Public Health Nutrition: 12(1), 59–63 doi:10.1017/S1368980008001924

A comparative study of selenium concentration and glutathione peroxidase activity in normal and breast cancer patients

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Submitted 8 May 2007: Accepted 5 January 2008: First published online 7 March 2008

Abstract

Objective: The present study was undertaken to compare plasma Se values and glutathione peroxidase (GPX) activity in normal and breast cancer patients.

Design: In a case–control study, forty-five breast cancer patients and the same number of healthy women were randomly selected from their population. Se was measured in plasma by atomic absorption spectrophotometry and GPX activity in erythrocytes was measured using a standard spectrophotometric method.

Results: Plasma Se concentration in healthy women and breast cancer patients was in the normal range, with no statistically significant difference observed between the two groups (138·40 (SD 40·36) μg/l *v.* 132·15 (SD 35·37) μg/l, respectively). Erythrocyte GPX activity was significantly ($P < 0·01$) higher in breast cancer patients (24·81 (SD 11·66) U/g Hb) compared with healthy women (20·29 (SD 4·24) U/g Hb).

Conclusion: The present study indicated that Se deficiency was not a problem in the participants, and sufficient quantity of this element could increase GPX activity to have a protective effect against oxidative damage.

Keywords
Antioxidant
Breast cancer
Glutathione peroxidase
Selenium

Breast cancer is the most common malignancy in women, with 1 million new cases worldwide each year, and comprises 18% of female cancers[1]. In the Middle East, breast cancer is the most common malignancy among women[2]. Similarly in Iran, breast cancer is the highest in rank among cancers of women and accounts for 21·4% of all malignancies in females[3]. There is evidence that Se as an essential trace element has anticancer properties[4–7]. Research spanning the last 35 years has established that Se is effective in the reduction of cancer incidence when provided to animals at non-toxic dose (five to ten times the nutritional requirement)[8,9]. But for researchers conducting studies on Se and cancer prevention, the most exciting news in recent years has been the finding of Clark *et al.*[10] indicating that the supplementation of free-living people with selenized brewer's yeast decreases overall cancer morbidity and mortality by nearly 50%.

Epidemiological data have also supported a protective effect of Se in man with regard to the prevention of both prostate cancer[11,12] and lung cancer[13]. The relationship between Se status and the incidence of breast cancer needs to be clarified, however, because one study indicates a protective effect[14] whereas other studies have not shown any protective effect[15–18].

Cellular oxidative damage is a well-established general mechanism for cell and tissue injury. Oxidative damage to cells is caused primarily by free radicals and reactive oxygen species. Free radicals have the ability to bind to most normal cellular components; they react with unsaturated bonds of membrane lipids, denature proteins and attack nucleic acids. Prime targets of reactive oxygen species are the PUFA in cell membranes, causing lipid peroxidation which may lead to damage of cellular structure and function[19]. Oxidative stress has been suggested to play a role in some physiological conditions and in many disease processes, including carcinogenesis. It has been proposed that Se exerts its chemoprevention effect in different ways, providing a protective effect against oxidative damage by decreasing the amount of free radicals and by increasing the synthesis of glutathione peroxidase (GPX)[4–7]. This cytosolic enzyme is the first and best-characterized selenoprotein[20]. In the cell cytosol, it functions as an antioxidant by directly reducing H_2O_2 and hydroperoxides to the corresponding alcohols and water. Therefore this enzyme can prevent

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the production of reactive oxygen radicals and thus may contribute to protection of the organism's macromolecules and biomembranes against oxidation.

The present study was conducted to compare plasma Se concentration and erythrocyte GPX activity in patients with breast cancer and in healthy women.

Materials and methods

Participants

The present study was a hospital-based case–control study. Forty-five women with breast cancer aged 26–70 years (mean 45 (SD 9) years) participated in the study.

The inclusion criteria for the patients were: (i) cases of breast cancer proved by histopathology/cytopathology; (ii) not having undergone any specific treatment for breast cancer; (iii) not suffering from concomitant diseases such as diabetes mellitus, rheumatoid arthritis or thyroid and liver disorders; and (iv) not having taken vitamin or mineral supplements during the past year.

In histopathological analyses, tumours were classified according to the WHO nomenclature classification[21]. By this means, eight patients were classified as stage I, twenty-three patients were classified as stage II and fourteen patients were classified as stage III. As a control group, forty-five healthy female volunteers aged 27–67 years (mean 44 (SD 8) years) were selected and included in the study. The last three inclusion criteria mentioned for breast cancer women were considered for healthy subjects as well.

The study protocol and ethical aspects were approved by the ethics committee of the Research Council of the Dean of Research Affairs of Shiraz University of Medical Sciences.

Background characteristics and food consumption assessment

Data on demographic characteristics, any concurrent illness history, medication, and vitamin and mineral supplementations were collected by interviews. Anthropometry including weight and height were measured for each participant. Body weight was measured to the nearest 0·1 kg using a Seca 713 scale while subjects were minimally clothed. Height was determined using a measuring tape on subjects without shoes. BMI was calculated by dividing weight (kg) by the square of height (m^2). The food consumption pattern was evaluated by a semi-quantitative FFQ. Macro- and micronutrient components were calculated by using Food Processor Software version 1 (Tehran University, Tehran, Iran) modified by incorporating the Iranian food table.

Biochemical analyses

Blood samples (5 ml) were taken by venous arm puncture and drawn into EDTA tubes. Plasma was separated by centrifugation at 1000***g*** for 10 min at 4°C and stored at −80°C until analysis. After plasma separation, the white buffy layer (leucocytes) was removed and the packed cells washed twice with physiological saline. A known volume of erythrocytes was lysed in 4 volumes of ice-cold HPLC-grade water and then centrifuged at 3000***g*** for 10 min at 4°C. The supernatant was collected and stored at −80°C until analysis.

Graphite furnace atomic absorption spectroscopy was used to determine the concentration of Se in plasma[22]. GPX activity in erythrocytes was measured using the spectrophotometric method described by Paglia and Valentine[23].

Statistical analyses

Data are expressed as means with their standard deviation. Comparison between parameters of breast cancer patients and controls was performed using the independent *t* test. A value $P < 0{\cdot}05$ was considered significant. All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS for Windows) statistical software package version 11 (SPSS Inc., Chicago, IL, USA).

Results

Table 1 presents details of the study population in terms of age, weight, BMI, age at first pregnancy, age at onset of

Table 1 Description of the study population: Iranian breast cancer patients and controls

		Cases		Controls		
Characteristic	*n*	Mean	SD	Mean	SD	Significance (*t* test)
Age (years)	45	45·0	9·0	44·3	8·7	NS
Weight (kg)	45	64·8	12·2	70·1	9·5	$P < 0{\cdot}02$
Height (cm)	45	156·5	5·3	157·4	4·8	NS
BMI (kg/m^2)	45	26·5	5·0	28·4	3·7	$P < 0{\cdot}05$
Age at first pregnancy (years)	41	22·0	6·0	18·5	3·6	$P < 0{\cdot}002$
Age at menarche onset (years)	45	13·3	1·6	13·3	1·1	NS
Number of children	41	3·7	1·8	3·8	1·8	NS
Age at menopause onset (years)	11	48·4	4·5	48·3	4·0	NS

https://doi.org/10.1017/S1368980008001924 Published online by Cambridge University Press

Table 2 Plasma selenium and erythrocyte glutathione peroxidase (GPX) activity in Iranian breast cancer patients and controls

		Cases		Controls		
Parameter	*n*	Mean	SD	Mean	SD	Significance (*t* test)
Plasma Se (μg/l)	45	132·15	35·37	138·40	40·36	NS
Erythrocyte GPX (U/g Hb)	45	24·81	11·66	20·29	4·24	$P<0·01$

Table 3 Plasma selenium and erythrocyte glutathione peroxidase (GPX) activity in Iranian breast cancer patients according to disease stage

		Plasma Se (μg/l)		Erythrocyte GPX (U/g Hb)	
Clinical stage	*n*	Mean	SD	Mean	SD
Stage I	8	120·87	36·03	25·33	17·29
Stage II	23	133·65	30·60	24·91	11·39
Stage III	14	125·07	46·52	24·36	8·90
Significance (*t* test)		NS		NS	

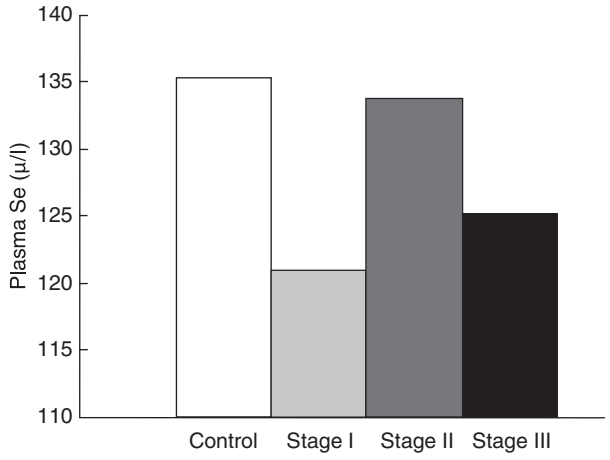

Fig. 1 Plasma selenium concentration in Iranian breast cancer patients according to disease stage

menarche, number of children and age at onset of menopause. The table shows that weight, BMI and age at first pregnancy were significantly different between the two groups ($P<0·05$).

The mean plasma Se concentration and erythrocyte GPX activity of the breast cancer patients and the control subjects are presented in Table 2. No significant difference was observed in Se levels of the two groups, but patients with breast cancer had a significantly higher erythrocyte GPX activity than did the control group ($P<0·01$).

According to the WHO classification, tumours were classified as stage I (eight patients), stage II (twenty-three patients) and stage III (fourteen patients). As Table 3, Fig. 1 and Fig. 2 show, there were no significant differences in plasma Se concentration and erythrocyte GPX activity between these disease subgroups.

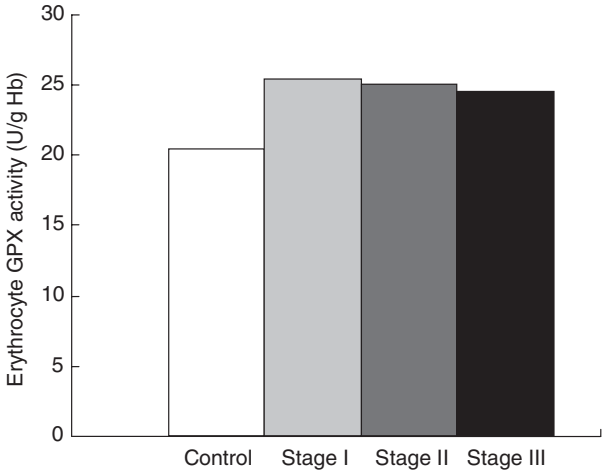

Fig. 2 Erythrocyte glutathione peroxidase (GPX) activity in Iranian breast cancer patients according to disease stage

Discussion

In recent years, many studies have shown that Se is a potent protective nutrient for some forms of cancer. Studies have also shown that breast cancer patients present serious disturbance in the status of trace elements, especially those involved in oxidant systems. Oxidative stress produced through either increased free radical generation and/or a decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis[24]. As far as breast cancer is concerned, enhanced oxidative stress in tissues and serum has been reported[25–27]; however, the aetiology of the majority of human breast cancer is still unclear. With regard to human tumours, the limited data available concerning serum levels of Se do not show similar trends. The results of the present study showed lower plasma Se levels in breast cancer patients compared with control subjects; however, the difference was not statistically significant. Also, no significant reduction in plasma Se was observed across three breast cancer disease stages. This may be due to the small numbers of patients in these subgroups.

A case–control study conducted in Spain revealed that the mean serum Se concentration was 81·1 μg/l in women with breast cancer and 98·5 μg/l in women with non-tumoural disease ($P<0·001$), but the difference between two subgroups (stage I–II and stage III–IV) was not significant[28]. Gupta *et al.*[29] also found that plasma

https://doi.org/10.1017/S1368980008001924 Published online by Cambridge University Press

Se concentration was decreased in patients with breast cancer but that Se level decreased with the progress of disease. Similar results were found by Piccinnini *et al.*[30] and Krsnjavi and Beker[31]. Willett *et al.*[32] reported that low serum Se levels existed before the cancers developed, thereby increasing a person's risk of developing cancer. However, the results obtained for Se in the present study are similar to others in that breast cancer was not found to be influenced by Se status; so it has been suggested that, in contrast to men, women do not appear to be as sensitive to Se[17,18,33–35].

It must be borne in mind that variability of serum Se may be due to factors other than cancer such as the Se contents of soil and products grown in a geographical area, age, sex, BMI, dietary habits, lifestyle (smoking etc.), concurrent disease and medications.

On the other hand, these studies are methodologically complex, and at present various types of investigation (prospective, environmental, epidemiological and case–control) have failed to provide conclusive results. So the explanation for these differences cannot be made at this time.

The products of lipid peroxidation reactions in the serum and tissue of breast cancer patients have been reported by Punnonen *et al.*[36]. Higher H_2O_2 production in breast cancer under oxidative stress conditions, and the increased concentrations of free radicals and reactive oxygen species may lead to damage of most biomolecules. Some enzymes such as GPX are considered antioxidant enzymes, since it is involved in direct elimination of these products. Se is a component of GPX, and acts as antitumour agent. The decrease in plasma Se may be the result of increased activity of GPX or increased tumoural mass, which in turn may increase the amount of free radicals in the tumoural tissue. These free radicals may attract greater amounts of Se through electrophilic mechanisms which results in the reduction of Se in plasma.

The present findings showed a highly significant elevation in erythrocyte GPX activity in breast cancer patients ($P < 0{\cdot}01$) compared with the control group. This may be due to the response to higher free radical production in breast cancer patients. This result is in good agreement with many other authors. Ray *et al.*[37] reported significantly increased GPX activity in breast cancer patients and significantly higher GPX activity in all subgroups (stage II, III and IV). Findings by Seven *et al.*[38] also suggest that there is a significant increase in erythrocyte GPX concentration ($P < 0{\cdot}01$) in breast cancer patients compared with patients with benign breast disease.

We suggest that patients with breast cancer have increased GPX activity due to increased formation of reactive oxygen species that causes increase in the antioxidant enzymes such as GPX, to improve the resistance of neoplastic cells to toxicity associated with tumour promotion. On the other hand, in our population study because Se deficiency was not a problem and its concentration was in the normal range, it was possible for cells to increase GPX.

Conclusion

Although Se deficiency was not a problem in the present study, it remains unclear whether low serum Se levels are induced by cancer or if they are a risk factor for cancer. If a low Se level is a risk factor for cancer, then Se administration may have a preventive effect.

Acknowledgements

Conflicts of interest: The authors declare that they have no conflicts of interest, financial, academic or otherwise.

Sources of funding: Funding through Shiraz University of Medical Sciences supported this study.

Author contributions: M.M. conceived and conducted the trial. M.M., M.H.E. and A.T. contributed to the study protocol; A.T. provided constant assistance at all stages of data gathering; M.M. and M.H.E. carried out the laboratory work. M.M. and A.R.F. conducted the statistical analysis; M.M., M.H.E. and A.T. contributed to the design of the analysis and interpretation of the results. M.M. drafted the first version of the manuscript and revised it with cooperation from M.H.E.

Acknowledgments: The authors wish to thank to the participants for their compliance and patience, and the Gasteroentrohepatic Research Center for facilitating the biomedical tests.

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