Exp Oncol 2013
35, 2, 97–100

Experimental ONCOLOGY

SERUM SELENIUM CONCENTRATION AND ANTIOXIDANT ACTIVITY IN CERVICAL CANCER PATIENTS BEFORE AND AFTER TREATMENT

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***Aim*: In the present study, the effect of chemo and radio therapies on serum trace elements content and antioxidant activity in blood serum of cervical cancer patients was evaluated. *Methods*: Among 104 cervical cancer patients selected for the present study, 54 and 50 patients were treated with chemo- and radiotherapy respectively. Plasma Se, Zn, Cu and some enzymatic antioxidants activities were estimated in serum before and after the treatment. *Results*: The decreased levels of serum trace elements, glutathione peroxidase activity and total antioxidant capacity, and increased malondialdehyde, glutathion reductase was observed in cervical cancer patients when compared to healthy controls. The increased concentration of serum Se, Zn was observed in patients treated with chemotherapy. Simultaneously there was a significant ($P < 0.001$) increase in glutathione peroxidase and total antioxidant capacity, and significant decrease ($P < 0.05$) in malondialdehyde and glutathion reductase levels in the serum of patients treated with chemotherapy compared to the patients treated with radiotherapy. *Conclusion*: The results demonstrated that chemotherapy but not radiotherapy results in significant increase in the trace elements levels and antioxidant activities in blood serum of cervical cancer patients.**
***Key Words*: cervical cancer, free radicals, glutathione peroxidase, selenium, chemotherapy, radiotherapy.**

Cervical cancer is one of the most prevalent cancers world-wide and the most recent compilation of data indicates that an estimated 4,70,000 new cervical cancer cases occur annually among women world-wide. Human papilloma virus is the main causative agent for the cervical cancer along with some other risk factors. It is acknowledged that reactive species (ROS) play an important role in many pathological conditions including cancer. Under normal conditions their harmful effects upon the cells are neutralized to a large extent by a cascade of antioxidant defense mechanisms of the body. When overwhelming generation of ROS malfunction of antioxidant balance occurs in the body, the system is considered to be in a state of oxidative stress [1]. It has been stated that generation of ROS during cancer development interferes with organ functions directly by damaging proteins, fats and nucleotides [2].

Antioxidant supplementation during radiation therapy possesses a conundrum for the radiation oncologist, as antioxidants that defend normal cells from reactive oxygen species may provide the same benefits to cancer cells and reduce the efficacy of treatment. Short- and long-term injury to normal healthy cells, including tissue damage and risk of oncogenic transformation [3], can be prevented by antioxidants. New findings that antioxidants induce apoptosis in tumor cells and protect patients from severe side effects of radiation treatment may prove these compounds useful in future adjuvant therapy.

At optimal doses, the radiation used in therapy depletes cellular alphatocopherol in normal cells, thereby increasing their risk of damage; animal studies show that whole-body exposure to X-ray irradiation decreases the concentrations of vitamins C and E in tissues [4]. A decline in tissue vitamin E and Se during radiation therapy for breast cancer and a fall in vitamins A, C, E, and Se during breast cancer treatment with ROS-producing adriamycine may increase normal tissue sensitivity to radiation damage.

Selenium is one of the key components of antioxidative mechanism which belongs to every cell, and there is evidence that this essential trace element have anticancer properties. The large body of evidence suggested the protective role of selenium (Se) in different cancers including cervical cancer. Se exerts its chemopreventive effect in different ways, such as a defense against oxidative damage by scavenging the ROS and improves the synthesis of enzymatic antioxidant glutathione peroxidase (GPx) [5, 6]. The cytosolic GPx is the best selenoprotein, characterized in mammalian cells, capable of reducing equivalents from glutathione to detoxify hydrogen and lipid peroxidation [7]. In relation to the fortification with Se, it has been demonstrated that Se and vitamin E (tocopherol) are interrelated in the *in vivo* antioxidant system by a double way, first, the formation of GPx contributes to the decomposition of lipid hydroperoxides into non-pro-oxidant species; second, tocopherol acts as a chain-breaking antioxidant [8, 9].

Therefore, the present study evaluated the effect of chemo and radiotherapy on serum trace element levels and antioxidant capacity in cervical cancer patients.

MATERIALS AND METHODS

Sample collection. Serum samples of cervical cancer patients were collected from the Government Maternity Hospital, Tirupati, and Department of Radiation Oncology, Government General Hospital, Guntur, Andhra Pradesh. The population in-

Received: March 5, 2013.
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Abbreviations used: Gpx – glutathione peroxidase; GR – glutathione reductase; MDA – malondialdehyde; ROS – reactive oxygen species.

cluded 104 (54+50) cervical cancer patients, aged 30 to 75 years (46.13±4 years), randomly collected from the hospitals. Serum samples from age matched 50 healthy donors (30–73 years) were collected as control samples. The inclusion criteria for the selection of cervical cancer patients were proven by the biopsy/histopathology, these patients had any other pathologies and were treated only with chemotherapy or radiotherapy.

For histopathological analysis, tumors were classified according to TNM classification system [10]. From 104 cervical cancer patients, 54 patients were treated with chemotherapy (cisplatin + mitomycin) and 50 patients were treated with radiotherapy (Cent Gy/sec).

***Ethical approval*.** The experimental protocol and ethical aspects were approved by the Institutional Ethical Committee (IEC), Sri Venkateswara Medical College, Tirupati along with the patient consent forms.

***Biochemical studies*.** Approximately 3 ml of blood was collected before and after treatment (chemo- or radiotherapy). Serum was separated from the blood and stored at -40 °C until analysis.

Atomic adsorption spectroscopy was used to analyze the concentration of serum trace elements like Se [11], zinc and copper [12]. The GPx activity [13] and total antioxidant ability [14] was measured by spectrophotometric method. Serum malondialdehyde (MDA) was determined by the method [15] and glutathione reductase (GR) activity was measured in the serum by the method described in [16].

Statistical analysis. The data were analysed using a statistical package for social sciences (SPSS 11.5 version). A one way ANOVA was performed followed by Tukey's test for comparison of results between control and experimental groups. All values were expressed as mean±S.E.

RESULTS

In this study, 104 cervical cancer patients were selected for therapy based on their tumor stage and clinicopathological indexes (Table 1). All these patients had decreased levels of trace elements, GPx and total antioxidant activity compared to the healthy controls (Table 2). Increased concentration of serum trace elements was observed in patients treated with chemotherapy ($P < 0.05$) versus patients treated with radiotherapy. Interestingly, there was significant ($P < 0.001$) increase in the activity of total antioxidant activity (2.09±0.12) and GPx (58.2±7.82) in patients treated with chemotherapy, and there is no significant change in these indexes (1.23±0.34 and 43.2±2.32) in radiotherapy treated patients.

The lipid peroxidation product, i.e. MDA levels, and GR were found to be increased (15.99±2.63 µl/l and 58.59±4.62 U/l) significantly in the serum of the patients with cervical cancer compared to the controls (11.86±1.89 µl/l and 48.63±2.32 U/l), but there is no significant changes in these indexes after the courses of chemotherapy or radiotherapy. However, after chemotherapy, the decreased MDA and GR levels were registered compared to radiotherapy-treated group.

Table 1. Characteristics of cervical cancer patients

№	Character	Patients, n
1	**Median age (range), years**	104 (30–75)
2	**Stage of cervical cancer**	
	IIA	16
	IIB	28
	IIIA	34
	IIIB	18
	IV	8
3	**Histological features**	
	SCC	64
	DSCC	12
	M.D/WDSCC	6/15
	Du SCC	4
	In. SCC	3
4	**Lymphnode involvement**	26
	No involvement of lymphnode	78
5	**Treatment**	
	Chemotherapy (cisplatin + mitomycin)	54
	Radiotherapy (80 centi Gy/30 sec – min. per cycle)	50

Notes: SCC – squamous cell carcinoma; DSCC – differentiated squamous cell carcinoma; M.D/WD SCC – moderately and well differentiated squamous cell carcinoma; Du SCC – ductal squamous cell carcinoma; In. SCC – intermediate squamous cell carcinoma.

Table 2. Serum levels of trace elements and antioxidant status of cervical cancer patients before and after treatment

No	Parameter	Healthy controls (n = 50)	Before treatment (n = 104)	After treatment	
				Chemotherapy (n = 54)	Radiotherapy (n = 50)
1	Se (µg/dl)	13.83 ± 0.21	7.32 ± 0.59*	11.16 ± 0.32*	8.90 ± 1.23*
2	Zn (µg/dl)	89.45 ± 0.32	56.32 ± 0.43*	83.62 ± 4.76*	59.98 ± 2.76*
3	Cu (µg/dl)	98.76 ± 2.43	72.15 ± 2.8*	73.13±2.6***	72.54±2.6***
4	Mn (µg/dl)	0.54 ± 0.12	0.23 ± 0.18*	0.49 ± 1.2*	0.32 ± 0.23*
5	GPx (nmol/ NADPH/min/ mg protein)	62.5 ± 3.82	42.4 ± 4.63*	58.2 ± 7.82*	43.2 ± 2.32**
6	MDA (µl/l)	8.89 ± 1.52	15.99 ± 2.63*	11.86±1.89**	14.78±0.54**
7	GR (U/l)	24.63 ± 0.46	58.59 ± 4.62*	48.63±2.32**	54.41±1.56**
8	TAC (mmol/l)	3.34 ± 0.23	1.02 ± 0.46*	2.89 ± 0.12*	1.23 ± 0.34**

Notes: Values are mean ± SE. Values with different superscripts differ significantly from values within the same row, by ANOVA and Tukey's tests. *Significant at $P < 0.001$; **significant at $P < 0.05$ and ***unsignificant; GPx – glutathione peroxidase; TAC – total antioxidant capacity; MDA – malondialdehyde, GR – glutathione reductase.

***Correlation between Se and GPx*.** Serum selenium was correlated with GPx at different levels, correlation values is 0.33 in healthy samples versus 0.55 and 0.63 in chemo- or radiotherapy treated patients respectively (Fig. 1 and 2). This correlation indicated that increased concentration of serum Se increases the antioxidant enzyme GPx, which is a selenium dependent antioxidant.

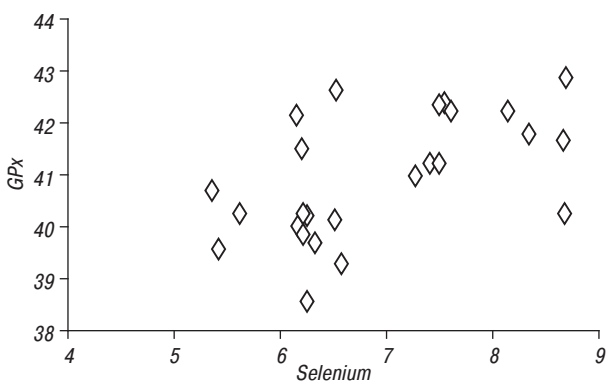

Fig. 1. Correlation curve between Se and GPx after the chemotherapy

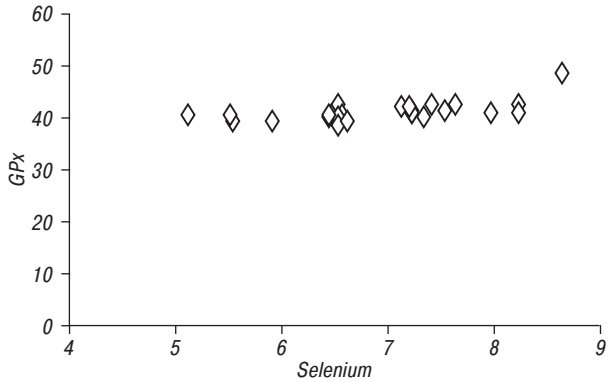

Fig. 2. Correlation curve between Se and GPx after the radiotherapy

DISCUSSION

Chemotherapy involves administration of cytotoxic drugs that prevents growth and proliferation of neoplastic cell. Se is an antioxidant agent: it can can reduce hydrogen peroxides, lipid and phospholipids hydroperoxides via GPx, thereby dampening the propagation of free radicals and ROS.

In the present study, decreased serum trace elements, especially Se levels were observed in cervical cancer patients as compared to healthy controls (Table 2). This is in accordance with observations that the patients with Crohns disease and cancer [17, 18] have significantly lowered serum Se than the controls. The epidemiological data suggest that cancer mortality is inversely correlated with Se consumption and that people living in geographic areas with low Se content have higher rate of malignancies, especially gastrointestinal and breast cancer [19].

There is no significant effect of radiotherapy on antioxidant and trace elements in cervical cancer patients. A recent study has demonstrated that there is no considerable change in serum antioxidant levels after radiotherapy in cervical cancer patients [20]. Our results were similar: no significant change in serum antioxidant levels especially GPx and total antioxidant activity after radiotherapy were found. However, after the chemotherapy, 80% of the patients have shown increased concentration of Se, Zn and Mn accompanied by GPx and total antioxidant activities. The similar findings were reported in the study [21] which indicated that GPx activity was significantly increased in breast cancer patients after chemotherapy. Fig. 1 and 2 show that there is correlation between serum Se and GPx in chemo and radiotherapy treated patients. The increased Se concentration may be attributed to the exogenous supplementation of drugs. Increased Se in serum of cancer patients induces the increased production of Se dependent antioxidant mechanism like GPx, hence in the present study GPx and total antioxidant activity was increased in chemotherapy treated patients compared to radiotherapy treated patients.

It is well established that antioxidant defense system scavenges the free radicals directly or interferes with the generation of free radical mediated events inhibiting the neoplastic process [22]. In the present study, the serum antioxidant enzyme, i.e. MDA and GR activity was found to be increased significantly in patients with cervical cancer. Similar reports of raised antioxidant enzyme activities have been reported in patients with prostate cancer [23]. Glutathione reductase, an oxidative stress inducible enzyme, plays a considerable role in the peroxyl scavenging mechanism and in maintaining the functional integration of the cell membranes. The rise in the activity of GR could be due to its induction to counter the effect of increased oxidative stress.

In the present study, a significant elevation of serum MDA in cervical cancer patients in comparison to healthy controls has been observed. The reason for increased lipid peroxidation could be due to increased generation of reactive oxygen species or suppression of the antioxidants defence mechanism in the metabolically active tissues. Recently it was reported the antioxidant defence was suppressed in various colorectal cancer tumors [24].

Selenium is an essential trace element for animals including humans, has been shown to affect the function of selenoproteins by being a part of active site in antioxidant enzymes like glutathione peroxidase and thioredoxin reductase. Furthermore Se can acts as anticancer agent through the plausible mechanisms like stimulation of immune system and inhibition of cell proliferation [25]. The sustainable persuasive evidence indicates that Se can certainly play an important role in cancer prevention [26].

Multiple lines of experimental evidence indicated that optimal supplementation with Se reduces pulmonary metastasis of melanoma in mice [27]. Results obtained with the gene therapy approach clearly demonstrated that CH_3SeH can inhibit tumor growth and prolong host survival rate [28]. The prevention of tumor cell adhesion and migration is related to inhibition of tumor cell invasion into basement membrane [29]. It was also documented that brief pre-exposure of HeLa cells to micromolar concentration of selenite resulted in a dose dependent decrease in the rate of their subsequent attachment to a solid matrix [30]. Further, the present findings also reveal a remarkable relationship between levels of trace elements versus antioxidants enzymatic system, especially Se versus GPx and the concentration of Se is directly proportional to the GPx activity. Consistent with the above findings it is important to maintain a constant balance in serum trace elements like Se in cancer patients which induces the enhancement of antioxidant enzymes.

In conclusion, we have registered the decreased concentration of trace elements, Se, GPx activity and increased activity of lipid peroxidation, GR in cervical cancer patients. However these indexes were significantly improved after chemotherapy but not radiotherapy. Hence, chemotherapy promotes an increase of the antioxidant defense.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. D.S. Raju Naidu and Dr. P. Chandra Sekharan for providing cervical cancer samples. The authors also thanks to Institutional Ethical committee (IEC), S.V. Medical College, for providing ethical clearance and UGC-BSR-Non-SAP for providing financial assistance.

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