Indian Journal of Clinical Biochemistry, 2004, 19 (2) 80-83

OXIDANT-ANTIOXIDANT STATUS IN COLORECTAL CANCER PATIENTS-BEFORE AND AFTER TREATMENT

Sharmila Upadhya*, Subramanya Upadhya, S. Krishna Mohan*, K. Vanajakshamma*, Mamatha Kunder*, Seema Mathias***

**Department of Biochemistry, Kasturba Medical College, Manipal - 576104*
***Department of Physiology, International Center for Health Sciences, Manipal - 576 104*

ABSTRACT

Erythrocyte antioxidant glutathione and malondialdehyde levels in erythrocytes and plasma glutathione S-transferase levels were estimated in patients with colorectal cancer and compared to controls. Further, the patients underwent four weeks of radiotherapy with adjuvant chemotherapy. The same parameters were estimated after four weeks of radiotherapy and compared with pretreatment levels. It was observed that there was a decrease in erythrocyte glutathione and malondialdehyde levels in patients with colorectal cancer compared to controls, but not in case of GST. However, after chemoradiotherapy, there were no statistically significant differences in all the parameters studied.

KEY WORDS

Glutathione (GSH), Malondialdehyde (MDA), Glutathione S-transferase (GST), Colorectal cancer.

INTRODUCTION

Colorectal cancers ranks third in frequency in men and second in women. The incidence is higher in developed countries than in developing countries (1). Alteration in the oxidant- antioxidant profile is known to occur in cancer (2, 3). Oxidative stress due to damage brought about by free radicals is also known to influence the response of these patients to therapy. Moreover the body's defence mechanisms would play a role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation.

In the present study, the following parameters were assessed in the erythrocytes and plasma to elucidate the oxidant-antioxidant status in patients with colorectal cancer. Erythrocyte glutathione (GSH) levels were estimated as an index of antioxidant status. Malondialdehyde (MDA) levels were measured as thiobarbituric acid reacting substances (TBARS) which served as an index of extent of lipid peroxidation. These parameters were estimated in RBC's to try to assess the disturbances in oxidant-antioxidant status and their effect on erythrocytes. Glutathione S-Transferase (GST) levels were estimated in plasma. GST is an enzyme involved in antioxidant defence and also involved in detoxification. It is used as a tumor marker in certain cancers such as oral cancer. Alterations in GST levels in tumor tissue have been reported by various studies (2, 3).

Author for correspondence :

Sharmila Upadhya
Associate Professor,
Department of Biochemistry,
Kasturba Medical College,
Manipal-576 104, INDIA
Email: shardhya@yahoo.com

MATERIALS AND METHODS

The study was conducted in patients with histologically proven colorectal carcinoma. Patients selected were in Duke's stage B of colorectal cancer. Twenty healthy individuals of matching age and sex were used as controls.

The controls and patients were divided into three groups:

Group 1: 20 healthy, age and sex matched controls.

Group 2: 17 patients with histologically proven colorectal carcinoma in Duke's stage B before starting combined chemotherapy and radiotherapy (pre treatment).

Group3 : 8 patients after four weeks of radiotherapy and adjuvant chemotherapy (post treatment).

The venous blood samples obtained from these

subjects were used for the estimation of GSH and MDA in erythrocytes and GST in plasma. GSH was estimated by the method of Beutler et al using dithio bis nitro benzoic acid (DTNB) (4), MDA was determined as the measure of TBARS (5) and GST was measured by using 1-chloro-2,4 dinitrobenzene (CDNB) (6). All reagents used were of analytical reagent grade. DTNB, CDNB and thiobarbituric acid were obtained from Sigma chemicals, St Louis, MO.

Statistical analysis between group 1 (controls) and group 2 (pretreatment) was performed by the unpaired t-test using the Statview package. Statistical analysis between group 2 (pretreatment) and group 3 (post treatment) was done by the unpaired t-test by using the same package.

RESULTS AND DISCUSSION

The mean ± SD of erythrocyte GSH and MDA are indicated in the table 1. There was a statistically significant decrease in the GSH and MDA levels in group 2 compared to group 1. The levels of plasma GST did not show any significant change between controls and in patients before treatment. Following four weeks of radiotherapy, the GSH, MDA and GST levels showed no statistical difference compared to pretreatment levels ($p > 0.05$).

In the present study, GSH, an antioxidant was significantly decreased in patients with colorectal carcinoma when compared to controls. The decrease in the GSH levels may be due to the increased turnover of GSH for preventing oxidative damage in these patients. Similar reports of lowered GSH levels in cancers have been reported earlier by Ahmed *et al.* (7) in patients with cervical cancer. They have observed that GSH levels were mainly reduced in poorly differentiated tumours than in well and moderately differentiated tumours. Reduced glutathione levels in cancer were also reported by Bhuvarahamurthy *et al.* (3) and Faber *et al.* (8). GSH depletion is reported in hepatoma at an advanced stage (9). An increase in tumour tissue glutathione levels in oral squamous cell carcinoma has been reported by Subapriya *et al.* (10).

In our study lipid peroxidation product i.e, malondialdehyde (MDA) has been decreased in the erythrocytes of patients with colorectal cancer. Gerber *et al.* (11) and Saintot *et al.* (12) have reported similar findings in patients with breast cancer. They have also observed a decrease in plasma MDA with tumor size and progression. A similar decrease in tissue lipid peroxide was observed in oral squamous cell carcinoma (10). Saroja *et al.* have also reported diminished lipid peroxidation in oral tumor tissue and a decrease in phospholipid content and an increase in cholesterol: phospholipid ratio (2).

MDA is reported to be an unstable intermediate in the peroxidation sequence of unsaturated fatty acids. It may be metabolised further or be transported (13). This may explain the observation of decrease in MDA in erythrocytes in our subset of patients. Skrzydlewska et al have reported increased activity of superoxide dismutase, glutathione peroxidase and glutathione reductase (enzymatic oxidant defence system) and a decrease in GSH content (non-enzymatic antioxidant parameter) in cancer tissue suggesting an increased defence against oxidant damage in cancer (14). However MDA levels have been reported to be higher in patients with malignant breast tumor (15,

Table 1. The mean ± SD values of glutathione (GSH), malondialdehyde (MDA) and glutathione S-transferase (GST) in controls & patients (Pre & Post Treatment) with colorectal cancer

Parameter	Group 1 (Controls) n=20	Group 2 (Pre-Treatment) n=17	Group 3 (Post-Treatment) n=8
GSH (mg/gm of Hb)	13.9 ± 4.9	6.7 ± 5.1*	6.1 ± 2.7^{NS}
MDA (nmoles/gm of Hb)	8.3 ± 3.4	5.4 ± 5.9**	3.4 ± 2.6^{NS}
GST (micromoles/ of Hb)	4.1 ± 1.0	5.1 ± 3.8^{ns}	4.9 ± 1.9^{NS}

*p < 0.0001 compared to controls
**p = 0.05 compared to controls.
ns - not significant when compared to controls (p > 0.05)
NS - not significant when compared to pre treatment (p > 0.05)

16), colorectal cancer tissue (17) and in erythrocytes of rats with hepatoma (9).

We have observed no significant difference in the levels of GST in controls and patients with colorectal cancer. The GST levels in tumor tissue of colorectal cancer were similar to the GST levels in the normal colorectal tissue of subjects with colorectal cancer (17). No significant difference in activity of GST was observed in human breast cancer tumor cell line and Adriamycin resistant cell line. This indicates that GST may not appear to play a role in drug resistance (18). GST levels were found to be decreased in uterine cervical carcinoma (3, 19) and in fibroadenoma and adenocarcinoma of breast (16). An increase in GST in oral tumor tissue has been reported by Saroja *et al.* (2, 10).

In our study eight patients with histologically proven colorectal cancer underwent 4 weeks of radiotherapy with adjuvant chemotherapy. The levels of GSH, MDA in erythrocytes and plasma GST were estimated in these patients.

No significant difference was observed between the pretreatment and post treatment levels. This may be because of the small sample size used for the follow-up study. GSH detoxification system is one of the defense systems against free radicals and carcinogens. Lower levels of GSH may favor an overproduction of free radicals and lipid peroxides which in turn may induce damage to the cell membrane and cellular molecules (DNA, RNA) leading to neoplasia. Similarly lower erythrocyte GSH levels in cervical cancer patients was reported by Mukundan *et al.* (20). They have hypothesised that there may be an impairment of the GSH scavenger system due to the carcinogenic process. Bhuvarahamurthy *et al.* have reported a decrease in GST and GSH levels in cervical cancer. They have reported a varying degree of normalisation of the biochemical parameters after therapy. They have observed that different types of therapy had different responses (3).

Antioxidants like vitamin E, vitamin C, glutathione and azelastine hydrochloride have been used in patients with head and neck tumors receiving combined radiotherapy and chemotherapy. Inclusion of azelastine had decreased the incidence of severe mucositis in the patients (21).

High levels of GSH cause drug resistance in the tumor tissue. Buthionine Sulfoximine (BSO) causes depletion of GSH and increases the susceptibility of the tumor tissue to anticancer drugs. The clinical trials for efficacy of BSO are being carried out. Inhibitors of GST are also reported to sensitize tumour cells to alkylating agents (22). The measurement of erythrocyte GSH levels may be used as indicators of intracellular GSH levels. Thus measurement of erythrocyte GSH levels may be used as an alternative to measure GSH levels in tumor tissues during BSO administration. This would provide an easier method to assess GSH and GST levels and their effects on therapy.

REFERENCES

1. Skibber, J.M., Minsky, B.D. and Hoff, P.M. (2001) Cancer of the colon. In: *Cancer: Principles and practice of oncology.* Devita, V.T., Hellman, S. and Rosenberg, S.A. (Eds.). Lippincott's Williams and Wilkins, Philadelphia. Sixth edition, p. 1216.

2. Saroja, M., Balasenthil, S. and Nagini, S. (1999) Tissue lipid peroxidation and glutathione dependant enzyme status in patients with oral squamous cell carcinoma. Cell Biochem. Funct. 17, 213-216.

3. Bhuvarahamurthy, V., Balasubramanian. N., and Govindasamy, S. (1996) Effect of radiotherapy and chemoradiotherapy on circulating antioxidant system of human uterine cervical carcinoma. Mol. Cell Biochem. 158, 17-23.

4. Fairbanks, V.F. and Klee, G.G. (1994) Biochemical aspects of hematology. In: *Tietz textbook of Clinical Chemistry.* Burtis, C.A. and Ashwood, E.R. (Eds.). W.B. Saunders Company. 2nd edition, 1990-1991.

5. Jain, S.K., Mcvie, R., Duett, J. and Herbst, J.J. (1989) Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 38, 1539-1542.

6. Warholm, M., Guthenberg, C., Christer von Bahr and Mannervik, B. (1985) Glutathione transferases from human liver. In: *Methods of enzymology.* Alton Meister (Ed.). Academic Press, Vol 113, p. 500-501.

7. Ahmed, M.I., Fayed, S.T., Hossein, H. and Tash, F.M. (1999) Lipid peroxidation and antioxidant status in human cervical carcinoma. Dis. Markers 15, 283-291.

8. Faber, M., Coudray, C., Hida, H., Mousseau, M. and Favier, A. (1995) Lipid peroxidation products and vitamin and trace element status in patients with cancer before and after chemotherapy, including adriamycin. A preliminary study. Biol. Trace Elem. Res. 47, 117-123.

9. Batko, J. (1997) The effect of experimental

neoplastic disease on malonyldialdehyde level and glutathione status in erythrocytes of rats. Acta. Biochim. Pol. 44, 767-769.

10. Subapriya, R., Kumaraguruparan, R., Ramachandran, C.R. and Nagini, S. (2002) Oxidant-antioxidant status in patients with oral squamous cell carcinoma at different intraoral sites. Clin. Biochem. 35, 489-493.

11. Gerber, M., Astre, C., Segala, C., Saintot, M., Scall, J., Simony-Lafontaine, J., Grenier, J. and Pujol, H. (1996) Oxidant-antioxidant status alterations in cancer patients: relationship to tumor progression. J. Nutr. 126, 1201S-1207S.

12. Saintot, M., Astre, C., Pujol, H. and Gerber, M. (1996) Tumor progression and oxidant and antioxidant status. Carcinogenesis 17, 1267-1271.

13. Roy, D. and Liehr, J.G. (1989) Changes in activities of free radical detoxifying enzymes in kidneys of male Syrian hamsters treated with estradiol. Cancer Res. 49, 1475-1480.

14. Skrzydlewska, E., Stankiewicz, A., Sulkowska, M., Sulkowski, S. and Kasacka, I. (2001) Antioxidant status and lipid peroxidation in colorectal cancer. J. Toxicol. Environ. Health A 64, 213-222.

15. Polat, M.F., Taysi, S., Gul, M., Yilmax, I., Bakan, E. and Erdogan, F. (2002) Oxidant-antioxidant status in blood of patients with malignant breast tumor and benign breast disease. Cell Biochem. Funct. 20, 327-331.

16. Kumaraguruparan, R., Subapriya, R., Kabalimoorthy, J. and Nagini, S. (2002) Antioxidant profile in circulation of patients with fibroadenoma and adenocarcinoma of breast. Clin. Biochem. 35, 275-279.

17. Ozdemirler, G., Pabuccuoglu, H., Bulut, T., Bugra, D., Uysal, M. and Toker, G. (1998) Increased lipid peroxide levels and antioxidant system in colorectal cancer. J. Cancer Res. Clin. Oncol. 124, 555-559.

18. Sinha, B.K., Mimnaugh, E.G., Rajagopalan, S. and Myers, C.E. (1989) Adriamycin activation and oxygen free radical formation in human breast tumor cells: Protective role of glutathione peroxidase in adriamycin resistance. Cancer Res. 49, 3844-3848.

19. Manju, V., Kalaivani, Sailaja, J. and Nalini, N. (2002) Circulating lipid peroxidation and antioxidant status in cervical cancer patients: a case control study. Clin. Biochem. 35, 621-625.

20. Mukundan, H., Bahadur, A.K., Kumar, A., Sardana, S., Naik, S.L., Ray, A. and Sharma, B.K. (1999) Indian J. Exp. Biol. 37, 859-364.

21. Osaki, T., Ueta, E., Yoneda, K., Hirota, J. and Yamomoto, T. (1994) Prophylaxis of oral mucositis associated with chemotherapy for oral carcinoma by azelastine hydrochloride with other antioxidants. Head Neck 16, 331-339.

22. Colvin, O.M. (2001) Antitumor alkylating agents. In: Cancer: Principles and practice of oncology. Devita, V.T., Hellman, S. and Rosenberg, S.A. (Eds.). Lippincott's Williams and Wilkins, Philadelphia. Sixth edition, pg 369.