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ORIGINAL PAPER

EFFECT OF BREAST CANCER AND BREAST CANCER TREATMENT ON THE BLOOD SERUM CONCENTRATIONS OF TRACE ELEMENTS AND SELENOPROTEINS*

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Running title: Selenoproteins and trace elements in breast cancer

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Abstract

Trace elements (TEs) playing critical roles in chemical events that occur at the cellular level in the body are necessary for biological processes in human health. The role of TEs and selenoproteins and their relationship with breast cancer (BC) have not been studied thoroughly and therefore remain relatively unknown. Our study aimed to investigate possible changes in the serum selenoproteins (Glutathione Peroxidase 1 (GPX1), Glutathione Peroxidase 6 (GPX6), Selenoprotein F (Sel-F), Selenoprotein H (Sel-H), Selenoprotein S (Sel-S), Selenoprotein V (Sel-V), Selenoprotein M (Sel-M)), and TEs (Se, Zn, Mn, Cu, and Fe) levels, and TEs ratios (Fe/Se, Fe/Zn, Fe/Mn, Cu/Se, Cu/Zn, and Cu/Mn) in patients with BC before and after treatment (surgery, radiotherapy, and chemotherapy), and to evaluate the results in the patient groups with healthy controls. A total of 35 patients with BC and 25 healthy subjects were included in the study. Blood samples were collected from the patient group on the day prior to treatment, and on the day treatment was completed. Serum GPX1, GPX6, Sel- F, Sel-H, and Sel-S levels were decreased in both before and after treatment groups compared to the control. The treatment of BC resulted in increasing the concentration of Sel-V compared to before treatment levels. The treatment of BC resulted in lowering serum Se, Zn, and Fe concentrations compared to before treatment levels. Also, serum Se, Zn, and Fe levels were decreased in both before and after treatment groups compared to the control. The ratios of Cu/Se, Cu/Zn, and Cu/Mn were increased after treatment compared to the values before treatment. Cu/Se and Cu/Zn ratios were increased, but Fe/Mn ratios were decreased after treatment compared to healthy control. This study indicates that changes in serum levels of TEs such as Zn, Mn, Cu, and Se, as well as their ratios and selenoproteins, may be related to the treatments of BC. Further studies are required to clarify the exact specific mechanisms involved in the status of TEs and selenoproteins in therapeutic strategies of BC.

Keywords: breast cancer, treatment, selenoproteins, selenium, zinc, copper, iron, manganese.

INTRODUCTION

Breast cancer (BC), the most common cancer in women worldwide, is multi--etiological and multifactorial. The major risk factors are age over 40 years, history of cancer in first-degree relatives, history of mammary gland disease, early menarche, and late childbearing. Local treatment for BC consists of surgery and/or radiation therapy as well as drug therapies such as chemotherapy, anti-hormone therapy, and targeted therapies (Harris et al. 1992, Mahata et al. 2003, Waks and Winer 2019).

Some trace elements (TEs) are necessary to biological processes and classified as essential. However, an overabundance or a deficiency of TEs may promote the development of cancers (Popescu, Stanescu 2019). Changes in levels of circulating TEs have been observed in BC patients in different countries and areas. However, the relationship between these changes and the metabolic and clinical course and outcome of BC is not fully understood. There is still a gap in our understanding of the relationship between the functions of TEs and the initiation, promotion, and inhibition of the carcinogenic process (Banas et al. 2010, Ding et al. 2015). Experimental and epidemiological studies suggest that the serum levels of TEs may be associated with BC risk but their role in mutagenesis has been under-investigated. Exposure to TEs mainly occurs through environmental contamination of food, drinking water, and air. The increasing urbanization and industrialization may lead to high exposure to heavy metals and consequently increased cancer risk (Lin et al. 2006).

Zinc (Zn) is an essential trace element, which plays an anti-carcinogenic role through the structural stabilization of DNA, ribonucleic acid (RNA), and ribosome. Zinc chloride was reported to significantly decrease DNA strand breaks in human cutaneous fibroblasts exposed to a mutagen. There is also limited evidence for an inverse association between Zn and BC, and again, prospective studies are needed to confirm it (Jackson 2009). Studies reported elevated copper (Cu) levels in BC, lymphoma, and thyroid gland carcinomas compared to benign disease groups. They have focused on the role of Cu in angiogenesis, a crucial process for cancer development, and by producing DNA damage via toxic free radicals (Dragutinović et al. 2014). The role of manganese (Mn) and iron (Fe) in carcinogenesis can be observed through their role in the inhibition of apoptosis, generation of oxygen radicals, and binding competition among metal ions at chromatin and other molecules. Animal models revealed the association between parenteral Fe application with tumor growth and higher tumor incidence (Lavilla et al. 2009).

Selenium (Se) is a trace element with the most important antioxidant properties and has an inhibitory effect on viral and chemical carcinogenesis by modulating cellular proliferation in both normal and neoplastic cells. Data from *in vitro* and animal model systems show that Se compounds exert protective effects throughout BC. Several cellular and molecular mechanisms

have been implicated in Se chemoprotection, chemoprevention, and chemotherapy effects in BC. Se is an essential micronutrient with promising BC prevention and treatment potential since it is proven with preclinical research that it may inhibit mammary carcinogenesis (Reid et al. 2002, Cox et al. 2016). But studies showed that Se supplementation decreased the risk of prostate, colon, and lung cancers, whereas it increased the risk of BC. The evidence currently available appears to support an inverse association between Se exposure and cancer risk, but the evidence is inconsistent, so additional prospective studies are needed (Reid et al. 2002, Duffield-Lillico et al. 2003, Thompson et al. 2016).

Se is the primary component of selenoproteins, which have roles in counteracting oxidative stress and regulating the redox status of other molecules (Goldhaber, 2003). Se exerts most of its biological effects through selenoproteins which it is incorporated into as selenocysteine (Steinbrecher et al. 2010). Glutathione peroxidases (GPXs) belong to the selenoprotein family related to antioxidant enzymes, involved in the reduction of hydrogen peroxide (H_2O_2), detoxification of hydroperoxides, and maintaining cellular redox homeostasis, Selenoprotein F (Sel-F) and Selenoprotein M (Sel-M) are involved in protein folding in the endoplasmic reticulum (ER), Selenoprotein S (Sel-S) related to the elimination of misfolded proteins from the ER, also Selenoprotein H (Sel-H), and Selenoprotein V (Sel-V) is one of the thioredoxin-like (Rdx) family proteins that protects against endoplasmic reticulum stress (Davis et al. 2012, Méplan, Hesketh, 2012, Labunskyy et al. 2014, Kang et al. 2020). In the processes of carcinogenesis, a reduced expression of the genes encoding selenocysteine-containing proteins leads to either inhibition of the oncotransformation or the return of the cell to the oncophenotype. It is reported that selenoproteins have been implicated in cancer development (Steinbrecher et al. 2010).

In light of the aforementioned information, there are studies in the literature on the action of TEs in BC (Wu et al. 2004, Siddiqui et al. 2006, Saleh et al. 2011, Rehman, Husnain 2014, Lappano et al. 2017, Choi et al. 2019). However, possible changes in selenoproteins and TEs with treatments have not been investigated in BC. Therefore, the present study aimed to determine the effect of treatment (surgery, radiotherapy, and chemotherapy) on serum Glutathione Peroxidase 1 (GPX1), Glutathione Peroxidase 6 (GPX6), Sel-F, Sel-H, Sel-S, Sel-V, Sel-M, Se, Zn, Mn, Cu, Fe levels, and TEs ratios (Fe/Se, Fe/Zn, Fe/Mn, Cu/Se, Cu/Zn, and Cu/Mn) in patients with BC. Also, TEs and selenoproteins levels were evaluated for before and after treatment and in control groups, and were discussed in terms of their potential role in therapeutic strategies of BC.

MATERIALS AND METHODS

Case selection

A total of 35 patients (range 30-68 years) diagnosed with BC were included in the study. The 25 healthy female volunteers of similar age (range 29-64 years), who did not receive any medication, constituted the control group. Diabetes, hypertension, chronic inflammatory diseases, infectious diseases, and distant metastases or other malignant diseases were designated as exclusion criteria for both the patients with BC and healthy control subjects. All patients gave written informed consent, and the present study was approved by the Local Ethics Committee (Approval number: E-54022451-050.05.04-38194) and performed under The Declaration of Helsinki.

Sample collection

Five milliliters of a venous blood sample were drawn after 12 h overnight fasting before treatment, and after the treatment from patients with BC. The same volume of blood samples was collected from the forearm antecubital veins of all cases to be included in the study before any treatment or from controls. Then the blood samples were separated by centrifugation at 3000 rpm for 10 min and the separated sera were put into capped plastic tubes and kept at −80°C until assays. Selenoproteins and TEs were determined in serum samples.

Measurement of selenoproteins

The levels of GPX1, GPX6, Sel-F, Sel-H, Sel-S, Sel-V, and Sel-M in serum samples were quantified according to the manufacturer's instructions and guidelines using ELISA kits (Abbkine Scientific Co., Ltd, Wuhan, Hubei, China). The intra-assay and interassay variabilities of the ELISA kit for GPX1, GPX6, Sel-F, Sel-H, Sel-S, Sel-V, and Sel-M were 6.9 and 7.8%; 6.6 and 6.9%; 6.6 and 7.3%; 5.5 and 5.8%; 5.3 and 5.9%; 5.8 and 6.2%; and 7.4 and 8.3%, respectively.

Measurement of the trace elements

The trace element levels were measured with an inductively coupled plasma optical emission spectrophotometer (ICP-OES, Thermo iCAP 6000, Cambridge, UK). The serum samples were diluted (1:9) with distilled water (Millipore, Bedford, MA, USA) containing 0.3% HNO_3 (Merck, Darmstadt, Germany). Calibration standards were prepared using the stock solution at a concentration of 1,000 mg L^{-1} (Chem-Lab, Belgium). Elemental solutions of 0.0025, 0.005, 0.010, 0.025, 0.05, 0.25, and 0.50 ppm concentrations were prepared by using stock solution and distilled water containing 0.3% HNO_3. The Se, Zn, Mn, Cu, and Fe element levels were determined at wavelengths of 196.026, 213.800, 257.610, 324.754, and 259.940 nm respectively. Results were presented as μg dl^{-1}.

Statistical evaluation

Data were presented as mean ± the standard deviation (SD). Statistical analysis was performed using the Wilcoxon signed-rank, and Mann-Whitney *U* tests. Statistical Package for the Social Sciences – SPSS 21.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for calculations. $P<0.05$ was considered to indicate a statistically significant difference.

RESULTS

Demographic data

Demographic data of patient and control groups are presented in Table 1. Besides, both the patients and controls did not report any occupational/accidental exposure to heavy metals or use of hormone replacement therapy. Moreover, no subjects reported impaired appetite, malnutrition, TEs supplementation, or unusual weight change. The age of patients was greater than 40 in 91.43% and 80.00% in the control group. Only one patient had lobular carcinoma and the rest were classified as invasive ductal carcinoma. The estrogen receptor status was negative in 2 patients while the progesterone receptor status was negative in 3 patients. CerB2 was positive in 4 patients. TNM status was 100% in M0. Patients' characteristics for T stages were 10 patients (28.58%) in T1, 20 patients (57.14%) in T2, and 5 patients (14.28%) in T3. Also, characteristics of patients according to N stages were 20 patients (57.14%) in N0, 11 patients (31.43%) in N1, 2 patients (5.71%) in N2, and 2 patients (5.71%) in N3. The treatment applied was surgery and radiotherapy in all cancer patients while only 13 patients (37.14%) also received chemotherapy. All our cases underwent a fine needle aspiration biopsy before surgery. Breast-conserving surgery was used to remove cancer in all patients.

Results of serum selenoproteins' measurement

Serum Sel-V levels were increased in the after treatment group compared to the before treatment group ($p<0.05$). GPX1, GPX6, Sel-F, Sel-H, Sel-S, and Sel-V levels were decreased in BC patients before treatment compared to the control group ($p<0.001$, $p<0.01$, $p<0.01$, $p<0.01$, $p<0.01$, and $p<0.01$ respectively). Similarly, GPX1, GPX6, Sel-F, Sel-H, and Sel-S levels were decreased in BC patients after treatment compared to the control group ($p<0.001$, $p<0.05$, $p<0.05$, $p<0.01$, and $p<0.01$ respectively) – Table 2.

Results of serum TEs levels and ratios in all groups

Mean serum Se, Zn, Fe, and Cu levels were within the normal range (normal range; Se: 7.0-15.0 μg dl^{-1}, Zn: 66.0-110.0 μg dl^{-1}, Fe: 40.0-155.0 μg dl^{-1}, and Cu: 63.7-140.12 μg dl^{-1}) in BC patients, but Se, Zn, and Fe levels were

Table 1

Demographic data of breast cancer and control groups

Variables	Breast cancer *n* (%)	Control *n* (%)
Age (Year)		
>40	32 (91.43)	20 (80.00)
≤40	3 (8.57)	5 (20.00)
Gender		
female	35(100)	25(100)
Alcohol consumption		
yes	-	-
no	35(100)	25(100)
Smoking		
yes	-	-
no	35(100)	25(100)
Breast cancer		
one side	35(100)	-
two sides	-	-
Biopsy before surgery		
yes	35(100)	-
no	-	-
Type of surgery		
breast-conserving surgery	35(100)	-
mastectomy	-	-
Histopathological types		
invasive ductal carcinoma	34 (97.14)	-
lobular carcinoma	1 (2.86)	-
TNM status		
T1	10 (28.58)	-
T2	20 (57.14)	-
T3	5 (14.28)	-
N0	20 (57.14)	-
N1	11 (31.43)	-
N2	2 (5.71)	-
N3	2 (5.71)	-
M0	35 (100)	-
Estrogen receptor status		
positive	33 (94.29)	-
negative	2 (5.71)	-
Progesterone receptor status		
positive	32 (91.43)	-
negative	3 (8.57)	-
CerbB2		
0	31 (88.57)	-
1	4 (11.42)	-
Treatment		
breast surgery	35 (100)	-
radiotherapy	35 (100)	-
chemotherapy	13(37.14)	-

Table 2

Serum selenoprotein levels of breast cancer and control groups

Selenoproteins	Before treatment (n:35)	After treatment (n:35)	Control (n:25)
GPX1 (pg ml^{-1})	5.327±1,302^{b***}	5.570±0.991^{c***}	7.342±2.016
GPX6 (pg ml^{-1})	1.237±0.334^{b**}	1.331±0.294^{c*}	1.617±0.466
Sel-F (pg ml^{-1})	5.344±2.077^{b**}	5.787±2.074^{c*}	7.122±1.615
Sel-H (pg ml^{-1})	45.250±13.090^{b**}	47.350±8.862^{c**}	62.270±25.180
Sel –S (pg ml^{-1})	37.360±9.616^{b**}	39.060±5.591^{c**}	45.890±11.260
Sel-V (pg ml^{-1})	6.431±2.488^{a*,b**}	7.200±2.369	8.583±2.410
Sel-M (pg ml^{-1})	16.340±3.652	16.240±3.444	16.810±3.794

All values were presented as the mean ± standard deviation. ^{a} before treatment vs. after treatment, ^{b} before treatment vs. control, ^{c} after treatment vs. control, * p<0.05, ** p<0.01, *** p<0.001.

decreased in the after treatment compared to the before treatment group (p<0.05, p<0.01, and p<0.05, respectively). Serum Cu/Se, Cu/Zn, and Cu/Mn ratios were increased in the after treatment group compared to the before treatment group (p<0.001, p<0.001, and p<0.05, respectively). There were no significant changes in Fe/Se, Fe/Zn, and Fe/Mn ratios between before treatment and after treatment groups (p>0.05) – Table 3.

Serum Se, Zn, and Fe concentrations were found within the normal range both in the before treatment group and the control group, but decreased in the before treatment group compared to the control group (p<0.01, and p<0.05, and p<0.001, respectively). The Cu/Se and Cu/Zn ratios were increa-

Table 3

Serum trace element levels and ratios of breast cancer and control groups

Elements	Before treatment (n:35)	After treatment (n:35)	Control (n:25)
Se (µg dl^{-1})	12.880±5.317^{a*,b**}	11.001±3.952^{c***}	17.052±5.395
Zn (µg dl^{-1})	90.410±22.270^{a***,b*}	82.130±21.340^{c***}	103.600±22.002
Mn (µg dl^{-1})	0.967±0.186	0.920±0.167^{c*}	1.056±0.215
Cu (µg dl^{-1})	97.660±23.370^{a*}	104.101±25.370^{c*}	88.260±20.220
Fe (µg dl^{-1})	92.140±27.240^{a**,b***}	84.640±24.740^{c***}	129.302±31.150
Cu/Se ratio	8.511±3.365^{a***,b**}	10.490±4.412^{c***}	5.660±1.992
Cu/Zn ratio	1.155±0.434^{a***,b*}	1.356±0.492^{c***}	0.897±0.318
Cu/Mn ratio	104.800±35.520^{a*}	114.900±41.330	111.500±33.160
Fe/Se ratio	8.223±3.735	8.595±3.762	8.617±3.838
Fe/Zn ratio	1.097±0.468	1.116±0.475	1.310±0.438
Fe/Mn ratio	99.280±39.660^{b***}	91.320±30.440^{c***}	165.20±58.270

All values were presented as the mean ± standard deviation. ^{a} before treatment vs. after treatment, ^{b} before treatment vs. control, ^{c} after treatment vs. control, * p<0.05, ** p<0.01, *** p<0.001.

sed, but the Fe/Mn ratio was decreased in the before treatment group compared to the control group ($p<0.05$, $p<0.05$, and $p<0.001$, respectively).

Serum Se, Zn, and Fe levels were decreased, but Cu levels were increased in the after treatment group compared to the control group ($p<0.001$, $p<0.001$, and $p<0.01$, respectively). Also, the Cu/Se and Cu/Zn ratios were increased, but the Fe/Mn ratios were decreased in the after treatment group compared to the control group ($p<0.001$ for all) – Table 3.

DISCUSSION

Oxidative stress induces a cellular redox imbalance that has been reported to be present in various cancer cells more than in normal cells; the redox imbalance thus may be related to oncogenic stimulation. In previous studies, some results have been suggested cancerogenesis associated with oxidative damage. It has been reported that oxidative stress and free radicals production increased in the various cancer types (Harris et al. 1992, Feng et al. 2012, Gan et al. 2014, Yang et al. 2015, Cox et al. 2016, El-Deeb et al. 2016). TEs such as Se, Mn, Zn, Cu, and Fe participate in the control of various metabolic and signaling pathways. Imbalance in the composition of TEs recognized to be essential to human homeostasis, besides the accumulation of potentially toxic or nonessential TEs may cause disease (Kiziler et al. 2010). In this study, levels of some TEs and selenoproteins in the patients and healthy controls were determined to understand their roles in the etiopathogenesis of BC and their changes with treatment. To our knowledge, our study is unique in examining serum Se, Mn, Zn, Cu, Fe, GPX1, GPX6, Sel-F, Sel H, Sel M, and Sel S levels in terms of the treatment of patients with BC.

Zn could act as an antioxidant, and it has the potential to replace redox-active metals (Fe, and Cu) from binding sites in proteins. Metallothionein as a metal transporter is involved in the interaction between Zn and other metal ions such as Cu. It also protects tissue from oxidative damage by binding to the sulfhydryl groups of proteins. Zn plays an important role in protecting cellular components from oxidation and damage to DNA (Lee 2018). Se is an element that plays an important role in antioxidant/redox and anti-inflammatory processes through various selenoproteins. It is stated that transition metals such as Cu and Fe play a role in the formation of oxidative damage in various types of cancer. Free metal ions such as Fe and Cu lead to molecular damage and alteration of cell homeostasis due to the formation of hydroxyl radical as a result of the Fenton reaction. Other means by which Cu and Fe can affect carcinogenesis may be related to their role in angiogenesis, that is, the formation of new blood vessels. Thus, high levels of Cu and Fe promote angiogenesis and thereby tumor growth. Alterations of levels of some TEs and oxidant/antioxidant balance in various cancer types have been reported. In the literature, there are studies, which have contradictory

results related to cancer etiopathogenesis and TEs levels. It has been suggested that a wide variety of factors may be the source of these conflicting findings (Kiziler et al. 2010, Cihan et al. 2011, Feng et al. 2012, El-Deeb et al. 2016, Zaichick et al. 2016). Selected populations, together with histopathological results, therapeutic effects, environmental, metabolic, hormonal, and genetic factors, are also important in this respect. Previous studies reported that some TEs status and oxidant/antioxidant balance in circulating changed in various cancer types. TEs have been found at different concentrations in patients with cancer of different primary locations, such as in BC (El-Deeb et al. 2016), lung cancer (Momen et al. 2015), prostate cancer (Kiziler et al. 2010, Zaichick et al. 2016), and colorectal cancer (CRC) (Stepien et al. 2017). Further studies are needed to answer the question: "Do elemental alterations have a role in cancerogenesis or are they a consequence of the disease itself?" (Juloski et al. 2020).

In previous studies, some explanations have been proposed for TEs and antioxidant status associated with BC. Wu et al. found that the serum levels of Se, Zn, and Mg were significantly lower, but Mn, cadmium (Cd), nickel (Ni), Fe, aluminum (Al), Cu, and chromium (Cr) levels were significantly higher in BC compared to healthy subjects (Wu et al. 2004). But Ding et al. informed that no significant differences between BC and control groups were apparent in the Zn, calcium (Ca), lead (Pb), Se, and Ni levels (Ding et al. 2015). Choi et al. found that serum Cu levels were found significantly higher in BC patients with distant metastasis, while Se levels were significantly lower (Choi et al. 2019). Siddiqui et al. showed that blood Pb, Fe, and Zn were significantly higher in the BC group compared to controls (Siddiqui et al. 2006). Rehman and Husnain found that the levels of Fe, Cu, and Zn in malignant breast tissues were estimated higher compared to benign tissues (Rehman, Husnain 2014). However, Saleh et al. reported that the blood levels of Cu, Zn, and Se were measured lower in BC patients as compared to controls (Saleh et al. 2011). Gecit et al. showed that Mn and Zn levels were lower in BC patients compared to healthy controls (Gecit et al. 2011). Apart from the TE levels, it has also been suggested that TE ratios can be used as biomarkers such as Cu/Zn ratio. It was reported that the serum Cu/Zn ratio was significantly higher in BC patients than in control (Zowczak et al. 2001, Golabek et al. 2012).

In our study, we found that serum Se, Zn, and Fe levels were decreased, but Cu levels were increased in the after treatment group compared to the before treatment group. Also, serum Se, Zn, and Fe levels were decreased, but Cu levels were increased in both before and after treatment groups compared to the control group. Increased levels of Cu, and decreased levels of Se, Zn, Fe, and Mn in the after treatment group caused the ratios of Cu/Se and Cu/Zn ratios were increased, but Fe/Mn ratios were decreased in the after tretment group compared to the control group (Table 3). It is reported that high levels of transition metals show pro-oxidant characteristics by genera-

ting reactive oxygen species (ROS) and, in that way, inducing cell damage (Kohzadi et al. 2017). It was argued that the tumor cells are rapidly growing cells and Cu is a promoter of cellular growth. Moreover, tissue necrosis could in turn increase the serum level by releasing Cu into the circulation by ceruloplasmin. Changes in the levels of these TEs in cancer patients may be due to inflammation and oxidative damage associated with the development and progression of cancer. Additionally, it is important that Fe/Se, Fe/Zn, Fe/Mn, Cu/Se, Cu/Zn, and Cu/Mn ratios might serve as factors for the increased severity of cancer progression and therapeutic effects.

We also investigated changes in GPX1, GPX6, Sel-F, Sel-H, Sel-S, Sel-V, and Sel-M levels in BC patients. Our examined selenoproteins' cellular functions were implicated in antioxidation (GPX1 and GPX6), redox regulation (Sel-H, Sel-V, and Sel-M), and protein folding (Sel-S and Sel-F). Our results showed that serum levels of GPX1, GPX6, Sel-F, Sel-H, and Sel-S were statistically decreased in both before treatment and after treatment groups compared to the control. Also, Sel-V increased in the after treatment compared to the before treatment group (Table 2). The decreased GPX1, GPX6, Sel-F, Sel-H, and Sel-S levels in the before and after treatment BC groups may explain the effects of selenoproteins on carcinogenesis or the treatment of cancer.

The actions of selenoproteins in carcinogenesis-associated processes have been explained by decreased expression of genes encoding selenocysteine-containing proteins (Varlamova et al. 2016). A study has been reported that selenoproteins, including Sel-P, GPXs, thioredoxin reductases (TXNRD) and Sel-F, can regulate tumorigenesis and progression through their effects on cancer-related signaling pathways (Jia et al. 2020). It is suggested the protective effect of GPX1 in oxidative stress, and GPX1 expression are reduced in many tumor types. GPX6 is another antioxidant enzyme that protects against oxidative damage, but its role in cancer mechanisms has not been fully elucidated (Gan et al. 2014, Yang et al. 2015, Varlamova et al. 2016). Short and Williams have been reported that Sel-F downregulation in cancer cell lines resulted with upregulation of the cell cycle inhibitors p21 and p27, and decreased proliferation and growth arrest in colon and liver cancer cells (Short, Williams 2017). Also, a study in cell culture shows that Sel-M supports intracellular calcium homeostasis, and protects against oxidative stress. Altered Sel-M expression levels in humans have been associated with hepatocellular carcinoma (Gong et al. 2016). It is reported that Sel-H regulates redox homeostasis and suppresses DNA damage during development and tumorigenesis. Sel-V has an important potential role in regulating tissue Se metabolism or maintaining body Se homeostasis (Chen et al. 2020). Se supplementation is known to affect selenoprotein expression, but chemopreventive effects of Se supplementation have produced conflicting results, possibly regarding a combination of different baseline Se status and/or genetic factors that modulate selenoprotein function. *In vivo* cancer studies using

transgenic mice demonstrated the role of selenoproteins in both preventing and promoting cancer (Cox et al. 2016).

In conclusion, we found decreased GPX1, GPX6, Sel-F, Sel-H, and Sel-S in before and after treatment BC patients compared to the healthy controls. Low levels of selenoproteins in patients with BC compared to controls could impact the development and the progression of cancer. Free radicals can damage DNA, proteins, lipids, and cell membranes in the body. The initial target of the oxidation varies depending on the cell, the type and location of oxidative stress and its severity, and the availability of metal ions. Changes in the serum levels of trace elements in BC patients might lead to increased oxidative stress levels and alteration of cell hemostasis by various cell pathways. However, further molecular studies are required to clarify the specific mechanisms involved in the interaction between TEs and selenoproteins status and therapeutic strategies in BC.

Conflict of interest

The authors declare that they have no conflict of interest.

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