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Can antioxidants predispose to cancer recurrence?

Krishnananda Prabhu*, Gummadi Maheshwar Reddy, Anjali Rao

Department of Biochemistry, Kasturba Medical College Manipal, India

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ABSTRACT

Objective: To measure and compare pre- and post-treatment levels of serum total glutathione -S-transferase (GST) in stage IV cervical cancer patients and erythrocytic glutathione (GSH) and malondialdehyde (MDA) and plasma ceruloplasmin (CP) and total GST in stage IV oral cancer patients and to correlate with the response to treatment during a two year follow-up period in respective cancer groups. **Methods:** Thirty-four biopsy-proven stage IV oral cancer and cervical cancer patients (n=17 in each group) who underwent same mode of treatment were chosen for this study. Erythrocytic MDA and GSH, CP and serum total GST were measured in all patients before the onset of treatment, and the GST level was only measured in cervical cancer patients after radiotherapy. The levels were compared with their respective prognosis in 2 years. **Results:** Oral cancer patients with higher pretreatment levels of GSH, CP and GST came with cancer recurrence within 2 years after the onset of treatment. Cervical cancer patients with higher post radiotherapy levels of serum total GST had higher recurrence tate. **Conclusions:** This study indicates there may be a role for these antioxidant parameters namely GST, GSH, CP in assessment of long term survival and prognosis of cancer patients.

1. Introduction

Oxidative challenge due to generation of free radicals is implicated in triggering or transforming non-malignant cells to malignant ones either by DNA damage or by modulating gene expression. Furthermore, lipid peroxidation is a significant characteristic of free radical reactivity which results in deleterious effect on cells leading to their damage[1].

Antioxidants like glutathione (GSH)[2], ceruloplasmin (CP)[3], antioxidant enzymes like glutathione-S-transferase (GST)[4] and lipid peroxidation product malondialdehyde (MDA)[5] have been implicated in pathogenesis and prognosis of various epithelial malignancies including oral cancer. But their role in overall prognosis and cancer recurrence is not clearly understood. Therefore, we estimated erythrocytic MDA and GSH, plasma CP and total GST in patients with biopsy proven oral squamous cell carcinoma and cervical cancer at stage Ⅳ(both n=17) before the onset of treatment and after radiotherapy only in cervical cancer patients. Levels were compared with their respective prognosis in 2 years.

2. Materials and methods

Approval for the study was given by the Institutional Ethics Committee. Informed consent was obtained from each patient before withdrawal of blood sample. All the subjects were from Udupi District, Costal Karnataka, India, and admitted between Feb 2006 to Jan 2009.

Seventeen oral cancer patients who were clinically and histologically diagnosed as squamous cell carcinoma of oral cavity (stage Ⅳ), and underwent same mode of treatment were chosen for this study. Patients with oral carcinoma were staged using International Union against Cancer (UICC) TNM classification (1974) in the study. None of the patients with oral cancer had any habit of smoking, tobacco, and alcohol consumption. All the cases were treated with combination of surgery followed by a course of radiotherapy around 23 fractions for 4 to 5 weeks.

Blood samples were taken from oral cancer patients before any definitive treatment using aseptic precautions. Blood was collected into ethylenediamine tetraacetate (EDTA) bottles and immediately centrifuged under refrigeration at 3 000 g for 10 minutes. Plasma was carefully removed and separated cells were washed thrice with cold phosphate buffer containing 0.15 mol/L NaCl, pH 7.4. The erythrocytes were suspended in an equal volume of physiological saline as 50% cell suspension[6]. Hemolysate was prepared from erythrocyte suspension by addition of distilled water and was used in the estimation of GSH[7] and MDA[8]. Hemoglobin content of erythrocytes was measured by cyanmethhemoglobin method[9]. Plasma was used for the estimation of CP[10] and total GST[11]. Cervical cancer

*Corresponding author: Dr. Krishnananda Prabhu, Associate Professor, Department of Biochemistry, Kastutrba Medical College, Manipal, India -576104.
Fax: 091 0820 2571927
E-mail: krishnakunj2000@yahoo.com

patients plain blood was collected twice before treatment and after radiotherapy and only serum GST was estimated. Statistical analysis was carried out using Mann-Whitney test and Independent Sample *t* test.

3. Results

Sinca all the oral cancer patients were at stage IV, only 36% of patients had no recurrence. All the above parameters namely erythrocytic GSH, MDA, plasma CP and total GST were higher in patients with recurrence (Table 1).

Out of 17 cervical cancer patients who received radiotherapy, 9 showed significant decrease [(6.60±0.72) IU/L VS (4.01±0.68) IU/L] ($P<0.01$) while 8 showed significant increase [(5.46±0.66) IU/L VS (6.67±0.78) IU/L] ($P<0.01$) in post radiotherapy GST as compared to pre treatment values, respectively. These patients were followed up for two years and 71% with significant increase in post radiotherapy values had relapse of cancer within 2 years where as 66% of those with significant decrease had no evidence of relapse.

Table 1
Comparison of GSH, CP, MDA and GST in oral cancer patients (Mean±SD).

Parameter	GSH (mg/grHb)	CP (mg/dL)	MDA (nM/grHb)	GST (IU/L)
Recurrent (n = 9)	5.51±3.76	38.88±26.70	4.53±2.22	6.45±3.97
Non-recurrent (n = 5)	5.26±2.63	32.05±21.55	3.97±1.51	3.30±2.20

4. Discussion

The generation of reactive oxygen radicals in mammalian cells profoundly affects numerous critical cellular functions, and the absence of efficient cellular detoxification mechanisms which remove these free radicals can result in several diseases. Reactive oxygen species (ROS) are tumorogenic by virtue of their ability to increase cell proliferation, survival, cellular migration, and also by inducing DNA damage, leading to genetic lesions that initiate tumorogenicity and sustain subsequent tumor progression[1].

Enhanced antioxidant capacity of tumor tissues make them less susceptible to oxidative stress conferring specific growth advantage[12] which has also been implicated in radio/chemo resistance leading to tumor recurrence[4,13]. In this study even though all the oral cancer patients were at stage IV, some had no recurrence. Then we grouped them into good and bad responders. It is observed that patients with higher pretreatment levels of GST, GSH, and CP had recurrence within 2 years after treatment. Radiation is known to induce oxidative stress, which may cause induction of antioxidant status of irradiated tumor[13]. This may influence the radiation response in some selected human carcinoma cells[14]. Our results show the association of GST level and radiation response in cervical cancer patients. This indicates high levels of the antioxidant produced in these cancer patients as an adaptive response to combat the free radicals, which are generated as a consequence of high metabolic rate, then to protect these cells against the cytotoxic effects of radio/chemotherapy and hence leading to recurrence.

Conflict of interest statement

We declare that we have no conflict of interest.

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