free radicals and hydroperoxides. Glutathione has important function such as storage and transport of cysteine, maintaining the reducing state of proteins and thiols, and protecting the cells from toxic compounds such as reactive oxygen species, drugs or heavy metal ions. Reduced glutathione (GSH) is highly abundant in all cell compartments and is the major soluble antioxidant. GSH/GSSG ratio is a major determinant of oxidative stress. GSH shows its antioxidant effects in several ways. It detoxifies hydrogen peroxide and lipid peroxides via action of glutathione peroxidase (GPx). GSH donates its electron to H_2O_2 to reduce it into H_2O and O_2. Oxidized glutathione (GSSG) is again reduced into GSH by Glutathione reductase that uses NAD(P)H as the electron donor [9]. GPxs levels in body are also related with glutathione which is the most important antioxidant present in cytoplasm of cells and protect cell membrane from lipid peroxidation.[10]. GSH donates protons to membrane lipids and protects them from oxidant attacks [11].

Numerous enzymatic and non-enzymatic mechanisms protect the cell against oxidative injury. The removal of damaging oxygen products is catalysed by antioxidant enzymes and they play a major role in protecting the cell from the oxidative damage caused by free radicals. Antioxidants have been shown to inhibit both

initiation and promotion in carcinogenesis and counteract cell immortalisation and transformation [12]. Since the deleterious effects produced by the free radicals depend upon the balance between the oxidant and antioxidant capacity of the system, it was decided to study the relationship between free radicals, antioxidant enzymes, malignancy and cytotoxic effect of radiation. The present study was taken up with a view of the paucity of Indian studies on the effects of radiotherapy on the oxidant-antioxidant status in brain cancer and the existing lacunae in the field of oxidative stress biomarkers of brain cancer ,therefore we assessed MDA, the marker of lipid peroxidation, and the antioxidants GPx and GR, in the blood of astrocytomas grade III brain tumor patients, before and after radiotherapy.

MATERIAL AND METHOD

Subject treatment and sample collection: The present study was carried out on 80 human subjects. Out of which 40 age matched normal healthy volunteers were considered as control group and 40 were anaplastic astrocytomas (WHO grade III) patients. These patients were diagnosed and confirmed for astrocytomas grade III brain tumor, based on CT, MRI and histopathological study (biopsy), were chosen for the study. The same patients were underwent surgery following the Radiotherapy regimen, by gamma rays (60Co) with radiation dose employed was 5000 cGY doses in 25 fractions over a 5 week period , 5 days in a week, daily single dose comprising of 200 cGY. Blood samples were collected in plain and K3-EDTA vacutainers, intravenously from normal healthy volunteer, and from patients prior to radiotherapy and one day after completion of the last dose of radiotherapy. The present study consists of three groups: (1) Group I, normal healthy control (HC), n=40; (2)Group II, astrocytomas grade III patients before radiation treatment (BRT), n=40; and (3) Group III, astrocytomas grade III patients after radiation treatment (ART) n =40.

Biochemical Assay

MDA levels were estimated in plasma by the method of **Jean C.D. et al (1983)**[13].TBA reagent (1ml TBA solution or stock + 0.5ml of 7% perchloric acid) was added to plasma sample and the mixture was heated in a boiling water bath for 30 minutes.After cooling, 3ml of n-Butanol was added.Mixed by shaking and centrifuge. Absorption of pink chromogen (allegedly a $(TBA)_2$-MDA adduct) supernatant was read spectrophotomically at 531 nm. Estimation of antioxidant enzymes were done in blood lysate preparation. glutathione peroxidase (GPx) was done by **Hafeman D.G. et al** method (1974)[14]. Glutathione peroxidase catalyzes the decomposition of hydrogen peroxide in presence of reduced glutathione forming oxidized glutathione and water. The final absorbance of the test solution and standard were read against blank at 412nm within 2 minutes.Glutathione reductase (GR) activity was measured in erythrocytes with a manually available kit (kit GR, Randox

Laboratories, Cat no: 2368) based on **Goldberg D.M. & Spooner R.J et al method (1983)**[15], by measuring the decrease of absorbance of NADPH. Glutathione reductase catalyses the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidized to $NADP^+$. The decrease in absorbance was measured at 340 nm.

Statistics

Student's t-test (paired & unpaired) were used in the statistical evaluation of the result using Graphpad Prism 3.0 software.

FIGURES & TABLES:

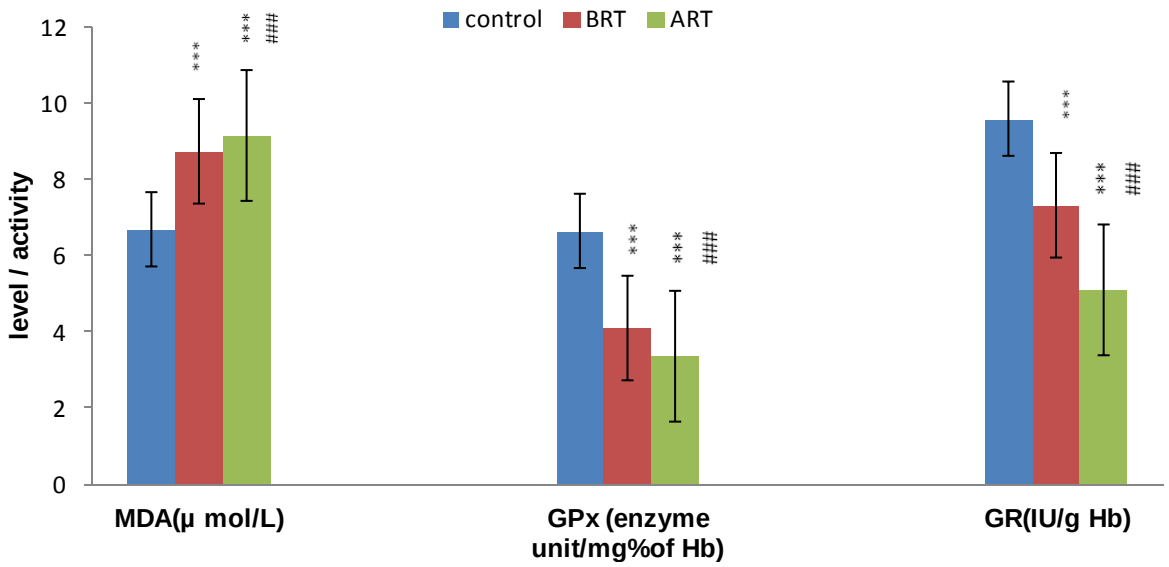

Figure1: Diagrammatic representation of Malondialdehyde (MDA)level and Glutathione Peroxidase(GPx) and Glutathione Reductase(GR) activity of healthy controls (HC) and astrocytomas grade III patients before (BRT)and after (ART) radiation treatment.(n=40 each group)

The values are expressed as (mean±SEM). Statistically significant variations are compared with the control by **unpaired t test** (***p<0.0001) and by **paired t test** (###p<0.0001) between patients before and after radiation treatment.

Table 1: Personal profile and physical parameters of healthy controls and anaplastic astrocytomas (WHO grade III) patients before (BRT) and after (ART) radiation treatment.

	Healthy controls(n=40)			Anaplastic astrocytomas (WHO grade III) patients (n=40)				
	Age	BMI	Weight	Age	BMI		Weight	
					BRT	ART	BRT	ART
Min	18	16.47	45	18	16.77	15.62	41	38
Max	72	32.35	87	74	26.67	25.15	73	67
Mean	32.43	21.89	60.82	32.63	21.37	19.40	57.1	51.83

Smoker	3	8
Non-smoker	37	32
Alchoholic	3	6
Non-alchoholic	37	34
Vegetarian	32	30
Non-vegetarian	8	10

Table2: The mean ± SD values of Malonaldialdehyde (MDA)level in plasma and Glutathione Peroxidase(GPx) and Glutathione Reductase(GR) activity in heamolysate of healthy controls (HC) and astrocytomas grade III patients before (BRT)and after (ART) radiation treatment.

	Malonaldialdehyde (MDA) (µ mol/L)	Glutathione Peroxidase (GPx) (enzyme unit/mg%of Hb)	Glutathione Reductase (GR) (IU/g Hb)
Healthy Controls (HC) (n=40)	6.676 ± 1.448	6.634 ± 1.091	9.581 ± 1.664
Astrocytomas grade III patients before radiation (BRT) (n=40)	8.724 ± 1.090***	4.084 ± 1.076***	7.307 ± 1.349***
Astrocytomas grade III patients after radiation (ART) (n=40)	9.140 ± 1.063***###	3.334 ± 0.0899***###	5.086 ± 1.743***###

***highly significant ($p < 0.0001$) when compared to healthy control

###highly significant ($p<0.0001$) when compared to astrocytomas grade III patients before radiotherapy

RESULTS & DISCUSSION

In the present study, significantly elevated concentration of lipid peroxidation products MDA were seen in astrocytomas grade III patients BRT when compared to the control group indicating susceptibility to free radical attack in these cases **(figure 1,table 1)**. Free radicals induce increased membrane permeability through membrane lipid peroxidation. There is interdependency between reactive oxygen species (ROS) and lipid peroxidation — ROS initiate the reaction of lipid peroxidation and are also produced in these reactions as intermediates. Some of the products of lipid peroxidation are diffusable and can spread the damage far beyond the site of original free radical attack [16]. The MDA level kept on elevating in the ART patients which showed that radiation induces free radicals which in turn induce MDA levels. Increase in MDA in anaplastic astrocytomas patients after radiotherapy might be due to the decomposition products of polyunsaturated fatty acids (PUFAs) of biomembranes. Similar observations have been reported in breast cancer patients [17].

We found highly significant decrease in GPx and GR activity in astrocytomas grade III patients BRT when compared to the control group **(table 2)**. Glutathione has got important role in neutralizing free radical and maintenance of protein bound thiols. According to previous studies low glutathione levels can predispose for development of neurogenerative disorders, cancer including brain tumor [18]. Radiotherapy is the major form of treatment which is available for brain cancer. Studies have shown varied findings with respect to the effect of radiotherapy on the oxidant-antioxidant status. Some authors have observed increased oxidative stress after radiotherapy and have suggested that radiation-induced free radicals cause oxidative damage to biomolecules [19].

Radiation has been shown to cause structural and functional alterations to membranes, as well as induce chemical changes to both their lipids and proteins [20]. Most of the enzyme's active sites contain -SH groups. They in turn alter the activity of antioxidant enzymes and render the system inefficient to manage free radical attack. Deficiency of all the antioxidant enzymes (eg.GPx, GR) in erythrocytes of untreated and CMF-treated breast cancer patients has been observed, which may be due to a poor antioxidant defense mechanism [21]. Our findings show a similar trend which is evident from Fig.1 the activity of GPx and GR decreased highly significantly ART as compared to patients BRT.

The decrease in GPx may be due to the ill effects of free radicals on the enzyme. Some study reported a decrease in GPx activity in blood, serum and cerebrospinal fluid in ischemic brain diseases[22]. A decrease in GPx activity may lead to a decrease in reduced glutathione. The GSH/GSSG in normal cells is kept high, because of the reduction of GSSG back to GSH by GPx enzyme. Reduced glutathione is a co-factor for several enzymes in different metabolic pathways.

Moreover, it acts as a scavenger of hydroxyl [OH] radical and singlet oxygen and it can reactivate some enzymes that have been inhibited by exposure to high oxygen concentration. Presumably the oxygen causes oxidation of essential –SH groups on the enzymes which are regenerated on incubation with GSH. GSSG inactivates a number of enzymes, probably by forming mixed disulfides. It has been shown to inhibit protein synthesis in animals cells [23]. If a tissue is exposed to a large flux of hydrogen peroxide and/or –OH, a point might be reached at which GSH/GSSG cannot be maintained at its normal ratio. This can aggravate the oxidative stress developed. Moreover, Buckmann and co-workers [24] have reported that cytotoxicity was potentiated by the inhibition of glutathione reductase.

In cancer, there is an enormous production of free radicals in the system [25]. Dormandy [26] has proposed a close relationship between free radical activity and malignancy. GPx and GR can act as scavenging enzymes, destroying the free radicals and H_2O_2. The activities of peroxidase and reductase both showed a greater decline, suggesting a greater accumulation of H_2O_2. This might also be responsible for degradative reactions in the tissues including membrane damage via lipid peroxidation [27].

In conclusion, we report here that there is an increase in MDA levels and a decrease in the levels of antioxidant enzymes in BRT and ART patients when compared with control subjects. This suggests that radiotherapy causes impairment of the ability of scavenging ROS in astrocytomas grade III patients than controls. Radiation damages cells by direct ionization of DNA and other cellular targets and by indirect effect through ROS. Exposure to ionizing radiation produces oxygen-derived free radicals in the tissue environment; these include hydroxyl radicals (the most damaging), superoxide anion radicals and other oxidants such as hydrogen peroxide. Additional destructive radicals are formed through various chemical interactions. Although effective in killing tumor cells, ROS produced in radiotherapy threaten the integrity and survival of surrounding normal cells before we know how it happens a lot of lacunae need to be filled by further research.

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