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Assessment of Blood Glutathione Peroxidase and Glutathione Reductase in Glioma Patients of Gwalior Chambal region, Pre and Post Radiotherapy

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Abstract

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 such as free radical generation. Exposure to ionization radiation inherent tissue sensitivity and repair, and modulating intracellular factors that include oxygen concentration, and level of antioxidants in the tissue environment. The brain has high content of peroxidizable unsaturated fatty acids, which is a key in lipid peroxidation and a shortage of antioxidant defense systems or more generation of ROS, or free radicals, can exceed the scavenging ability of endogenous antioxidants, resulting in a shift of the redox state of the brain to the oxidative state. Gliomas are the most common intra-axial tumors arising from the central nervous system. The effect of radiation on the antioxidant enzyme GPx and GR in glioma patients of Gwalior Chambal region has been presented in this work. Several studies implicated the association of dysfunctional GPx and GR and cancer risk and in our work we have also find highly significant decrease in the levels of GPx and GR in the haemolysate of glioma patients pre and post radiotherapy which postulates lack of antioxidant defence system and radiation somehow making it more inefficient.

Key words: *Glioma, Glutathione peroxidase, Glutathione reductase, Lipid peroxidation, Radiotherapy.*

Introduction

Gliomas are the most common intra-axial tumors arising from the central nervous system [1]. Diffuse, infiltrative gliomas are the primary intracranial neoplasms, accounting for 40% of all primary and 78% of all malignant central nervous system tumors [2]. They are graded from low to high grade tumors by their histological 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 [1]. Radiation therapy has been used in cancer treatment from many decades; it is used to eradicate cancer and as a palliative to relieve pain associated with metastases [3,4,5]. 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[6]. Radiation chemistry reperesents a method to selectively generate an study on individual reactive free radical in order to evaluate theit potential involvement in biochemical cytotoxicity [7], gamma irradiation of erythrocytes induces alterations at three different functional units of the membrane:

a. the lipid bilayer

b. the protein component of glycocalyx

c. the cytoskeleton at the membrane surfaces [8]

Lipid peroxidation is initiated by superoxide and hydroxyl radicals and by H_2O_2. The peroxidation of unsaturated lipds in natural membrane at moderate radiation does increases rigidity of the hydrophobic region of the lipid bilayer and thus increases permeability to different solutes, while as at high doses, it leads to destruction of the membrane structure with consequent erythrocyte haemolysis [9, 10]

To overcome these consequences cells have an antioxidant defence system which scavenges the free oxygen radicals and suppresses the free radical chain peroxidation of the lipids. It is achieved by number of enzymes and compounds present in the cell [11]. The role of oxidative stress in the genesis of various types of cancers is well established. [12]. Brain tumor development involves not only oxidative aggression but also a reduced response of antioxidant defense [13].Several chemical, cell culture and animal studies also indicate that antioxidants may slow or even prevent the development of cancer [14].

During prolonged oxidative stress, changes in brain antioxidant enzymes activities, including superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione transferases (GTSs) appear. These enzymes normally act to prevent or decrease brain damages caused by free radicals in excess [13]. GPx and GR act antioxidatively. Reduced glutathione (GSH) present in most cells, can chemically detoxify H_2O_2 and forms oxidized glutathione (GSSG), catalyzed by GPx. GR reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH and thus regenerate the antioxidative agent again [15, 16, 17].

Several studies implicated the association of dysfunctional GPx and GR and cancer risk [18]. It is proposed that oxygen-derived free radicals play a key role in human cancer development [19]. Altered activity of GPx has been reported in various cancer patients [20,21,22,23,24,25]. A significant drop in GRx activity has been observed in carcinoma of the uterine cervix [26].Since there are low activities of protective antioxidant enzymes accompanied with a high ratio of membrane surface area compared to cytoplasm and non replicability of neuronal cells in comparison with other organs of the body, the nervous system may be especially vulnerable to free radical mediated injury [27].

Very few reports are available on the involvement of free radicals in intracranial tumor development and on the vulnerability of the brain to free radical ill-effects. Therefore the present study was undertaken to assess the erythrocyte GPX and GR levels in glioma patients.

Materials and methods

In the present study estimation of blood levels of enzyme glutathione peroxidase (GPx) and glutathione reductase (GR) in glioma patients was carried out in the department of biochemistry and department of radiation oncology , J A group of hospitals G R medical college Gwalior.

For this study , n=124 glioma patients in the age range of (36.64±14.22) years having clinically confirmed glioma , planned to undergo surgery followimg the radiotherapy by using cobalt 60 with radiation dose 50 gray in 25 fractions in a period of 5 weeks. Inclusion criterion considered for selection, patients who were not receiving any other mode of treatment, except radiation and also without any known systemic disorders. We excluded glioma patients with known clinical

conditions associated with altered lipid peroxidation and antioxidant status such as hypertension, artherits, diabetes mellitus, or with cancer in regions other than cerebral hemisphere of brain. Hundred age and sex matched healthy controls (n=100, 35.19±14.14 years) belonging to the same socio-economic status and sane geographical area were also included in the study.

Intravenous Blood samples were collected aseptically once from healthy controls and twice from glioma patients pre and post radiotherapy and were further investigated. The preparation of haemolysate was done by the method of McCord and Fridovich [28]. Blood samples collected in K3-EDTA vial centrifuged at 3000 rpm for 15 minutes. Plasma was removed from packed cell volume (PCV). Packed Cell Volume was washed 3 times with normal saline. Cell were lysed by adding 1ml of D.W. Mixture was refrigerated for 10 minutes and then vigorously shaken in vertex for 2 minute, then 0.5ml chloroform was added as a preservative. Mixture was centrifuged at 3000rpm for 20 minutes. The whole mixture was clearly separated into three layers, lower most layers was chloroform, middle layer was cell stroma (mucous) and upper most layer was clear haemolysate solution. This haemolysate solution was pipetted out and collected in microcentrifuge tube, which was finally utilized for the estimation of Hemoglobin and antioxidant enzymes like glutathione peroxidase (GPx) by Hafeman DG, et al. method 1974 [29] and glutathione reductase (GR) by Randox kit (Randox cat no.gr 2368) based on Goldberg DM & Spooner RJ, et al. method 1983 [30].

Results

The results we found after conducting our study on healthy controls (n=100) and glioma patients (n=124) to understand the effects and alteration in GPx and GR levels pre and post radiotherapy showed highly significant changes. In case of GPx there is highly significant ($p<0.0001$) decrease in the erythrocytic GPx levels and activity when compared between healthy controls (Mean± SD ; 6.637±1.008) and glioma patients prior to radiation treatment (4.065 ±1.054). The radiation treatment seemed to continue the decrease as the values of the GPX in the same glioma patients post radiotherapy reduced to (3.351±0.863) **[Fig 1A].** Similarly in the case of GR there was a significant decrease ($p<0.001$). The values kept on decreasing from healthy controls (9.719± 1.653) to PreRT glioma patients (7.292± 1.302) to PostRT glioma patients (5.102± 1.697) [**Fig 1B].**

The statistical analysis were performed using Prism3.0 software.

Fig 1A

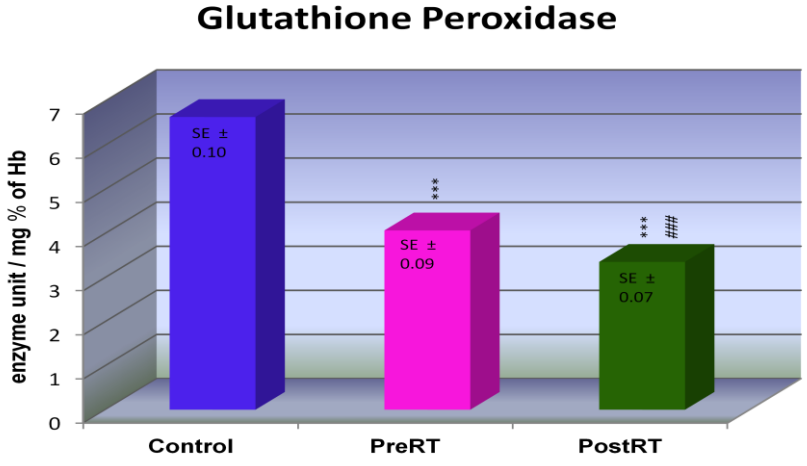

Fig 1B

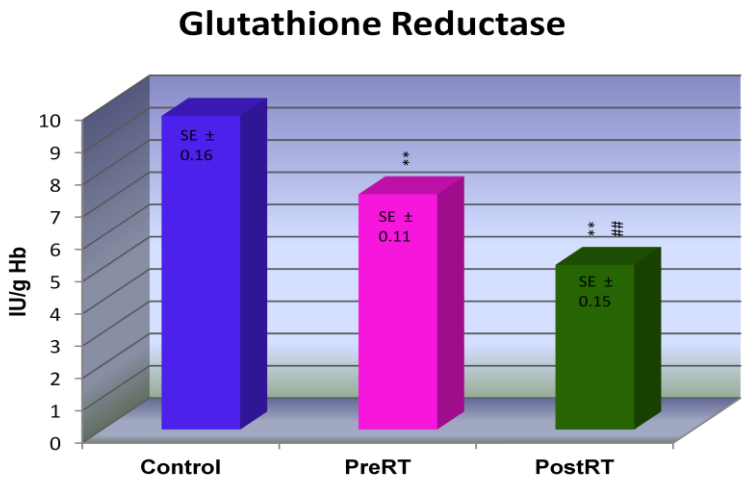

Figure1(A&B): Diagrammatic representation of Glutathione Peroxidase (GPx) and Glutathione Reductase(GR) activity of healthy controls (HC) and glioma patients Pre (PreRT)and Post (PostRT) radiation treatment.(n=100 for control, n=124 for PreRT and PostRT group).The values are expressed as (mean±SEM). Statistically significant variations are found with the comparision between control and patient groups by **unpaired t test** (**p<0.001**)** (***p<0.0001) and by **paired t test** (##p<0.001) (###p<0.0001) between patients pre and post radiation treatment.

Discussion and Conclusion

Radiotherapy is the primary modality of treatment of brain tumor [31]. There is no single marker available to predict the response to radiotherapy. Here we estimated antioxidant enzymes (GPx and GR) status, before and just after the completion of radiotherapy to predict the response of radiotherapy in glioma patients. Radiation is known to induce oxidative stress, which may cause fluctuation of antioxidant status of irradiated tumor. Therefore in the present study we are trying to evaluate whether post radiotherapy antioxidant enzymes values are helpful in predicting the radiation response or not.

Detrimental effects caused by reactive oxygen species occur as a consequence of an imbalance between the formation and inactivation of these species. Oxidative damage may be involved in the pathogenesis of major diseases such as cancer, atherosclerosis [32] and certain neurological disorders [33]. Inactivation and removal of reactive oxygen species depend on reactions involving the antioxidative defense system. The capacity is determined by a dynamic interaction between individual components. Some of the most important enzymatic antioxidants are glutathione peroxidase (GSH-Px; EC1.11.1.9) and glutathione reductase (GR; EC 1.6.4.2). GPx reduces organic peroxides into their corresponding alcohols using GSH as a hydrogen donor whereby GSH is oxidized. GPx is a widely distributed in erythrocytes and cardiac tissue. Most of the blood GPx activity is in erythrocytes and only 1-2 % in plasma. Erythrocytes are continuously exposed to oxidative stress. Although the reducing capacity of normal erythrocytes is greater than its oxidising potential, lack of antioxidant defence system causes increased lipid peroxidation which leads to membrane damage [34].The GSH/GSSG in normal cells is kept high, because of the reduction of GSSG back to GSH by GRx 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 [35]. Dzhandzhgava and Shakarishivilli [36] reported a decrease in GRx activity in blood, serum and cerebrospinal fluid in

ischemic brain diseases. A decrease in GRx activity may lead to a decrease in reduced glutathione. GPx and GR decrease and protein oxidation increases in patients with glioblastoma multiforme and transitional meningioma and clear different oxidative status was found in the two kinds of tumors which represent specially one of the most malignant and most benign tumors respectively [37].

There are several studies which shows antioxidant enzyme deficience in different cancer conditions. Erythrocyte antioxidant enzymes deficiency have been reported in lymphoma patients [38], reduced GPx in patients with malignant diseases [39], pre and post radiotherapy treated oral cancer patients [40]. The observations stands true in decreased erythrocytic GPx and GR levels and with other enzymes prior and after radiotherapy as compared to control groups.

We also compared levels of GPx and GR enzymes in cases of glioma patients, and we confirm the results of other authors [41] that levels of these enzymes were significantly less in glioma patients than in controls and the levels kept on decreasing in patients post radiotherapy.The low levels of antioxidants in glioma patients could be as a result of this increased oxidative damage; as in cancer their is an enormous production of free radicalsin the system. Dormandy has proposed a close relationship between free radical activity and malignancy[42],the low levels of GPX represents greater accumulation of H2O2 which in turn causing degradative membrane lipid peroxidation.

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. Important finding of this study is the highly significant reduced levels of antioxidant enzymes GPx and GR activities of glioma patients pre and post radiotherapy. The possible reason for this decrease may be due to the elevated level of ROS generation (due to radiation effect), the exhaustion of the antioxidant defense system in removing ROS, rapid depletion of the antioxidant enzyme pool of the cells, stressful disease condition, poor defense mechanism of body and the effect of radiotherapy on the patients.

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 glioma patients than controls, because of the lowered levels of antioxidants which predispose the patient to harmful effects of carcinogens and radiation therapy tend to increase the free radical generation and damage the normal tissue also as a major side effect , that is needed to be controlled. Before we know exactly how it happens, a lot of lacunae need to be filled by further research.

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