Serum and urinary cadmium and zinc profiles in breast cancer patients and their association with estrogen and HER-2 receptors, and redox status

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Abstract

Background: Cadmium, a metal implicated in environmental toxicity, is linked to tumor growth and cancer. On the other hand, zinc plays an essential function in oxidative stress and can counteract cadmium toxicity and carcinogenicity.

This research aims to evaluate the urine and serum values of cadmium and zinc in breast cancer (BC) patients and their association with estrogen (ER) and HER-2 receptors, and redox status.

Methods: Forty BC patients and thirty healthy subjects participated in this study. Cadmium and zinc levels were measured in serum and urine samples by atomic absorption spectrophotometer. Redox status markers were determined by calorimetric methods.

Results: The amount of cadmium in the BC patients was substantially greater than in the healthy subjects. Zinc levels were significantly lower in patients with BC compared to controls. Breast cancer patients with ER-positive tumors had significantly higher urinary cadmium concentrations (U-Cd) compared to patients with ER-negative tumors. There was no significant difference between the parameters of redox status and the value of cadmium and zinc between patients with BC in the HER-2 subgroup. Malondialdehyde levels in the serum were substantially greater in BC patients than in healthy subjects. Total thiol level and the activity of catalase and superoxide dismutase in serum were considerably lower in BC patients than in healthy subjects.

Conclusions: Breast cancer etiology may be influenced by disturbing redox state and element levels. Increasing U-Cd and lowering zinc levels in the serum could be the risk factors for BC.

1. Introduction

Breast cancer (BC) is the most prevalent cancer, and it is also the leading cause of cancer-related death in women around the world. Breast cancer is on the rise in developed countries, according to statistics. In Iran, the average age of women diagnosed with BC is ten years younger than in western women [1, 2]. This underlines the need to comprehend the factors that may indicate the disease's onset. According to numerous research, BC is caused by a combination of genetic and environmental factors. Increasing epidemiological evidence reveals a substantial link between heavy metal exposure, such as cadmium, with the development, progression, and promotion of BC [3, 4].

Cadmium is a member of a new class of powerful xenoestrogen known as metallo-estrogens. According to experiments, cadmium like estradiol stimulates estrogen receptors (ER) through a high-affinity interaction with the receptor's hormone-binding domain [5].

There is an association or inverse relationship between trace elements and cancer risks [6, 7]. Zinc is the most critical element for cellular growth and acts as a cofactor for superoxide dismutase, which shields cells from free radicals. As a result, zinc deficiency can raise the risk of BC by causing oxidative...
alterations [8]. Due to a deficit of zinc, oxidative stress induces alterations in mammary gland tissue and increased macrophage infiltration, resulting in a toxic microenvironment, which is associated with increased expression of alpha estrogen receptors, ductal modifications in the organization, and advanced fibrosis in the mammary glands [9, 10].

On the one hand, cadmium exposure disrupts zinc levels in the body, while on the other hand, dietary zinc intake has a significant effect on cadmium absorption, accumulation, and toxicity. In connection with the development of cadmium toxicity, the body's zinc status is critical [11].

The interaction of reactive oxygen species with biological macromolecules such as proteins, lipids, and DNA causes oxidative damage [12]. When reactive oxygen species (ROS) react with polyunsaturated fatty acids, they produce toxic and reactive aldehyde metabolites like malondialdehyde (MDA), which is one of the end products of lipid peroxidation. MDA may promote tumor growth by interfering with several biological processes. The most important antioxidants in cells are total thiol (TT) such as glutathione and enzymes such as superoxide dismutase (SOD), and catalase (CAT), which protect cells from ROS-induced damage [13].

In the current study, we investigated the roles of urinary and serum cadmium and zinc profiles and redox status markers in BC patients vs. healthy controls.

2. Methods

2.1. Subjects

Forty women with BC were selected from the Oncology Clinic in Ahvaz, Iran. The 40 BC patients were newly diagnosed cases, before receiving any medical cancer intervention.

The control group also included 30 healthy individuals in the age range of cancer patients. Exclusion criteria in the study groups included people with chronic diseases such as diabetes, cardiovascular disease, liver and kidney diseases, malnutrition, and inflammatory diseases. Figure 1 shows the flowchart of study participants.

The questionnaires were administered at baseline and completed by participants. Demographic and clinical data of the patients included body mass index (BMI), age, genealogical history, medical history, reproductive history, the status of estrogen and progesterone receptors, human epidermal growth factor receptors, and other related data were recorded for each participant. The clinical stage of each BC patient was determined by the oncologist using the tumor-nodes-metastasis (TNM) classification method. The ethical committee of Ahvaz Jundishapur University of Medical Sciences approved this study (Ethics code: IR. AJUMS.REC.1399.065), and all participants gave informed written consent.

2.2. Sample collection and preservation
For each participant, a 10 mL fasting blood sample was collected in a sterile non-heparinized Vacutainer tube. The samples were allowed to clot for 5–10 min and then centrifuged for 10 min at 3000 × g. Clear serum from each sample was transferred to microtubes and labeled. The specimens were frozen at −20°C for 2 hours to transfer to −80°C.

Spot urine samples were collected from all the participants. Urine samples were stored at −80°C. The Jaffe method [14] was used to detect creatinine in urine to monitor kidney function [15].

### 2.3. Determination of cadmium and zinc level

The calibration curve was prepared using cadmium and zinc standard solutions (Merck Co., Germany). They were made by dilutions with distilled water from a stock solution of 1 g/L for each metal.

Microwave (Milestone, Italy) was used to digest of the serum and urine samples. To digest samples, 5.0 ml of serum or urine sample and 10.0 ml of a 1:1 mixture of concentrated hydrochloric and nitric acids (Sigma-Aldrich, USA) were transferred into a 125 ml pressure-resistant bottle. The samples were digested either at 300 W for 4 min, which removed the interfering matrix within the samples. The resulting solution was digested until colorless, evaporated almost to dryness to remove excess acid, and then diluted to 25.0 ml with double deionized water [16, 17]. Each sample was measured in triplicate and data were offered as the means of three measurements. The accuracy of the method was verified by analysis of the serum and urine samples spiked with known amounts of cadmium and zinc. Recovery percentages ranged from 93 for Cd to 108% for Zn. The detection limit, calculated in accordance with IUPAC recommendations, cadmium and zinc were 0.038 (µg/l) and 0.352 (µg/l), respectively [18]. The precision was measured as the coefficient of variation for replicate measurements and was < 5%.

The urine and serum cadmium and zinc concentrations were measured by atomic absorption spectrometer (AA240FS–Varian, Australia) equipped with pyrolytic coated graphite tubes, autosampler PSD 3000, and deuterium background effect correction.

### 2.4. Determination of serum redox biomarkers

Lipid peroxidation in serum was estimated by the thiobarbituric acid test for MDA [18]. In summary, 2-ml TCA-TBA-HCl reagent [15% (w/v) TCA, 0.375% (w/v) TBA in 0.2 N HCl, (Sigma-Aldrich, USA)] was mixed with a 1 ml serum sample and heated at 100°C for 15 min. After cooling the solution, it was centrifuged at 3000 × g for 10 min. The absorbance was measured at 535 nm.

Catalase activity (CAT) was measured using H₂O₂ as substrate. The disappearance of H₂O₂ was followed at 240 nm [19].

Total thiol levels were determined using Ellman's method [20]. In this method, residues of TT reduce the DTNB (5,5′-dithiobis-(2-nitrobenzoic acid) molecules to NTB (2-nitro-5-benzoic acid), which has an absorbance at 412 nm.
Superoxide dismutase (SOD) activity, was determined using a commercially available kit (ZellBio GmbH, Germany ZB-SOD- 96A) based on the colorimetric method (420 nm).

2.5. Statistical analysis

SPSS software was used to analyze the data (version 24). The data was provided as a mean ± SD or number (percentage). The Kolmogorov-Smirnov test was used to determine whether the data were normality. An independent t-test was used to compare the differences between the groups. The correlations between Cadmium and zinc concentrations and values of redox status markers were assessed using Pearson. The multiple logistic regression model compares standardized biological variables between the two groups. For each variable, z-scores were utilized to interpret the results of standard deviation units. Odds ratios and 95% confidence intervals (95% CI) were expressed per standard deviation unit. The significance level was considered to be < 0.05.

3. Results

The descriptive statistics for both cases and controls are shown in Table 1. The mean BMI between the two groups was significant (P < 0.05). Histologically confirmed BC type was invasive ductal carcinoma (IDC) in all participants in this investigation. Grade I, II, and III BC were observed in 10 (25%), 24 (67.5%), and 3 (7.5%) patients, respectively.
### Table 1
Characteristics of a study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Breast cancer patients (n = 40)</th>
<th>Controls (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>45.7 ± 8.3</td>
<td>44.1 ± 8.6</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>29.08 ± 5.2</td>
<td>25.84 ± 3.7</td>
</tr>
<tr>
<td>Pregnancy history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37(%92.5)</td>
<td>27(%90)</td>
</tr>
<tr>
<td>No</td>
<td>3(%7.5)</td>
<td>3(%10)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4(%10)</td>
<td>5(%16.7)</td>
</tr>
<tr>
<td>Negative</td>
<td>36(%90)</td>
<td>25(%83.3)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>32(%80)</td>
<td>26 (%86.7)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>8(%20)</td>
<td>4 (%13.3)</td>
</tr>
<tr>
<td>Hookah smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (%5)</td>
<td>3 (%10)</td>
</tr>
<tr>
<td>No</td>
<td>38(%95)</td>
<td>27 (%90)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or lower</td>
<td>31(%77.5)</td>
<td>8 (%26.7)</td>
</tr>
<tr>
<td>College or above</td>
<td>9(%22.5)</td>
<td>22 (%73.3)</td>
</tr>
</tbody>
</table>

The data are shown as mean ± SD or number (%)

BMI: Body mass index, Hookah smoking: A hookah is a water pipe that is used to smoke sweetened and flavored tobacco.

* P < 0.05.

Data on the cadmium and zinc profile in BC patients and healthy subjects are summarized in Table 2. The mean serum (S-Cd) and urine cadmium (U-Cd) concentrations were both significantly elevated in BC patients (P = 0.03, P = 0.01, respectively). Cadmium levels in BC patients were twice as high as the healthy subjects. The mean value of serum zinc (S-Zn) in patients with BC was lower than in the healthy subjects (P < 0.001). The mean urine zinc (U-Zn) concentration was lower than otherwise healthy individuals, but this difference was not statistically significant (P = 0.58). The patients with BC were further assigned into three subgroups based on their clinical grades. Multiple comparisons were made between patients with
different clinical grades of BC. No differences were found between the levels of cadmium and zinc and the grade of patients.

**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Cadmium (µg/dl; mean ± SD)</th>
<th>Urine(^a) Cadmium (µg/g; mean ± SD)</th>
<th>Serum Zinc (µg/dl; mean ± SD)</th>
<th>Urine(^a) Zinc (µg/dl; mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.029 ± 0.006</td>
<td>1.37 ± 0.6</td>
<td>67.14 ± 1.06</td>
<td>366.3 ± 6.08</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>0.057 ± 0.01</td>
<td>3.9 ± 2.8</td>
<td>60.67 ± 0.82</td>
<td>348 ± 5.05</td>
</tr>
<tr>
<td>P-value</td>
<td>0.03</td>
<td>&lt;0.0001</td>
<td>0.001</td>
<td>0.58</td>
</tr>
</tbody>
</table>

\(^a\) concentration in urine adjusted by creatinine

ER+/ PR+, ER+/PR-, ER-/PR+, and ER-/PR- were specified for 45, 10, 7.5, and 37.5% of BC patients, respectively. A significant difference was found between the mean cadmium value in subgroups of BC patients with ER + and ER-. The mean cadmium concentration in the ER + subgroup (including ER+/ PR+, ER+/PR-) was 5.2 and 0.07 for urine and serum, respectively. The mean concentration was 2.1 and 0.03 for urine and serum in the ER- subgroup (including ER-/PR+, and ER-/PR-). The details of comparing the mean of estrogen/progesterone and HER-2 subgroups with the studied variables are shown in Table 4.

**Table 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/ml)</th>
<th>CAT (U/ml)</th>
<th>SOD (U/ml)</th>
<th>TT (µmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.7 ± 1.9</td>
<td>76.3 ± 0.6</td>
<td>39.7 ± 4.1</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>29.8 ± 3.8</td>
<td>65.2 ± 1.1</td>
<td>22.7 ± 2.5</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.011</td>
<td>0.016</td>
</tr>
</tbody>
</table>

MDA: Malondialdehyde, CAT: Catalase, SOD: Superoxide dismutase, TT: Total thiol
Table 4
Relation between estrogen receptor and HER-2 with levels of cadmium, zinc, and redox status parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>ER-positive (mean ± SD)</th>
<th>ER-negative (mean ± SD)</th>
<th>P value</th>
<th>HER-2positive (mean ± SD)</th>
<th>HER-2 negative (mean ± SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>4.5 ± 1.6</td>
<td>5.8 ± 1.9</td>
<td>0.06</td>
<td>4.7 ± 0.7</td>
<td>5.2 ± 0.4</td>
<td>0.56</td>
</tr>
<tr>
<td>SOD</td>
<td>20.7 ± 13.7</td>
<td>25 ± 4.1</td>
<td>0.38</td>
<td>28.57 ± 5.6</td>
<td>19.33 ± 2.2</td>
<td>0.08</td>
</tr>
<tr>
<td>MDA</td>
<td>29.5 ± 5</td>
<td>21.8 ± 6</td>
<td>0.93</td>
<td>21.7 ± 6</td>
<td>34.5 ± 4.7</td>
<td>0.1</td>
</tr>
<tr>
<td>CAT</td>
<td>65.7 ± 1.6</td>
<td>64.8 ± 1.8</td>
<td>0.71</td>
<td>66.1 ± 1.3</td>
<td>64.8 ± 1.7</td>
<td>0.6</td>
</tr>
<tr>
<td>S-Zn</td>
<td>60 ± 1.03</td>
<td>61.5 ± 1.4</td>
<td>0.37</td>
<td>60.3 ± 1.4</td>
<td>60.9 ± 1.1</td>
<td>0.76</td>
</tr>
<tr>
<td>U-Zn</td>
<td>325 ± 56.1</td>
<td>374 ± 89</td>
<td>0.63</td>
<td>405 ± 108</td>
<td>316 ± 52.1</td>
<td>0.41</td>
</tr>
<tr>
<td>S-Cd</td>
<td>0.07 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.04</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.67</td>
</tr>
<tr>
<td>U-Cd</td>
<td>5.2 ± 3.3</td>
<td>2.1 ± 1.9</td>
<td>0.009</td>
<td>3.2 ± 3.6</td>
<td>4.9 ± 4.1</td>
<td>0.37</td>
</tr>
</tbody>
</table>


35% of patients were HER-2 positive, and 65% were HER-2 negative. Cadmium and zinc concentrations were compared between HER-2+ and HER-2- patients with BC, no significant difference was observed (Table 4).

Our finding demonstrated that urine cadmium and serum zinc were linked to the risk of having BC (Table 4). In particular, controlling for age and BMI (95% CI: 1.03–4.9), the association of cadmium was stronger than that of zinc, considering that for a standard deviation unit, there was a 2x higher likelihood of U-Cd and a 0.25x lower likelihood of S-Zn in patients with BC compared to healthy subjects (Table 5).
Table 5
Multiple logistic regressions modeling comparing standardized cadmium, zinc, and redox status markers in breast cancer patients and healthy subjects, including age, BMI, hookah smoking, family history of BC, menopausal status and education as covariates.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-Cd</td>
<td>2.25*</td>
<td>1.03–4.90</td>
</tr>
<tr>
<td>S-Cd</td>
<td>1.67</td>
<td>0.71–3.93</td>
</tr>
<tr>
<td>S-Zn</td>
<td>0.25*</td>
<td>0.11–0.54</td>
</tr>
<tr>
<td>SOD</td>
<td>0.38*</td>
<td>0.17–0.83</td>
</tr>
<tr>
<td>MDA</td>
<td>2.17*</td>
<td>1.03–1.36</td>
</tr>
<tr>
<td>CAT</td>
<td>0.71</td>
<td>0.39–1.33</td>
</tr>
<tr>
<td>TT</td>
<td>0.71</td>
<td>0.36–1.36</td>
</tr>
</tbody>
</table>

Estimates are adjusted for age, and body mass index. Z-scores used for all variables. * P < 0.05.


Serum MDA levels in of BC patients were signicantly higher than the healthy subjects (P < 0.0001). Breast cancer patients showed significantly lower CAT, SOD activity, and TT levels than the control group (Table 3). Based on the results of the logistic regression model, MDA level and SOD activity were associated with overall cancer incidence (Table 5).

Using bivariate correlation analysis of the measured parameters, negative correlations were found between serum MDA and TT levels (r = −0.332, P < 0.05), S-Cd and CAT levels (r = −0.262, P < 0.05), S-Zn and S-Cd (r = −0.393, P < 0.01), serum MDA and S-Zn (r = −0.125, P < 0.05) and a direct correlation was seen between TT and SOD levels (r = 0.331, P < 0.01), MDA and U-Cd (r = 0.335, P < 0.01), S-Zn and SOD levels (r = 0.319, P < 0.05).

4. Discussion

Breast cancer is a multifaceted disease with multiple etiologies. The role of metals in cancer is complicated and leaves many questions unanswered. Numerous investigations have found an apparent link between heavy metals and a range of cancers. In BC, like other malignancies, trace element levels are thought to increase or decrease. On the other hand, the etiological effects of heavy metals on BC remain unproven [21].

Primary and secondary inhalation of cigarette smoke, food, water, and ambient air are the main sources of cadmium in the general population, especially in urban areas and near industrial settings [22]. Around 75% of dietary cadmium intake comes from vegetables, with cereals accounting for the most [23].
Cadmium accumulates in the kidneys due to glomerular filtration and its extended half-life in the human body [24].

The amount of cadmium excreted in the urine is related to body burden and is used to measure of lifetime cadmium exposure. In contrast, the concentration in the blood represents acute exposure [23].

According to our findings that the concentrations of U-Cd and S-Cd in BC patients were greater than healthy subjects, and with an increase of U-Cd concentration in 1 µg/g creatinine, the odds of cancer incidence increased 2-fold. In agreement with these results, Nagata et al. observed that ORs of BC according to the tertile of the creatinine-adjusted cadmium levels in newly diagnosed women compared with controls were in high level. With an increase in 1µg/g creatinine, cadmium exposure was related with a 67% increased risk of BC [25].

In a study of Wisconsin women, McElroy et al. found that the risk of BC increased nearly twofold in women in the highest quartile of U-Cd levels compared to women in the lowest quartile, and the risk of BC increased 2.09-fold for every 1.0 µg/g of creatinine [4].

According to Strumylait et al., a considerable direct relationship between urine and blood cadmium concentrations and BC risk when compared to the controls [23].

Gallagher et al. discovered a substantial increase in the risk of BC with increasing U-Cd concentrations in cases living on Long Island, New York, and other US women compared with controls [26].

Subgroup analysis indicated that BC patients with ER-positive had higher cadmium content in urine and serum compared with ER-negative BC patients. Some experimental research suggests that cadmium can form a high-affinity complex with the hormone-binding domain of the estrogen receptors, and so, functionally, acts like steroidal estrogens in BC cells, causing them to proliferate [23, 27].

Our findings revealed that there was no significant link between hookah smoking status and BC, which may be due to a limited sample size of smokers in the study. As previously reported [28, 29], the zinc concentration in cancer patients was lower than in healthy individuals, which was consistent with the findings of our study, where serum zinc concentrations were significantly lower in BC patients’ serum samples as compared to healthy subjects. The odds ratio of cancer increases proportionally with decreased serum zinc levels.

The mean U-Zn levels differ in BC patients and healthy subjects, are not significant statistically.

Zinc plays an important role in stabilizing the structure of DNA, RNA, and ribosomes. Zinc is also essential for the function of transcription factors and proteins that control gene transcription. Zinc also protects against free radical damage [8]. Therefore, a decrease in zinc content in BC patients could disrupt any of the above processes, potentially functioning as a causal factor for cancer. In our research, MDA levels in blood serum were considerably higher in BC patients than in the control group. Because MDA is a marker for lipid peroxidation, it could play a role in breast carcinogenesis. Our results are in
agreement with previous studies [30, 31]. The most significant changes (OR = 2.17) were associated with MDA levels, indicating the importance of MDA as a marker for assessing redox status in patients with BC. In terms of TT levels in the serum, the current study found that BC patients had considerably lower amounts of TT than healthy participants. This suggests that serum TT can be used to distinguish patients with BC from controls. The findings of the current investigation were consistent with those of Yeh et al. [32], who found that TT levels in the blood of BC patients were considerably lower than in controls. TT should be considered an essential biomarker for the detection of BC, according to the researchers. These findings reinforce the theory that TT protects cancer patients from oxidative stress caused by reactive oxygen species. There has been accumulating evidence that shows the reduced level of SOD and CAT in BC patients [33, 34]. The results of these studies are in accordance with the results obtained in our research, which shows a low level of antioxidant status in women with BC.

There were certain limitations to our research. The first was the inability to measure other trace elements, such as iron, which may influence cadmium absorption and accumulation in the body. The latter was a lack of cadmium testing in foods and soil, as well as a limited sample size. Because the U-Cd levels in cases and control were higher than the critical concentration, it is proposed that measures be put in place to prevent cadmium from entering the environment, as well as more experimental and prospective epidemiologic studies to discover likely cadmium sources. Our findings, on the other hand, add to current concerns regarding cadmium exposure as a separate and significant risk factor for BC.

In conclusion, decreased zinc levels in the serum and increasing cadmium levels may be a risk factors for BC. It appears that supplementing zinc in individuals with low serum levels may lower the incidence rate of BC. These findings also suggest that oxidative stress markers, especially MDA, may be linked with BC or potentially increase the risk of developing the disease. Additional pragmatic study in this area is needed to draw definitive conclusions. Furthermore, the function of pathogenic and genetic variables in the therapy process, as well as their relationship with these aspects, should be evaluated.

Declarations

Declaration of Competing Interest

There are no conflicts of interest to declare.

Acknowledgments

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References


**Figures**

**Figure 1**

Flowchart of study participants