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Effect of Oxidative Stress Injury and Antioxidant Status in Malignancy

Arpita Chatterjee* and Gopeswar Mukherjee[1]

Department of Botany, Barasat College, Kolkata - 700 126, India
[1]Department of Research and Development, Barasat Cancer Research and Welfare Centre
Banamalipur, Barasat, Kolkata - 700 126, India
✉ arpita10c@gmail.com

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Abstract: Oxidative stress injury induced lipid peroxidation and the impairment of the antioxidant system has a potential role in the pathogenesis of cancer. Various pollutants like environmental toxicants and industrial wastages elevate the levels of lipid peroxidation products (LPP) with a decline in antioxidant status. During malignancy the level of antioxidant was low and LPP was more. The tissue damage (as evident from levels of C-reactive protein, CRP) in cancer patients was significantly higher. After radiation treatment the overall scenarios was altered as LPP and CRP level decreased and antioxidant level increased. Free radicals in the form of oxidative damage causes fatal diseases and oxyradical-induced cytotoxicity arises from both chronic and acute increases in reactive oxygen species, which give rise to subsequent lipid peroxidation. Antioxidants act as free radical scavengers and hence prevent and repair damage done by the free radicals.

Key words: Antioxidant, C-reactive protein, lipid peroxidation products, oxidative damage, malignancy, pollution.

Introduction

Reactive oxygen species play an important role in various kinds of cell toxicity, including cytotoxicity, chromosomal aberrations, sister chromatid exchanges, lipid peroxidation as well as carcinogenesis (Loft et al., 1993). Majority of carcinogens are found either to be free radicals involved in free radical reaction or ones that generate free radical intermediates (Rongliang et al., 1991). Free radicals induced by radiation, damage the sugar phosphate chains of DNA and cause strand breaks (Zhizhina et al., 1990). The free radicals generated by metal ions also promote lipid peroxidation and DNA damage (Sahu and Washington, 1991). It has been postulated that antioxidants like superoxide dismutase (SOD) significantly protect the DNA damage caused by reactive oxygen species (Subramanian et al., 1994). Studies showed that exposure to various pollutants, viz., environmental toxicants and industrial wastages elevate the levels of lipid peroxidation products (LPP), lipids and lipoproteins, with a decline in antioxidant status (Reddy et al., 1994). Antioxidant systems either prevent the formation of these reactive species or remove them before they can damage vital component of the cell (Kelly et al., 2003) by starting chemical chain reaction such as lipid peroxidation or by oxidizing DNA or protein. Dietary supplementation with antioxidants may provide a safe and effective means of enhancing the response to chemotherapy or radiotherapy and improve quality of life by reducing or preventing side effects and thus also influence the efficacy of cancer treatments (Norman et al., 2003).

Oxidative stress injury is very much related with pollution toxicity in nature. By reacting with polyunsaturated fatty acids in the various cellular membranes, oxy-radicals such as hydroxyl and peroxynitrite give rise

*Corresponding Author

to a variety of LPP. Once formed, these peroxidation metabolites demonstrate multiple interactions with cellular components, cause physio-pathological alterations and cyto-toxicity as cellular pollutants (Keller and Mattson, 1998). To understand the extent of tissue damage the C-reactive protein (CRP) level, an acute phase protein produced by liver or adipocytes, present in the patient's serum is an important marker (Pepys and Hirschfield, 2003). The present investigation was directed towards a mechanistic understanding of oxidative stress injury profile in malignancy by comparing the anti-oxidant, CRP and LPP level of a neoplasia group.

Materials and Methodology

Cases and Sample Preparation

A total of 25 cases (12 male and 13 female) belonging to age group 25–90 were enrolled for each investigation from patients attending to Barasat Cancer Research and Welfare Centre, a prime health centre covering many districts of West Bengal. The malignancy was diagnosed by various investigations like radio-imaging, cytology and histo-pathological examinations by physicians. Controls ($n = 25$) where participants frequency matched to cases by age, sex, and selected from hospital admission lists during the same time period. Demographic as well as environmental data was recorded. Written informed consent was obtained from all in accordance with the guidelines from hospital centre review board. Blood samples were collected from cases and controls before, during and after the commencement of radiation treatment. The blood samples (1 ml) were centrifuged at 3000 rpm for 15 minutes and clear serum was collected.

Determination of Antioxidant Level

Antioxidant level was analysed by evaluating the activity of SOD in malignant condition as well as during and after radiotherapy treatment (Reddy et al., 1998). The assay medium contained 50 mM sodium carbonate, bicarbonate buffer (pH 9.8), 0.1 mM EDTA, 0.6 mm adrenaline in a total volume of 3 ml. Adrenaline was the last component to be added and the adrenochrome formed in the four minutes was recorded at 470 nm. One unit of SOD activity was defined as the amount of enzyme needed to cause 50% inhibition of adrenaline auto-xidation at pH 9.8.

Determination of LPP Level

To determinate the LPP level, thiobarbituric acid was added to plasma sample under acidic condition, and the absorption of colour that developed after heating was estimated spectrophotometrically at 535 nm (Reddy et al., 1998). 1, 1, 3, 3-tetraethoxypropane was used as internal standard and the concentration was expressed in nmol of malondialdehyde per ml of plasma.

Determination of CRP Level

To estimate tissue damage by radiation therapy CRP level in the serum samples was assayed using turbidimetric immunoassay, based on the principle of agglutination reaction, as per manufacturer instruction (Tulip Diagnostics, India). Activation buffer (500 µl) and latex reagent (50 µl) were mixed properly, incubated at 37 ºC for 10 minutes and used as working solution. The serum sample (3 µl) was added to the working solution and the CRP concentration was estimated by spectrophotometric reading at 546 nm.

Results and Discussions

The present investigation revealed that antioxidant level was less during malignant condition as compared with the normal one (Table 1). All the patients showed further decrease of antioxidant level during radiation treatment, but after treatment the level of antioxidant increases much more even greater than the normal level. Reversely LPP level in blood serum was greater than normal level at the malignant stage; during radiation therapy this increase was much higher in all the patients. After radiation treatment the LPP level gradually decreased and this value was much less than the initial LPP value of the corresponding patients. These finding highlighted that malignancy may directly affect tissue damage which is reflected by the level of CRP in patients. The con-centration of CRP was greater in majority of the cancer patients (72%) as compared with the healthy one (Table 1). The study revealed decrease of CRP level in majority of the patients (56%) during radiotherapy, but more increase in serum CRP level was also observed in some cases. After radiation therapy the CRP level reduces in 80% of the patients, and this reduction was even less than the corresponding levels of CRP before treatment in them.

LPP is the marker for the damage of lipid plasma membrane of the cell, which further highlights the tissue damage and establish a framework for its involvement in diseased condition (Keller and Mattson, 1998). This inference was also supported by the increased CRP level in malignant condition. After radiation treatment the over all scenario was altered as the LPP and CRP level decreased and antioxidant level increased. This investigation suggests a potential role of oxidative stress induced lipid peroxidation and the impairment of the

Table 1: Level of antioxidant, LPP and CRP in malignant patients before, during and after radiation therapy

Types of Cancer	*Age (yr.)*	*Sex*	*Antioxidant level (unit/ml)*			*LPP level (μ/ml)*			*CRP level (mg/dl)*		
			Before	*During*	*After*	*Before*	*During*	*After*	*Before*	*During*	*After*
Ca–Ethimoid	25	Female	184	158	210	1.91	2.02	1.67	1.2	1.7	0.6
Ca–Breast	50	Female	178	162	208	1.89	2.17	1.64	0.8	1.4	0.5
Ca–Vocal cord	67	Male	169	148	219	1.72	2.13	1.51	1.6	0.9	0.4
Ca–Buccal mucosa	59	Male	173	147	224	1.81	2.19	1.60	0.9	0.8	0.5
Ca–Vocal cord	38	Male	175	146	202	1.70	2.30	1.52	0.7	1.1	1.3
Ca–Vocal cord	90	Male	164	147	209	1.69	1.97	1.45	1.2	0.6	1.6
Ca–Prostate	62	Male	171	153	217	1.64	2.31	1.39	2.3	0.7	0.4
Ca–Rectum	45	Female	166	144	216	1.72	2.00	1.46	2.5	1.9	0.5
Ca–NHL	47	Female	168	146	208	1.87	2.23	1.52	0.5	0.8	0.3
Ca–Breast	30	Female	164	150	223	1.75	2.11	1.41	1.1	0.6	0.5
Ca–Laryngopharynx	60	Female	184	138	219	1.81	2.04	1.53	0.5	0.8	0.8
Ca–Skin	60	Male	179	152	210	1.86	2.12	1.50	1.5	0.9	0.5
Ca–Brain	27	Male	181	173	202	1.92	1.92	1.42	0.6	0.7	0.4
Ca–Seminal vesicles	35	Male	176	143	208	1.71	1.86	1.46	0.4	0.7	0.5
Ca–Penis	48	Male	188	152	206	1.81	2.25	1.52	1.4	1.1	0.6
Ca–Cervix	35	Female	184	150	208	1.72	2.34	1.61	0.5	1.2	0.9
Ca–Oesophagus	60	Female	192	168	210	1.77	2.30	1.51	1.7	1.1	0.6
Ca–Lung	50	Male	196	146	211	1.81	1.91	1.57	0.5	0.6	0.4
Ca–Body uterus	63	Female	176	148	218	1.84	2.42	1.56	0.5	0.7	0.5
Ca–Gall bladder	35	Male	169	135	225	1.86	2.26	1.64	2.4	0.6	0.9
Ca–Breast	45	Female	174	141	220	1.78	2.18	1.57	1.9	0.8	0.3
Ca–Buccal mucosa	38	Female	171	153	218	1.67	2.14	1.39	2.2	0.6	0.5
Ca–Alveolus	50	Male	168	140	210	1.81	2.10	1.41	1.8	1.1	0.6
Ca–Labia majore	56	Female	158	134	223	1.72	2.16	1.51	1.2	0.6	0.5
Ca–Cervix	62	Female	184	151	229	1.76	2.27	1.41	0.5	0.5	0.7
Mean value (±SD)			175.7 (±9.14)	149.0 (±9.37)	214.1 (±6.97)	1.78 (±0.07)	2.15 (±0.14)	1.51 (±0.83)	1.2 (± 0.4)	0.9 (± 0.7)	0.6 (±0.2)
Control (±SD)			207.5 (±20.3)			0.94 (±0.06)			0.6 (±0.2)		
p value*			< 0.05			< 0.05			< 0.05		

* p value < 0.05 indicates the results are significant.

antioxidant system in the pathogenesis of cancer. The antioxidant factor plays a major role for the prevention of cancer. Free radicals in the form of oxidative damage causes fatal diseases and oxyradical-induced cytotoxicity arises from both chronic and acute increases in reactive oxygen species which give rise to subsequent lipid peroxidation (Keller and Mattson, 1998). Antioxidants act as free radical scavengers and hence prevent and repair damage done by the free radicals. Dietary supplements rich in antioxidant could help to reduce the effects of pollution on health and help the body to prevent damage from toxins and pollutants (Kelly et al., 2003).

Results revealed that CRP levels of cancer patients were significantly higher than those of the healthy subjects, which is at par as reported in early studies (Erlinger, 2004). CRP is a member of the class of acute phase reactants as its level rise dramatically during inflammatory processes occurring in the body (Erlinger, 2004). This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes (Pepys and Hirschfield, 2003). The present investigation revealed that cancer causes massive tissue damage which is evident from the elevated level of CRP in the serum of the patients. At this state of decreased antioxidant level and increased levels of LPP and CRP, patients may be prone to other bacterial infection, immune complex diseases or it may also induce a state of secondary immuno-deficiency leading to an unwanted consequence. This observation was in line with the fact that somatic state may result from the life threatening neoplastic disease causing an impairment of immune function (Stone et al., 1994).

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