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CAN SERUM GLUTATHIONE-S-TRANSFERASE LEVELS IN CARCINOMA CERVIX BE A PREDICTOR OF RADIATION RESPONSE?

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ABSTRACT

We conducted a pilot study wherein serum Glutathione-S-transferase levels were measured before and after radiotherapy in carcinoma cervix patients and correlated with response to treatment during a two-year follow-up period. Out of 17 patients who received radiotherapy, 9 showed a significant decrease ($p< 0.005$) while 8 showed significant increase ($p< 0.004$) in post radiotherapy glutathione-S-transferase values as compared to pre treatment values respectively. These patients were followed up for two years and we observed that 71% who had significant increase in post radiotherapy values had relapse of cancer within 2 years where as 66% of those who had significant decrease in post radiotherapy values had no evidence of relapse. This shows that alterations in serum Glutathione-S-transferase levels may help us to predict radiation response

KEY WORDS

Glutathione-s-transferase, Radiotherapy, Carcinoma cervix

INTRODUCTION

Alterations in circulating antioxidants and free radical scavengers have been linked with various epithelial malignancies(1-4). Many studies have shown a link between altered expression of glutathione-S-transferase (GST) in plasma as well as in tissue biopsy samples from carcinoma cervix (5-9). Radiotherapy (RT) is an important modality of treatment in carcinoma cervix. Response to RT may be good (with tumor regression and no relapse) or poor with relapse. We conducted a pilot study wherein we measured serum GST levels before and after RT in carcinoma cervix patients(10-15). The aim was to determine whether post RT GST levels as compared to pre RT levels had a relevance to relapse during the two year follow up period.

MATERIALS

Reduced glutathione $C_{10}H_{17}O_6S$ and 1-chloro-2,4,dinitro benzene (CDNB) were purchased from Sigma Chemical Company. All other reagents used were of Reagent grade. Deionized water was used throughout the study.

Serum GST levels were measured in biopsy proven 37 cases of carcinoma cervix and in age matched 10 healthy woman volunteers after obtaining proper permission. In cases blood was withdrawn just before initiation of any definitive treatment. In patients who received RT (n=17), GST was again estimated one day after the last dose. Clinical response to treatment was evaluated in terms of tumor regression and evidence of relapse in 2-year follow up period.

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METHODS

Serum GST was estimated by CDNB method (16-18).

Reagents

a. Phosphate Buffer: 0.1M, pH-6.5 prepared with deionized water and stored in brown bottle in refrigerator.

b. CDNB: 20 mM in 95% ethanol, stored in brown bottle in fridge.

c. GSH: 20mM in deionized water freshly prepared just before the assay.

d. Serum: Blood was collected without any anticoagulant and allowed to clot for 1 hr. Clotted sample was centrifuged at 3500 rpm x 30 min at 4°C (in cold centrifuge). Serum was separated and stored at 4°C until use.

Assay

GST was estimated in 1ml of incubation mixture containing 850 □l of 0.1 M phosphate, buffer pH 6.5 CDNB reagent (20 mM) 50 □l, preincubated at 37°C for

10 min. Reaction was started by adding 50□l of 20 mM GSH and 50□l of serum. Reaction was followed at 1 min interval for 5 min by measuring absorption at 340 nm. Simultaneously, blank was run by substituting deoinized water for serum. Then O.D change/min was calculated. GST was estimated by using the molar extinction coefficient [9.6 mM^{-1} cm^{-1}] of GST (17).

Formula:

$$\frac{\text{O.D or test} - \text{O.D of blank}}{9.6 \times 0.05} \times 1000 \text{ IU/ltr}$$

RESULTS

The serum GST was not significantly altered in cases as compared to controls (Table-1). Out of 17 patients who received RT, 9 showed significant decrease ($p<0.005$), while others (n=8) significant increase ($p<0.004$) in post RT GST levels as compared to pre RT levels respectively (Table-2). We followed up these patients for next 2 years. We observed that, 71% of patients, who had significant increase in post RT GST, had relapse within 2 years and 66% of patients who had significant fall in post RT GST, had no evidence of relapse.

DISCUSSION

GST was chosen because of its proven association with poor prognosis and therapy resistance in other malignancies (19-28). Relation between drug resistance and GST levels has been reported by many studies in various cancers (4,11,15,19,26,27). Association of GST and radiation resistance was reported by Agarwal et al (24), Khil et al (25) and many others (11,14,19,20,22,23,28). Radiotherapy is the primary modality of treatment in carcinoma cervix. There is no single marker available to predict the response to radiotherapy. Here we estimated GST before and just after the completion of radiotherapy. Radiation is known to induce oxidative stress, which may cause induction of antioxidant status of irradiated tumor (24). This may influence the radiation response in some selected human carcinoma cells (25). Our results show an association of GST level and radiation response. But this cannot be generalized because our study group was small. We are planning to continue this study further to evaluate whether post radiotherapy GST values are helpful in predicting the radiation response.

REFERENCES

1. 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(4), 283-291.
2. Nagini, S., Manoharan, S., and Ramachandran, C.R. (1998) Lipid peroxidation and antioxidants in oral squamous cell carcinoma. Clin. Chim. Acta. 273(1), 95-98.
3. Grignon, D.J., Abdel-Malak, M., Mertens, W.C., Sakr, W.A. and Shepherd, R.R. (1994) Glutathione S-transferase expression in renal cell carcinoma: a new marker of differentiation. Mod. Pathol. 7(2), 186-189.
4. Cao, W., Zuo, J., Meng, Y., Wei Q., Shi, Z.H., Ju, L.M. and Fang, F.D. (2003) anticancer drug resistance of HeLa cells transfected with rat glutathione S-transferase pi gene. Biomed. Envion. Sci. 16(2), 157-162.
5. Manoharan, S., Kolanjiappan, K., Kayalvizhi, M., and Sethupathy, S. (2002) Lipid peroxidation and antioxidant status in cervical cancer patients. J. Biochem. Mol. Biol. Biophys. 6(3), 225-227.
6. Balasubramaniyan, N., Subramanian, S., and Govindasamy, S. (1994) Status of antioxidant systems in human carcinoma of uterine cervix. Cancer Lett. 87(2), 187-192.
7. Martens, J.E., Smedts, F., ter Harmsel, B., Helmerhorst, T.J. and Ramaekers, F.D. (1997) Glutathione-S-transferase- pi is expressed in (pre) neoplastic lesions of the human uterine cervix irrespective of their degree of severity. Anticancer Res. 17(6D), 4305-4309.
8. Shiratori, Y., Soma, Y., Maruyama, H., Sato, S.,

Table 1. Serum total GST values in healthy controls and patients suffering from cancer cervix.

Group	Number of samples (n)	Mean GST± SEM	p value
Control	10	5.2±0 .67 IU/L	Not significant
Cases	37	5.94±0.29 IU/L	

Table 2. Comparison of pre and post radiotherapy (RT) values of GST in patients.

No. of Patients	Pre RT (GST IU/L)	Post RT (GST IU/L)	p value
9	6.6 ± 0.72	4.01 ± 0.58	< 0.005
8	5.46 ± 0.66	6.67 ± 0.78	< 0.004

Takano, A., and Sato, K. (1987) Immunohistochemical detection of the placental form of glutathione S-transferase in dysplastic and neoplastic human uterine cervix lesions. Cancer Res. 47(24 Pt 1), 6806-6809.

9. Randall, B.J., Angus, B., Akiba, R., Hall, A., Cattan, A.R., Proctor, S.J., Jones, R.A. and Horne, C.H. (1990) Glutathione-S-transferase (placental) as a marker of transformation in the human cervix uteri: an immuno-histochemical study. Br. J. Cancer 62(4), 614-618.

10. 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(1), 17-23.

11. Vural, E., Alexiou, M., Hunt, J 2nd, Koroutian, S., and Hanna, E. (2001) Is glutathione-S-transferase-pi expression a reliable predictor of chemoradiation response in cancer of the head and neck? Am. J. Otolaryngol. 22(4), 257-260.

12. Mukundan, H., Bahadur, A.K., Kumar, A., Sardana, S., Naik, S.L., Ray, A. and Sharma, B.K. (1999) Glutathione level and its relation to radiation therapy in patients with cancer of uterine cervix. Indian J. Exp. Biol. 37(9), 859-864.

13. Sabitha, K.E. and Shyamaladevi, C.S. (1999) Oxidant and antioxidant activity changes in patients with oral cancer and treated with radiotherapy. Oral Oncol. 35(3), 273-277.

14. Silvestrini, R., Veneroni, S., Benini, E., Daidone, M.G., Luisi, A., Leutner, M., Maucione, A., Kenda, R., Zucali, R. and Veronesi, U. (1997) Expression of p53, glutathione S-transferase-pi, and Bcl-2 proteins and benefit from adjuvant radiotherapy in breast cancer. J. Natl. Cancer Inst. 89(9), 605-607.

15. Hamada, S., Kamada, M., Furumoto, H., Hirao, T., and Aono, T. (1994) Expression of glutathione-S-transferase-pi in human ovarian cancer as an indicator of resistance to chemotherapy. Gynecol Oncol. 52(3), 313-319.

16. Beutler, E. (1984), Red cell metabolism. A manual of biochemical methods, 3rd Ed, Grune and Startron, Inc: London, pp.8-78.

17. Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974). Glutathione-S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

18. Harvey, J.W. and Beutler, E. (1982). Binding of heme by glutathione-S-transferases – A possible role of erythrocyte enzyme. Blood, 60(5): 1227-1230.

19. Preisegger, K.I.I., Beham, A., Kopp S., Jessernigg, G., Gugl, A., and Stammberger, II. (2001). Prognostic impact of molecular analyses in adenoid cystic carcinomas of the salivary gland. Onkologie. 24(3), 273-277.

20. Terakado, N., Shintani, S., Nakahara, Y., Mihara, M., Tomizawa, K., Suzuki, K., Taniguchi, N., and Matsumura, T. (2000). Expression of Cu, Zn-SOD, Mn-SOD and GST-pi in oral cancer treated with preoperative radiation therapy. Oncol Rep. 7(5), 1113-1117.

21. Tanita, J. (1992). Immunohistochemical detection of glutathione-S-transferase (GST)-pi in head and neck carcinoma and its changes by radiotherapy. Nippon Jibiinkoka Gakkai Kaiho. 95(9), 1421-1429.

22. Miura, K., Suzuki, S., Tanita, J., Shinkawa, H., Satoh, K., and Tsuchida, S. (1997). Correlated expression of glutathione-S-transerase-pi and c-Jun or other oncogene products in human squamous cell carcinomas of the head and neck: relevance to relapse after radiation therapy. Jpn J Cancer Res. 88(2), 143-151.

23. Tanita, J., Tsuchida, S., Hozawa, J., and Sato, K. (1993). Expression of glutathione-S-transferase-pi in human squamous cell carcinomas of the pharynx and larynx. Loss after radiation therapy. Cancer. 72(2), 569-576.

24. Agrawal, A., Choudhary, D., Upreti, M., Rath, P.C., and Kale, R.K. (2001). Radiation induced oxidative stress: I. Studies in Ehrlich solid tumor in mice. Mol Cell Biochem. 223(1-2), 71-80.

25. Khil, M.S., Kim, S.H., Pinto, J.T., and Kim, J.H. (1996). Ethacrynic acid: a novel radiation enhancer in human carcinoma cells. Int J Radiat Oncol Biol Phys. 34(2), 375-380.

26. Cheng, X., Kigawa, J., Minagawa, Y., Kanamori, Y., Itamochi, H.,Okada, M., and Terakawa, N. (1997). Glutathione-s-transferase-pi and glutathione concentration in ovarian carcinoma before and after chemotherapy. Cancer. 79(3), 521-527.

27. Bay, F., Nakanishi, Y., Kawasaki, M., Tokuyama, K., Yatsunami, J., Pei, X.H., Tsuruta, N., Wakamatsu, K., and Hara, N. (1996) Immunohistochemical expression of glutathione-s-transferase-pi can predict chemotherapy response in patients with nonsmall cell lung carcinoma. Cancer. 78(3), 416-421.