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Analysis of plasma and erythrocyte zinc levels in premenopausal women with breast cancer

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Abstract

Introduction: **Zinc deficiency has been associated with damage and oxidative changes in DNA that may increase an individual's risk of cancer. Furthermore, zinc metabolism may be affected in cancer patients, leading to alterations in its distribution that would favor carcinogenesis. Plasma and erythrocyte zinc levels in women with breast cancer were evaluated in this cross-sectional, controlled study.**

Material and methods: **Fifty-five premenopausal women of 25 to 49 years of age with and without breast cancer were divided into two groups: Group A, composed of women without breast cancer (controls, n = 26) and Group B, composed of women with breast cancer (cases, n = 29). Plasma and erythrocyte zinc levels were measured by flame atomic absorption spectrophotometry at γ = 213.9 nm. Diet was assessed using the 3-day diet recall method and analyzed using the NutWin software program, version 1.5. Student's t-test was used to compare means and significance was established at $p < 0.05$.**

Results: **Mean plasma zinc levels were 69.69 ± 9.00 g/dL in the breast cancer patients and 65.93 ± 12.44 g/dL in the controls ($p = 0.201$). Mean erythrocyte zinc level was 41.86 ± 8.28 μgZn/gHb in the cases and 47.93 ± 7.00 μgZn/gHb in the controls ($p < 0.05$). In both groups, dietary zinc levels were above the estimated average requirement.**

Conclusions: **The present results suggest that zinc levels are lower in the erythrocyte compartment of premenopausal women with breast cancer.**

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Key words: *Breast cancer. Zinc. Nutritional status. Erythrocytes. Plasma.*

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ANÁLISIS DE LOS NIVELES DE ZINC EN PLASMA Y ERITROCITOS EN MUJERES PREMENOPÁUSICAS CON CÁNCER DE MAMA

Resumen

Introducción: **La deficiencia de zinc se relacionada con daños y modificaciones oxidativas del DNA, lo que puede favorecer el riesgo de cáncer. Sin embargo, en pacientes con cáncer, puede haber alteraciones en el metabolismo del zinc con alteración en su distribución, favoreciendo la cancinogénesis. Por lo tanto, el objetivo de este estudio fue evaluar las concentraciones plasmáticas y eritrocitarias de zinc en mujeres con cáncer de mama.**

Material y métodos: **estudio de naturaleza transversal, del tipo caso y control llevado a cabo en 55 mujeres premenospáusicas con y sin cáncer de mama con un rango de edades situado entre 25 y 49 años. Las pacientes fueron distribuidas en dos grupos: Grupo A, sin cáncer de mama (control, n = 26) y Grupo B, con cáncer de mama (caso, n = 29). El análisis de las concentraciones de zinc plasmático y eritrocitario fue realizado según el método de espectofotometría de absorción atómica de llama γ = 213,9 nm. La evaluación de la dieta fue determinada utilizando el registro alimentario de tres días y el análisis por el software NutWin versión 1.5. Para el análisis de las medias fue utilizado el test de estudios t de Student ($p < 0,05$)**

Resultados: **la media de las concentraciones plasmáticas de zinc fue 69,69 ± 9,0 μg/dL y 65,93 ± 12,44 μg/dL en las pacientes casos (cáncer) y controles, respectivamente ($p = 0,201$). La media de zinc eritrocitaria fue 41,86 ± 8,28 μgZn/gHb en las pacientes casos y 47,93 ± 7,00 μgZn/gHb en los controles ($p < 0,05$). Ambos grupos tenían concentración de zinc, en la dieta, superior a la recomendada.**

Conclusiones: **Los resultados del presente estudio indican que mujeres menospáusicas con cáncer de mama presentan menor concentración de zinc en el compartimiento eritrocitario, lo que puede constituirlo en un nuevo biomarcador pronóstico y posible diana terapéutica del cáncer de mama**

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Palabras clave: *Cáncer de mama. Zinc. Estado nutricional. Eritrocitos. Plasma.*

Abbreviations

DNA: Deoxyribonucleic acid.
RNA: Ribonucleic acid.
FSH: Follicle-Stimulating Hormone.
µg: Microgram.
ml: Milliliter.
EAR: Estimated Average Requirement.
DRIs: Dietary Reference Intakes.
AM: Morning/For numbered the morning hours.
g: Gram.
°C: Degree Celsius/Means the unit of temperature.
Zn: Zinc.
Hb: Hemoglobin.
Zip: Zinc influx transporter.

Introduction

Over the past few decades, several studies have been conducted to investigate the participation of micronutrients in the antioxidant and anticarcinogenesis mechanisms that have been implicated in the development of cancer. In particular, zinc has attracted great interest from the majority of investigators for its association in biochemical processes and antioxidant defense. In addition, zinc is known to function as a transcription factor and to play a role in the activities of enzymes involved in the synthesis of DNA and RNA. It would therefore appear to exert an inhibitory effect on neoplastic cell growth.[1,2,3]

Lower plasma zinc levels have been found in patients with neoplasia compared to healthy individuals.[4,5,6] Likewise, Kuo et al.[7] reported significantly lower serum zinc levels in breast cancer patients compared to a control group and suggested that plasma zinc could be used as a possible prognostic and therapeutic marker in breast cancer. Furthermore, the positive effect of zinc supplementation on reducing oxidative stress and improving the immune response of cancer patients has already been demonstrated.[8.]

According to Oyama et al.,[9] plasma zinc, copper and selenium levels could be considered relevant markers for evaluating prognosis in cancer, since their analysis is simple and inexpensive compared to measuring the activity of their respective enzymes. In addition, metallothionein, a low molecular weight protein that results from the binding of thionein to zinc, iron or cadmium, has been shown to act as a biomarker of poorly differentiated and more aggressive breast carcinomas.[10]

The association between plasma zinc levels and cancer risk has also been evaluated by Gupta et al.[11] and more recently by Adzersen.[12] These studies showed the existence of significant inverse associations between plasma and dietary zinc and breast cancer risk that may be important for the development of strategies to prevent this disease. Some investigators have also suggested analyzing zinc levels in the plasma compartment as a marker of therapeutic and prognostic response.[9,13,14,15] Nevertheless, others have considered analysis of zinc levels in the erythrocyte compartment to be more precise.[16]

The mechanism by which zinc affects carcinogenesis is controversial. When tumors are present, an alteration may occur in zinc distribution, compromising its function. A reduction in zinc levels in the plasma or erythrocyte compartments may result from an increase in the expression of the codifying genes of the zinc transporter proteins that promote the transportation of this mineral from the blood compartments to tumor tissues.[17,18] Therefore, zinc levels may indeed represent an important prognostic marker and also a therapeutic target in breast cancer patients, the possibility of which led us to design the present study.

Patients and methods

This cross-sectional, analytical, case-control study involved 55 premenopausal women of 25 to 49 years of age. The patients were divided into two groups: a control group of women without breast cancer (n = 26) and the study group composed of breast cancer patients (n = 29). The women with breast cancer were recruited at the mastology clinic of the Department of Gynecology and Obstetrics, *Getúlio Vargas* Hospital, Federal University of Piauí. The project was approved by the Internal Review Board of the university and all patients signed an informed consent form prior to inclusion in the study. Women with serum FSH levels > 30 µg/ml and patients with a history of previous treatment for the disease were excluded from the study. Women in use of medication or vitamin/mineral supplements and those with acute or chronic diseases that could affect normal zinc metabolism were also excluded.

Evaluation of dietary zinc intake

Dietary zinc intake was evaluated using a questionnaire based on the 3-day dietary recall technique. The questionnaires were analyzed using the NutWin computer software program, version 1.5.[19] To verify zinc levels in the participants' diets, the Estimated Average Requirement (EAR), as defined in the Dietary Reference Intakes (DRIs), was used.[20]

Biochemical parameters for measuring plasma and erythrocyte zinc levels

Blood samples (12 mL) taken between 7:30 and 9:00 AM following at least 12 hours of fasting were split into two glass tubes as follows: 1) a tube containing 30% sodium citrate as an anticoagulant (10 mg/mL of blood) for zinc analysis and 2) a tube with no anticoagulant for the measurement of FSH levels.

Plasma was separated from the whole blood by centrifugation at 3000 x g for 15 minutes at 4ºC (SIGMA 2K15 centrifuge). To separate the erythrocytes and then measure zinc levels, the method described by Whitehouse et al. was used.[21] The erythrocyte mass obtained was washed three times with 5 mL of 0.9% saline, slowly homogenized by inversion and centrifuged again at 10,000 x g for 10 minutes (Sorvall® RC-SB) at 4ºC, after which the supernatant was discarded. Following the final centrifugation, the saline solution was aspirated and the erythrocyte mass was carefully extracted using a micropipette, transferred into demineralized Eppendorf tubes and stored at -20ºC until measurement of zinc levels.

Plasma zinc levels were measured by atomic absorption spectrophotometry according to the method proposed by Rodriguez et al.[22]. Two aliquots were taken from each plasma sample, diluted in Milli-Q® water at 1:4 and aspirated directly on the flame of the device. Tryptizol® (Merck), diluted in MILLIQ® water with 3% glycerol, was used as standard at concentrations of 0.1, 0.2, 0.3, 0.5 and 1.0 µg/mL.

Erythrocyte zinc levels were measured by atomic absorption spectrophotometry (Whitehouse et al.),[21] according to the methodology standardized by Cordeiro.[23] This technique guaranteed the desired level of analysis precision with no matrix interference.

Aliquots of 500 µL of erythrocyte mass were diluted 40-fold in Milli-Q® water. First, the 500 µL aliquot was diluted at 1:4 (lysate 1). Subsequently, triplicate 200 µL-aliquots of lysate 1 were further diluted at 1:10 (lysate 2). Following homogenization, lysate-2 samples were then directly aspirated in the atomic absorption spectrophotometer. Tryptizol® (Merck) diluted in Milli-Q® water at the concentrations of 0.1, 0.2, 0.3, 0.5 and 1.0 µg/mL was used as standard.

Hemoglobin was measured using a Senta spectrophotometer 700-S, at a wavelength of 540nm. Results were expressed as mgZn/g Hb.[24]

Statistical analysis

A univariate descriptive analysis was performed for the study groups. The data were analyzed using the S-PLUS software program, version 3.2, and Minitab Release, version 11.0 for Windows 9.0. Student's t-test was used to compare the variables studied. Significance level was defined as $p < 0.05$.

Results and discussion

Mean zinc level found in the diet of patients with breast cancer was 10.47 ± 3.89 mg/day compared to 9.39 ± 1.76 mg/day for women in the control group ($p = 0.187$) (table I). The mean plasma zinc levels were 69.69 ± 9.00 µg/dL in the breast cancer patients and 65.93 ± 12.44 µg/dL in the controls ($p = 0.201$). Mean erythrocyte zinc levels were 41.86 ± 8.28 µgZn/gHb in the breast cancer group and 47.93 ± 7.00 µgZn/gHb in the control group ($p < 0.05$) (table II).

Table I
Zinc levels in the diet of breast cancer patients and controls

Nutrient	*Study Group Mean ± SD*	*Control Group Mean ± SD*
Zinc (mg/day)	10.47 ± 3.89	9.39 ± 1.76

Reference values for zinc ingestion: EAR = 6.8 mg/day (Institute of Medicine, 2001)[20].
There was no statistically significant difference between the groups. Student's t-test ($p > 0.05$).

Table II
Plasma and erythrocyte zinc levels in breast cancer and control groups

Parameters	*Study Group*		*Control Group*	
	Mean	*± SD*	*Mean*	*± SD*
Plasma (µg/dL)	69.69	± 9.00	65.93	± 12.44
Erythrocyte (µgZn/gHb)	41.86*	± 8.28	47.93*	± 7.00

Reference values for plasma zinc levels: 70-110 µg/dL (Gibson, 1990)[16].
Reference values for erythrocyte zinc levels: 40-44 µgZn/gHb (Guthrie; Picciano, 1994)[34].
*Statistically significant difference between breast cancer patients and controls. Student's t-test ($p < 0.05$).

There were no significant differences in the mean plasma zinc levels found in the groups of women with or without breast cancer in the present study. These findings are in agreement with those reported by Huang et al.,[25] who also failed to detect any difference in plasma zinc levels in breast cancer patients compared to a control group.

The findings of studies that have used plasma for identifying zinc metabolism in breast cancer patients have indeed been contradictory and fairly limited.[26,27] This could be explained by considering the fact that as a parameter for evaluating this trace element, the dynamics of plasma are fast, maintaining it under homeostatic control and rendering it vulnerable to numerous physiopathological effects in response to various circumstances such as stress, infection, catabolism, hormones and diet.[28,29,30]

With the objective of improving understanding of the metabolic component of zinc in breast cancer, various studies have been performed using erythrocytes as a marker of zinc nutritional status. In the present study, unlike the results obtained in plasma, the mean erythrocyte levels of zinc in the women with breast cancer were significantly lower than those of the women in the control group. These findings are in agreement with the results of Sharma et al.,[31] who also reported hypozincemia in women with breast cancer.

Some hypotheses have been raised in the literature on the possible mechanisms leading to a reduction in ery-

throcyte zinc levels in breast cancer patients. It is presumed that following the onset of carcinogenesis, a redistribution of zinc would occur in these patients through the passage of this mineral from the erythrocyte to the interior of the tumor cells, consequently reducing its levels in the erythrocyte compartment.[17,18,32,33]

Kagara et al.,[17] Taylor[18] and Louis and Cousins[34] demonstrated an increase in zinc levels in the tumor tissue of breast cancer patients that was associated with an increase in the expression of the transport proteins Zip 10, Zip 7 and Zip 6. These investigators attributed the reduction in erythrocyte zinc levels in cancer patients to the overexpression of the codifying genes of zinc transporter proteins that would transfer this mineral from the erythrocytes to the interior of the tumor.

In the present study, zinc levels consumed by the patients were found to be high considering that the recommended level of this mineral according to the Estimated Average Requirement (EAR) of zinc for women is 6.8 mg/day.[20] Therefore, dietary zinc intake would not have contributed to the reduced erythrocyte zinc levels found in the present study. Thus, it is probable that the low zinc levels in the erythrocyte compartment indeed resulted from an increase in the expression of the zinc transporter proteins, as shown by Kagara et al.,[17] Taylor[18] and Louis and Cousins.[34]

Conclusions

There were no significant differences in the mean plasma zinc levels found in the groups of women with or without breast cancer in the present study, but the zinc levels are lower in the erythrocyte compartment of premenopausal women with breast cancer.

Considering the findings of the present study, it is clear that there is a need to conduct further investigation to evaluate the mechanisms involved in the distribution and compartmentalization of zinc in breast cancer patients and to assess the consequences of changes in nutritional status and the possibility of using this mineral as a prognostic marker and target for new therapeutic strategies to combat this disease.

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