Relationship between zincemia, superoxide dismutase activity and marker of oxidative stress in women with breast cancer

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Abstract

Introduction: studies show changes in zinc metabolism in women with breast cancer. This mineral has antioxidant action, and disorders in its biochemical parameters are related to poor prognosis of the disease and increase in the carcinogenic process.

Objective: this study evaluated the activity of enzyme superoxide dismutase and biochemical parameters related to zinc, and investigated the existence of correlation between these variables and the marker of oxidative stress in these patients.

Methods: this was a case-control study with 66 women aged between 20 and 50 years old, distributed into: case group (women with breast cancer, n = 34) and control group (healthy women, n = 32). Zinc intake was analyzed by three-day food diary, using Nutwin software, version 1.5. Plasma and erythrocyte zinc concentrations were determined by flame atomic absorption spectrophotometry method (λ = 213.9). Superoxide dismutase activity was assessed by Griess colorimetric method, and plasma thiobarbituric acid reactive substances (TBARS) were analyzed.

Results and discussion: mean levels of zinc intake, superoxide dismutase and TBARS were higher than recommended for the study participants with statistical difference for enzyme superoxide dismutase (p < 0.05). Mean plasma and erythrocyte concentrations of zinc were reduced in both groups (p > 0.05).

Conclusions: therefore, it can be assumed that zinc intake in women with breast cancer does not impact plasma and erythrocyte concentrations of this mineral. High superoxide dismutase activity in women with breast cancer may be related to increased oxidative stress in these patients.

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The relationship between oxidative stress and breast cancer has been widely investigated. Some carcinogens favor the production of free radicals that damage cells, predisposing to malignant transformation. The increased intracellular concentration of reactive oxygen species, as well as the impairment in DNA repair, makes this nucleic acid to be susceptible to oxidative damage, which contributes significantly to mammary carcinogenesis. Reactive oxygen species participate in inflammatory reactions and in signal transduction mechanisms, acting as second messengers to maintain different cellular functions. This inflammatory response, in turn, stimulates the production of new free radicals, and thus, the progression of tissue injury.

Also, in the antioxidant defense, specific enzymes attack reactive oxygen species, which prevent, by their own reduction, cellular oxidative change and minimize the toxicity caused by free radicals; and may also induce selectively apoptosis in cancer cells and inhibit cell proliferation. The enzyme superoxide dismutase has anti-carcinogenic action, inhibiting the initiation, promotion and progression phase in breast carcinogenesis. From this perspective, zinc acts as a co-factor of this enzyme with an important role in several biological processes, inhibiting or activating enzymatic reactions and competing with other elements, changing cell permeability and thus, having direct or indirect action in the carcinogenic process.

Several studies have been conducted to elucidate the association between zinc and breast cancer. In 2002, Kuo et al., found reduced serum zinc concentrations in patients with breast cancer, suggesting that plasma zinc could be used as a possible prognostic and therapeutic marker in this type of cancer.

Reduced serum zinc concentrations increase lipid peroxidation in mitochondrial and microsomal membranes, and also the osmotic fragility of erythrocyte membranes. However, at normal levels, zinc prevents lipid peroxidation, playing an important role in protecting cells against redox imbalance.

Although some studies have already showed the important role of zinc as an antioxidant nutrient in several mechanisms involved in the pathogenesis of breast cancer; data regarding nutritional status of this mineral in women with breast cancer are scarce. The determination of plasma and erythrocyte zinc concentrations, as well as investigation of a potential relationship between these variables and the activity of enzyme superoxide dismutase and plasma thiobarbituric acid reactive substances (TBARS) contributes to the understanding of the role of this mineral in mechanisms involved in metabolic disorders associated with breast cancer.

Therefore, this study aims to evaluate the activity of enzyme superoxide dismutase and biochemical parameters related to zinc, as well as to investigate the existence of correlation between these variables and a marker of oxidative stress in these patients.

**Methods**

Case-control study involving 66 premenopausal women, aged between 25-50 years old, divided into 2 groups: Case (with breast cancer, n=34) and control (without breast cancer, n=32). Women recruited for inclusion in the study sample were interviewed for clarification of study protocol. Patients with serum levels of follicle stimulating hormone (FSH) > 30 μg/mL and those with chronic diseases such as diabetes and hypertension and history of previous treatment for the disease were excluded from the study. Patients using medications and vitamin / mineral supplements that could interfere with zinc metabolism were also excluded.
Control group consisted of female, premenopausal volunteers with no breast cancer diagnosis, FSH not greater than 30 μg/mL, no chronic diseases and no prior history of treatment for the disease. The study was approved by the Institutional Review Board of the Federal University of Piauí under number CAAE 15861213.2.0000.5214.

Dietary Intake

Food intake was assessed using a three-day food diary, including two days during the week and one day on the weekend (Saturday or Sunday). Nutritional assessment was performed using Nutwin software, version 1.5. Methods and recommendations described in the Dietary Reference were used to estimate the zinc intake.

Determination of Plasma and Erythrocyte Zinc

Plasma zinc concentration was determined by atomic absorption spectrophotometry, according to the method proposed by Rodrigues et al. (1989). Two aliquots of each plasma sample were separated, diluted in Milli-Q® water at a 1:5 ratio and aspirated directly into the flame of the apparatus. Tritizol® (MERCK) prepared by dilution in Milli-Q® water with 3% glycerol, at concentrations of 0.1; 0.2; 0.3; 0.5 and 1.0 μg/mL was used as standard solution. Results, calculated from the absorbance values obtained, were expressed in μg/dL, representing the average concentrations of the samples prepared in duplicate.

Erythrocyte zinc concentration was determined by atomic absorption spectrophotometry following the standard methodology by Cordeiro (1994), in which a desirable precision level in the analyses and non-interference matrix in this biological material were found. A 400-μL aliquot of red cell mass was diluted with 1200μL of Milli-Q® water. This dilution was performed in two steps, named lysate 1 and 2, which corresponded, respectively, to a first dilution of 400-μL aliquot at a 1:4 ratio; and a second dilution in which 200-μL of lysate 1 was pipetted in triplicate and diluted again at a 1:4 ratio.

After homogenization, samples of lysate 2 were aspirated directly into the atomic absorption spectrophotometer. Tritizol® (MERCK), prepared by dilution with Milli Q® water, at concentrations of 0.1; 0.2; 0.3; 0.5 and 1.0 μg/mL, was used as standard solution. To express results of zinc/hemoglobin mass, hemoglobin concentration in lysate 1 was determined in parallel to hemoglobin concentration in lysate 1, and dilution was adjusted in the final calculation of the analysis. A 20-μL aliquot of this lysate was diluted in 5 mL of Drazkin’s solution to determine hemoglobin using cyanometahemoglobin method.

UV-visible spectrophotometer (FEMTO 700S Model) was used for hemoglobin reading, at a 540-nm wavelength. Based on zinc and hemoglobin concentrations, zinc concentration was calculated and expressed in μgZn/gHb.

Determination of the Activity of Erythrocyte Enzyme Superoxide Dismutase

Activity of erythrocyte superoxide dismutase was determined by the amount of enzyme capable to inhibit by 50% the formation of nitrite. Nitric oxide was quantified by nitrite concentration in erythrocytes using Griess colorimetric method.

Activity of enzyme superoxide dismutase was determined in erythrocytes, prepared by in vitro method. Whole blood samples were centrifuged for plasma separation at 3000 x g for 10 minutes at 4°C; plasma was subsequently extracted with automatic pipette and transferred into polypropylene Eppendorf tubes previously demineralized.

Then, erythrocytes were rinsed with 5 mL of 0.9% saline, and homogenized thoroughly by inversion and centrifuged. After the last centrifugation, saline was aspirated and discarded, and erythrocyte mass was extracted thoroughly with the help of an automatic pipette, placed in demineralized polypropylene Eppendorf tubes, which were kept at -80°C.

To analyze the activity of erythrocyte enzyme superoxide dismutase, one 100-μL aliquot of erythrocytes was diluted with 1110 μL of phosphate buffer, 75 μL of L-methionine, 40 μL of Triton X-100, 75 μL of hydroxylamine hydrochloride and 100 μL of EDTA. Samples were incubated in water bath at 37° C for 5 minutes; after this process, 80 μL of riboflavin were added and exposed to light for 10 minutes. Finally, 1 mL of Griess reagent was added and reading was performed at λ = 543 nm. Results were, then, used to calculate the enzymatic activity through the amount of superoxide dismutase capable of inhibiting by 50% the formation of nitrite; in this case, 36 ng of superoxide dismutase inhibit 50% of nitrite formation.

Determination of thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) in plasma were determined using the method suggested by Ohkawa; Ohishi; Yagi. Prior to sample processing, analytical calibration curve was prepared at concentrations of 0.5; 1.0; 2.0; 4.0 and 8.0 nmol/mL, using 1,1,3,3-tetraethoxypropane as standard. Absorbance was read using a UV/Vis Bel Photonics spectrophotometer, SP 1102 model (Osasco, SP, Brazil) at a wavelength of 532 nm.

Statistical Analysis

Data were exported to SPSS software (for Windows® version 18.0) for statistical analysis of the re-
results. Chi-square test was used to check data normality. Given that variables were normally distributed, Student’s t-test was used for comparison between study groups and Pearson’s linear correlation coefficient was used for correlation analysis. Difference was statistically significant when \( p \)-value <0.05, adopting a 95% confidence interval.

In order to reduce errors associated with dietary assessment, consumption was adjusted for total energy intake using residual method, and intra-individual variation, calculated by the mean of components for analysis of variance.

**Results**

The average age was 40.91±5.98 years old and 36.41±7.23 years old for women with breast cancer (n=34) and control (n=32) group, respectively. Mean body mass index was 26.17±4.91 kg/m² for women with breast cancer and 25.20±4.16 kg/m² for control group, and mean waist circumference was 83.62±10.83 cm and 80.24±9.97 cm for case and control groups, respectively.

Table I shows the average daily intake of energy, macronutrients and zinc based on the diet consumed by the patients with breast cancer and control group. Dietary intake of carbohydrate, protein, lipid and zinc was adequate.

Table II shows mean plasma and erythrocyte zinc concentrations for patients with breast cancer and control group. Table III shows mean and standard deviation of the activity of enzyme superoxide dismutase in erythrocytes and plasma concentrations of TBARS for patients with breast cancer and control group. Table IV shows the results of correlation analysis between biochemical parameters for zinc, superoxide dismutase and plasma TBARS in patients with breast cancer and control group.

**Discussion**

This study determined the biochemical parameters related to zinc and also investigated the existence of

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**Table I**

<table>
<thead>
<tr>
<th>Energy/Nutrient</th>
<th>Case group (n=34)</th>
<th>Control group (n=32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>1420.20 (437.12)</td>
<td>2064.41 (536.17)*</td>
<td>0.000</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>55.51 (7.34)</td>
<td>53.60 (9.35)</td>
<td>0.358</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>23.90 (8.72)</td>
<td>19.55 (8.38)*</td>
<td>0.043</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>25.70 (4.63)</td>
<td>26.16 (6.84)</td>
<td>0.745</td>
</tr>
<tr>
<td>Zinc (mg/day)</td>
<td>7.87 (2.84)</td>
<td>8.33 (3.82)</td>
<td>0.585</td>
</tr>
</tbody>
</table>

*Values significantly different among women with breast cancer and control group. Student’s t-test (p<0.05). *Raw values of zinc from case group were adjusted by energy and corrected by intra- and inter-individual variance; SD = standard deviation; Reference range: 10-35% protein, 20-35% lipids and 45-65% carbohydrates; and EAR = 6.8 mg Zn/day, age range between 19-70 years for females.

**Table II**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case group (n=34)</th>
<th>Control Group (n=32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Zinc (µg/dL)</td>
<td>60.45 (7.46)</td>
<td>64.59 (10.07)</td>
<td>0.065</td>
</tr>
<tr>
<td>Erythrocyte Zinc (µg Zn/g Hb)</td>
<td>37.51 (8.26)</td>
<td>35.97 (5.57)</td>
<td>0.383</td>
</tr>
</tbody>
</table>

No significant difference between women with breast cancer and control. Student’s t-test (p>0.05). Reference range in plasma: 70-110 µg/dL; Reference range in erythrocytes: 40-44 µg Zn/g Hb; SD = standard deviation.
correlation between these variables and concentrations of enzyme superoxide dismutase and plasma TBARS in women with breast cancer.

With regard to zinc intake found in the participants of this study, there was no significant statistical difference between the study groups, and the intake of this mineral was higher than the recommended daily intake, according to Estimated Average Requirement (EAR). It is important to highlight the significant amount of food, source of this nutrient, found in the diet of study participants and, additionally, an increased intake of protein was seen in both groups, which is normally accompanied by a considerable content of zinc, such as, red meat, liver and fish.

With regard to plasma zinc concentrations, they were reduced compared to normal values in both groups, with no significant statistical difference. Some factors could have influenced the zinc levels found in plasma of the study participants, such as, the dietary intake of this mineral. However, both the amount and bioavailability of zinc found in the diets were high, and thus, they apparently did not affect zinc plasma concentrations.

Another important point that could explain the reduced plasma zinc concentrations is related to changes in the compartmentalization of this trace element in patients with breast cancer. Upon establishment of carcinogenesis, a re-distribution of zinc would occur in these patients, through the influx of this mineral from plasma compartment into the tumor cells, consequently reducing the zinc levels in this compartment.

Regarding this matter, it should be highlighted the involvement of zinc transport proteins, playing an important role in the distribution of this mineral in the tissues. Thus, the presence of breast tumor leads to overexpression of genes encoding these proteins (Zip6, Zip7 and Zip10), which promote zinc inflow to

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case Group (n=34)</th>
<th>Mean (SD)</th>
<th>Control Group (n=32)</th>
<th>Mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (μgSOD/gHb)</td>
<td>8924.32 (3829.35)</td>
<td></td>
<td>7170.14 (3132.32)*</td>
<td></td>
<td>0.047</td>
</tr>
<tr>
<td>Plasma TBARS (μmol/L)</td>
<td>5.02 (4.55)</td>
<td></td>
<td>3.95 (1.85)</td>
<td></td>
<td>0.212</td>
</tr>
</tbody>
</table>

*Values significantly different between women with breast cancer and control group. Student’s t-test (p<0.05). Reference range for SOD: 6500-14500 μgSOD/gHb [25]. Reference range for TBARS: 1-3 μmol/L. SOD = superoxide dismutase. SD = standard deviation. TBARS = thiobarbituric acid reactive substances.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma Zn (μg/dL)</strong></td>
<td>r p r p</td>
<td>r p r p</td>
</tr>
<tr>
<td><strong>Erythrocyte Zn (μgZn/gHb)</strong></td>
<td>-0.260 0.137</td>
<td>-0.134 0.450</td>
</tr>
<tr>
<td><strong>Dietary Zn (g/day)</strong></td>
<td>-0.139 0.435</td>
<td>0.128 0.487</td>
</tr>
<tr>
<td><strong>Plasma Zn (μg/dL)</strong></td>
<td>r p r p</td>
<td>r p r p</td>
</tr>
<tr>
<td><strong>Erythrocyte Zn (μgZn/gHb)</strong></td>
<td>0.089 0.615</td>
<td>0.165 0.352</td>
</tr>
<tr>
<td><strong>Dietary Zn (g/day)</strong></td>
<td>0.109 0.554</td>
<td>-0.135 0.461</td>
</tr>
</tbody>
</table>

Zn = zinc. SOD = superoxide dismutase. TBARS = thiobarbituric acid reactive substances.
the tumor cells and inhibit transmembrane transporters of this mineral to other cells, predisposing to malignant transformation and, suggesting that tumor cells selectively increase zinc absorption in the disease. Thus, reduced plasma zinc is associated with greater risk of developing breast cancer, suggesting that this is a prognostic and therapeutic factor for the disease.

Regarding erythrocyte zinc, the mean levels found in this study were reduced in both groups, also with no significant statistical difference. It is noteworthy that, in the circulation, about 80% of zinc is found in erythrocytes, mainly bound to carbonic anhydrase, and only 16% are found in plasma. In an overall analysis, taking into account that the half-life of erythrocyte is 120 days, erythrocyte zinc becomes a parameter for the nutritional status of this micronutrient in the long term, not reflecting recent changes in the concentrations of this mineral in one individual, and also not being affected by the zinc content found in the diet of the patients assessed in this study.

With regard to concentrations of enzyme superoxide dismutase found in erythrocytes, it was observed that they were adequate in both groups, and higher in patients with breast cancer, with significant statistical difference between groups. These results corroborate the findings by Tas et al., Yeh et al., and Rajnesh et al., who also found high levels of this enzyme in women with breast cancer.

From this perspective, it should be noted that increased activity of the enzymatic antioxidant defense system in women with breast cancer appears to be the result of a compensatory regulation mechanism that occurs in response to oxidative damage found in these women. In this sense, reactive oxygen species act stimulating the expression of genes encoding enzymes involved in this system, which may have occurred among the female participants in this study.

The results found in the analysis of plasma concentrations of TBARS do not show statistical difference between the study groups, although the levels found in patients with breast cancer were higher. Thus, it is appropriate to draw attention to the fact that excessive production of free radicals can cause oxidative damage in the cell membrane, resulting in lipid peroxidation, mutagenesis and, consequently, could worsen the carcinogenesis process found in these patients.

Another hypothesis that can support the increased plasma levels of TBARS is the obesity and overweight found among patients with breast cancer. Thus, it is worth mentioning that obesity contributes to increased mitochondrial respiration and excessive oxygen consumption, which favors the production of reactive oxygen species, such as anion superoxide and hydrogen peroxide. The second mechanism by which overweight and obesity can increase lipid peroxidation is through progressive and cumulative cell injury related to increased body mass. Cell injury, in turn, releases cytokines, such as tumor necrosis factor, which generates reactive oxygen species.

Another important aspect in this discussion is the fact that, despite patients with breast cancer have high zinc intake, plasma levels of TBARS were more increased in these patients compared to control group, which may indicate the inability of zinc to act as a component of the antioxidant defense system, probably due to reduced concentrations of this mineral in the blood components assessed.

Based on the results of this study, it can be assumed that reduced zinc concentrations found in women with breast cancer, both in plasma and erythrocytes, can be due to increased expression of genes encoding proteins that promote zinc influx to the tumor tissue, which may explain the changes found in the parameters studied.

Conclusion

Female participants with breast cancer of this study have an adequate dietary zinc intake. Nevertheless, the biochemical parameters of zinc show reduced concentrations of this mineral in plasma and erythrocytes. The activity of enzyme superoxide dismutase in the erythrocytes and plasma concentrations of TBARS are adequate in both groups; however, the enzymatic activity was significantly higher in women with cancer.

The study did not show that zinc affects activity of enzyme superoxide dismutase and plasma levels of TBARS in women with breast cancer, since correlation analysis did not show a statistically significant difference.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

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