Evaluation of Zinc, Copper and Cu/Zn ratio in sera of women with breast cancer in Kirkuk City

Ali Yakub Majid [(1),] Usama Salman Al timari[(2),] Lubna Hussein Murshed [(3)]

(1) Poisoning Consultation Center /Specialized Surgeries Hospital, Medical City, Baghdad, Iraq. Email: doctoralicc@yahoo.com
(2) Department of medical analysis/medical and health of technical college/ Middle Technical University, Baghdad, Iraq. usamaaltimari@yahoo.com
(3) Medical Laboratory Science Technology, Azadi Teaching Hospital, Kirkuk, Iraq, Email: lubna8889@gmail.com

Abstract

The aim of this study was evaluating Zinc, Copper and Cu/Zn ratio in sera of women with breast cancer (BC) in Kirkuk City. The study involved 60 breast cancer women (20 on chemotherapy, 20 pre-operation, and 20 post-operation) and 20 women with benign breast tumors (BBT) as a disease control, all compared to 20 apparently healthy control women. Serum used to evaluate the levels of zinc and copper in control and patient groups, Cu/Zn ratio was also calculated. The results showed a highly significant ($P<0.01$) reduction in the mean serum zinc level of the total BC group (77.65 ± 8.49 µg/dl) and in its subgroups (on chemotherapy, pre- and post-operation) (78.85 ± 5.26; 72.8 ± 10.08; 81.3 ± 7.37 µg/dl) respectively and in benign breast tumor (82.55 ± 4.9 µg/dl), compared to that of healthy control (HC) group (93.55 ± 12.88 µg/dl).While a highly significant ($P<0.01$) elevation of mean serum copper concentration was found in total BC (150.92 ± 14.78 µg/dl) and in (Chemotherapy, Pre & Post-operation) breast cancer subgroups (148.65 ± 13.16, 154.9 ± 15.16, 149.2 ± 15.39 µg/dl) respectively, and in BBT (148.45±16.57 µg/dl) compared to HC (122.5 ± 13.33 µg/dl). In addition to a highly significant elevation in the mean serum Cu/Zn ratio in the total BC group (1.96 ± 0.36) and in breast cancer patients on Chemotherapy, Pre-operation and Post-operation (1.88 ± 0.19), (2.17 ± 0.52), (1.83 ± 0.13) respectively and in benign breast tumor (1.79 ± 0.22) compared to that of the HC group (1.32 ± 0.21) ($p< 0.01$). It is concluded from this study that the elevation of copper and Cu/Zn ratio may indicate increased oxidative stress in breast cancer which implicated in the etiology of breast cancer.

Keywords: Zinc, Copper, Cu/Zn, breast cancer, benign breast tumor.

تقويم الزنك والنحاس ونسبة النحاس إلى الزنك في أمصال النساء المصابات بسرطان الثدي في مدينة كركوك

الخلاصة

كان الهدف من هذه الدراسة هو تقويم الزنك والنحاس و نسبة النحاس إلى الزنك في أمصال النساء المصابات بسرطان الثدي في مدينة كركوك. اشتملت الدراسة على 60 امرأة مصابة بسرطان الثدي (20 على العلاج الكيميائي ، 20 قبل العملية ، و 20 بعد العملية) و 20 امرأة مصابات بأورام الثدي الحميدة كسيطرة مرضية ، وتم مقارنة جميع هذه المجاميع مع 20 امرأة أصحاء كسيطرة. يستخدم المصل لتقييم مستويات الزنك والنحاس في مجموعات التحكم والمرضى ، كما تم حساب نسبة النحاس إلى الزنك أيضا. أظهرت النتائج انخفاضاً معنوياً كبيرا (P <0.01) في متوسط مستوى الزنك في المصل في مجموع مرضى سرطان الثدي (77.65±8.49 ميكروغرام / ديسيلتر) وفي مجموعاتها الفرعية (على العلاج الكيميائي, قبل وبعد العملية) (78.85 ± 5.26 ؛ 72.8 ± 10.08 ؛ 81.3 ± 7.37 ميكروغرام / ديسيلتر) على التوالي وفي أورام الثدي الحميدة (82.55 ± 4.9 ميكروغرام / ديسيلتر) ، مقارنة مع مجموعة التحكم الصحية (12.88 ± 93.55 ميكروغرام / ديسيلتر) (P <0.01). بينما تم العثور على ارتفاع معنوي كبير في متوسط تركيز النحاس في أمصال مجموع مرضى سرطان الثدي (150.921 ±14.78 ميكروغرام / ديسيلتر) وفي المجموعات الفرعية لسرطان الثدي (العلاج الكيميائي, قبل وبعد العملية) (148.65 ± 13.16 ، 154.9 ± 15.16 ، 149.2 ± 15.39 ميكروغرام / ديسيلتر) على التوالي ، وفي مرضى الثدي الحميدة (148.45±16.57 ميكروغرام / ديسيلتر) مقارنة بالأصحاء (122.5±13.33 ميكروغرام / ديسيلتر). بالإضافة إلى ارتفاع معنوي كبير في نسبة النحاس إلى الزنك في أمصال مجموع مرضى سرطان الثدي (1.96±0.36) وفي مرضى سرطان الثدي على العلاج الكيميائي ، ما قبل العملية وبعد العملية (1.88 ± 0.19) ، (2.17 ± 0.52) ، (1.83 ± 0.13) على التوالي وفي أورام الثدي الحميدة (1.79 ± 0.22) مقارنة مع مجموعة الأصحاء (1.32±0.21) (P<0.01). أستنتجت من هذه الدراسة أن ارتفاع النحاس ونسبة النحاس إلى الزنك قد يشير إلى زيادة الإجهاد التأكسدي في سرطان الثدي الذي يتورط في مسببات سرطان الثدي.

الكلمات المفتاحية: الزنك, النحاس, نسبة النحاس إلى الزنك, سرطان الثدي, ورم الثدي الحميدة.

Introduction

Breast cancer is worldwide cancer and the most frequently diagnosed life-threatening and the leading cause of cancer death among women particularly Iraqi once (1). The risk factors for breast cancer involve gender, age, benign breast disease, family history of breast cancer or other cancers types, females with inherited mutations in the genes *BRCA1* or *BRCA2* (2). Moreover, obesity, diet, low physical activity, using of exogenous hormones or oral contraceptives, early age at menarche, late age at menopause, late age at first full-term pregnancy, consumption of alcohol, smoking and exposure to high dose of radiation during

early life are among the risk factors for breast cancer (3). Nevertheless, all of these risk factors have been appeared to have different relationships with breast cancer among different ethnic populations around the globe (4).

Zinc and copper are essential trace elements for healthy growth and function of the human body (5, 6). They are an integral part of as many as 40 metalloenzymes, including Cu/Zn superoxide dismutase with antioxidant and anti-inflammatory activity (7). Zinc is enriched in cancer tissue containing tumor cells in contrast to normal stroma and significantly lowers in sera of these patients due to high levels of zinc importers (Zip) in breast cancer which import zinc from extracellular matrix and fluids into breast cancer cells (8). Zinc is known to promote cell proliferation and may play a role in tumor growth (9). However, the zinc transporter ZnT and metallothionein MT are thought to be overexpressed in malignant breast cells, which protect them from over accumulations of zinc and prevent tumor cell apoptosis by inhibiting free radicals. Moreover, metallothionein interacts with zinc ions and involved in the regulation of several transcription factors that participate in carcinogenesis (10). Otherwise, when zinc concentration is either in excess or deficient it acts as a pro-oxidant and turn into pro-inflammatory and pro-apoptotic. Excess zinc provokes copper deficiency, resulting in decreased expression of copper-dependent antioxidant enzymes, such as superoxide dismutase and ceruloplasmin (11). However, when copper concentration excess it binds to genetic material activating organic peroxides which generate free radicals initiating oxidative damages to cellular macromolecules and DNA may induce mutation (12), after Cu entering the cell, it is transported to ATPases in the Golgi apparatus by Cu chaperone antioxidant-1(Atox1) and incorporate into Cu-dependent enzymes that plays an essential role in breast cancer cell migration by lamellipodia (13).

Methodology

The sample collection for this study was conducted at Azadi Teaching Hospital/ Oncology center and the main center for early detection of breast tumors, in addition to General Kirkuk Hospital during the period from the first of October/2017 to the first of February/2018. While analysis of these samples was conducted at the poisoning consultation center/specialized surgeries hospital. A total of 100 subjects were enrolled in this study and divided into three groups. The first group included 60 patients women aged between 23-68 years and divided into three subgroups (20 on Chemotherapy, 20 Pre-operation, and 20 Post-operation breast cancer women). The second group included 20 women with benign breast tumors and the third group

included 20 healthy females as a control group who have normal breast tissue and without any previous history of any systemic diseases. Zinc and copper were estimated for all cases by using the Flame Atomic Absorption Spectrophotometer. Cu/Zn ratio was calculated by dividing the Cu level to Zn level for each individual.

Statistical Analysis

Data were analyzed using SPSS version 18. Data of 3 groups were compared by the ANOVA test. If the ANOVA (F test) showed an overall difference, least significant difference (LSD) test was used to evaluate the significance of the differences. A two-tailed P value of less than 0.05 was considered statistically significant for all comparisons.

Results

Results in the table (1) are expressed as mean ± SD and represented the mean value of serum zinc levels in total breast cancer and its subgroups compared with benign breast tumor and healthy control groups. The results showed that the mean serum zinc concentration in total breast cancer group (77.65 ± 8.49 µg/dl) and in subdivision cases (on chemotherapy, pre and post operation) of breast cancer group (78.85 ± 5.26, 72.8 ± 10.08 and 81.3 ± 7.37 µg/dl) respectively and in benign breast tumors (82.55 ± 4.9), was highly significantly lower than that of healthy control group (93.55 ± 12.88 µg/dl) ($p< 0.01$) and only in pre-operation breast cancer subgroups was significantly lower than that of benign breast tumors ($P<0.05$), while mean serum zinc level in pre-operation BC cases (72.8±10.08 µg/dl) was significantly lower than that of chemotherapy cases (78.85±5.26 µg/dl) ($P< 0.05$), also a significant decrease in mean serum Zn level was observed in total breast cancer group when compared with benign breast tumor group ($P<0.05$) as shown in figure (1).

Table (1): Comparison of Serum Zinc concentrations (µg/dl) between the studied groups

Studied groups	No.	Zn (µg/dl) Mean±SD.	Test of Sig.	
			ANOVA test	LSD test
Healthy control	20	93.55± 12.88	F^a= 23.461 P=0.000 (HS)	P^1=0.000 (HS)
Disease control (BBT)	20	82.55 ± 4.9		P^2=0.038 (S)
				P^3=0.001 (HS)
Total BC	60	77.65±8.49		P^4=0.649 (NS)
Pre-operation BC	20	72.8 ± 10.08	F^b=15.288 P=0.000 (HS)	P^5=0.170 (NS)
Post-operation BC	20	81.3 ± 7.37		P^6=0.002 (HS)
Chemotherapy BC	20	78.85 ± 5.26		P^7=0.029 (S)
				P^8=0.37 (NS)

No= Number, Zn= Zinc, SD= Standard deviation, Sig= Significance, BC= Breast cancer, BBT= Benign breast tumor, HS= highly significant, S= Significant, NS= Non-Significant, LSD= Least significant difference, ANOVA= Analysis of variance, F= Fischer test, P=probability.

[a]= ANOVA test among healthy control, BBT, and Total BC groups, [b]= ANOVA test among Pre & Post-operation BC, Chemotherapy, BBT, and healthy control groups, [1]= Healthy control VS. each of other groups, [2]= BBT VS. Total BC, [3]= BBT VS. Pre-operation, [4] =BBT VS. Post-operation, [5]= BBT VS. Chemotherapy, [6]= Pre VS. Post-operation BC, [7]=Pre-operation VS. Chemotherapy, [8]= post-operation VS. Chemotherapy.

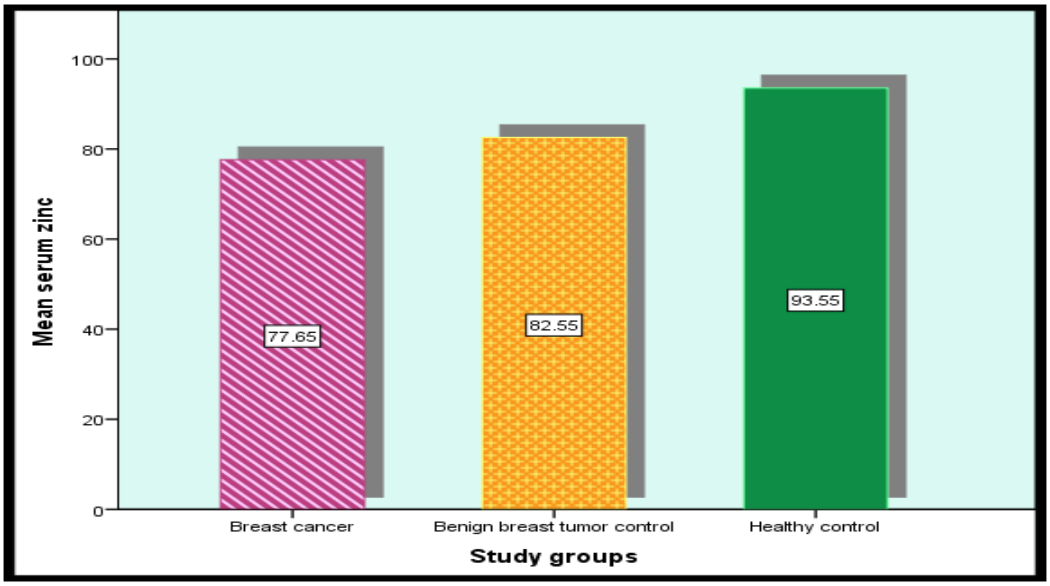

Figure (1): Mean serum zinc concentration (µg/dl) of the studied groups.

The results in table (2) and figure (2) revealed that the mean serum copper concentration in total breast cancer group (150.92±14.78 µg/dl) and in (Chemotherapy, Pre & Post-operation) breast cancer subgroups (148.65 ±13.16, 154.9 ±15.16, 149.2±15.39 µg/dl) respectively, and in benign breast tumor control (148.45±16.57 µg/dl) was a highly significantly elevated compared to healthy control (122.5 ± 13.33 µg/dl) ($p< 0.01$),while the mean serum copper concentration of Pre-operation cases (154.9±15.61 µg/dl) was a non-significantly higher than that of Chemotherapy cases(148.65 ± 13.16 µg/dl) ($p>0.05$).

Table (2): Comparison of Serum Copper concentration (µg/dl) between the studied groups

Studied groups	No.	CU (µg/dl) (Mean±SD.)	Test of Sig.	
			ANOVA test	LSD test
Healthy control	20	122.5±13.33	F^a= 28.102 P=0.000 (HS)	P^1=0.000 (HS)
Disease control (BBT)	20	148.45±16.57		P^2=0.523 (NS)
				P^3=0.174 (NS)
Total BC	60	150.92±14.78		P^4=0.874 (NS)
Pre-operation BC	20	154.9±15.61	F^b=14.616 P=0.000 (HS) p<0.01	P^5=0.174 (NS)
Post-operation BC	20	149.2±15.39		P^6=0.229 (NS)
Chemotherapy BC	20	148.65±13.16		P^7=0.187 (NS)
				P^8=0.907 (NS)

Cu= copper, BBT= benign breast tumor, BC= breast cancer, NS=non-significant

[a]= ANOVA test among healthy control, BBT, and Total BC groups, [b]= ANOVA test among Pre & Post-operation BC, Chemotherapy, BBT, and healthy control groups, [1]= Healthy control VS. each of other groups, [2]= BBT VS. Total BC, [3]= BBT VS. Pre-operation, [4] =BBT VS. Post-operation, [5]= BBT VS. Chemotherapy, [6]= Pre VS. Post-operation BC, [7]=Pre-operation VS. Chemotherapy, [8]= post-operation VS. Chemotherapy.

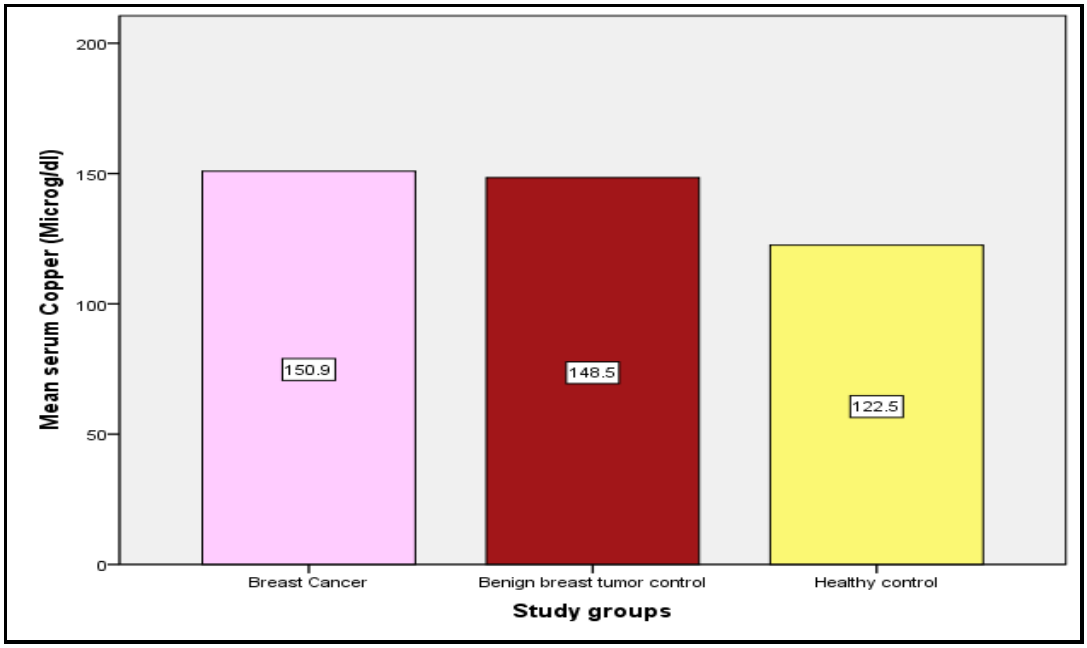

Figure (2): Mean serum copper concentration (µg/dl) among the studied groups.

As shown in the table (3), the results revealed that the mean serum Cu/Zn ratio in the total breast cancer group (1.96 ± 0.36) and in breast cancer patients on Chemotherapy, Pre-operation and Post-operation (1.88 ± 0.19), (2.17±0.52), (1.83±0.13) respectively and in benign breast

tumor control group (1.79±0.22), was a highly significantly elevated compared to that of a healthy control group (1.32 ± 0.21) (p< 0.01), in addition to a highly significant elevation of the mean Cu/Zn in Pre-operation breast cancer subgroup compared to benign breast tumor control and post-operation breast cancer subgroup, while no significant differences were seen in the mean value of Cu/Zn ratio for benign breast tumors (1.79±0.22) when compared to that of chemotherapy (1.88 ± 0.19) and post-operation breast cancer groups (1.83±0.13), and between breast cancer patients on chemotherapy and post-operation (p>0.05). However, a significant increase in the mean Cu/Zn ratio was seen in total breast cancer patients (1.96 ± 0.36) when compared to benign breast tumors (1.79±0.22) (P<0.05).

Table (3): Comparison of serum Cu/Zn ratio between the studied groups

Studied groups	No.	Cu/Zn (Mean±SD.)	Test of Sig.	
			ANOVA test	LSD test
Healthy control	20	1.32 ± 0.21	F^a=31.171 P=0.000 (HS)	P^1=0.000 (HS)
Disease control (BBT)	20	1.79 ± 0.22		P^2=0.042 (S)
				P^3=0.707 (NS)
Pre-operation BC	20	2.17 ± 0.52	F^b=21.854 P=0.000 (HS)	P^4=0.348 (NS)
Post-operation BC	20	1.83 ± 0.13		P^5=0.002 (HS)
Chemotherapy BC	20	1.88 ± 0.19		P^6=0.573 (NS)

No= Number, Cu/Zn= Copper to zinc ratio, SD= Standard deviation, Sig= Significance, BC= Breast cancer, BBT= Benign breast tumor, HS= highly significant, S= Significant, NS= Non-Significant, LSD= Least significant difference, ANOVA= Analysis of variance, F= Fischer test, P=probability

[a]ANOVA test between Total BC, BBT and Healthy groups, [b]ANOVA test between Chemotherapy, Pre &Post-operation, BBT and Healthy groups**,** [1]= Healthy control VS each of other groups, and Pre-operation VS. BBT and Post-operation BC, [2]= BBT VS. Total BC, [3] =BBT VS. Post-operation, [4]= BBT VS. Chemotherapy, [5]=Pre-operation VS. Chemotherapy, [6]= post-operation VS Chemotherapy.

Discussion

The previous study has recorded an increased breast cancer risk with decreased serum zinc level and elevated serum copper level. These results were compatible with a study in Iraq by Sahan *et al*, who reported a highly significantly reduction in the serum zinc concentration and a statistically significantly increase in copper concentration in newly diagnosed and old breast cancer patients in comparison to a healthy control (14) and Jasim, who showed that the

zinc level was significantly and highly significantly decreased in sera of breast cancer patients compared with the control group regardless of the period of time for suffering from this disease (15). Zn deficiency has been related to destruction and oxidative alterations in DNA that may raise an individual's risk of cancer (16). Many factors could be responsible for low zinc concentration in sera of breast cancer patients as increased Zn loss via the urine and increased uptake of Zn by cancer cells to be utilized for proliferation (17) and interference of zinc with intestinal absorption of copper which has high affinity to metallothionein protein (18). Conversely to these findings, El-Deeb *et al*., who reported a significant increase in serum zinc level in breast cancer groups as compared to the control group, while agreed with them in concerning of copper who showed a significantly increase in serum copper level in three groups (newly diagnosed BC, patients with metastases BC and benign breast tumors) compared with healthy control (19). The reason may be related to the differences in diet habit and the sample size of both studies.

low serum zinc concentration in preoperative breast cancer patients compared to postoperative and patients on chemotherapy treatment may be due to increased zinc uptake from blood circulation to cancer cells by high levels of Zips that are present in breast cancer cells (10) and consumption of zinc by tumor cells (20) which use it as cofactor for survival and replication (21). These results also were consistent with the results of Ali *et al*., who found that the plasma zinc and copper concentrations were significantly affected by chemotherapy in women with breast cancer compared to healthy control (22). However, our results were different from the results of Feng *et al*, who indicated that the zinc levels in breast cancer patients were significantly higher than in the control group (23).

The results of this study were agreed with Pavithra *et al*, who reported a statistically significant elevation in serum copper of patients with breast cancer when compared to controls (24). Copper in trace amount is a cofactor during redox reaction in tissue cells; however, elevation of serum Cu concentration increases the risk of BC because it activates several organic peroxides generating reactive oxygen species (ROS) which induce mutations through damaging genetic material (24). Furthermore, the low zinc level might elevate serum copper concentration (25) because low zinc levels may decrease the biosynthesis of an intracellular ligand metallothionein in enterocytes that binds Cu with high affinity and reduce the amount of copper absorbed by enterocyte (26). It is found that exhaustion of Cu possibly inhibits the manufacture and release of endothelial progenitor cells from the bone marrow, resulting in a repressed angiogenic switch and keeping tumor cell dormant (27).

The elevation of copper level in the Pre-operation breast cancer patients compared to the Post-operation group may be due to its function as an angiogenic factor in tumors, and that surgical removal of tumor decreases copper concentration due to lack of tumor cells so lack of angiogenic function (28). In contrast to our results, a study by Arooj *et al*, who showed a decrease in mean serum copper level in females with breast cancer compared to healthy control (29) and Sanjeev *et al.*, who reported a highly significant increase in mean serum copper levels in breast cancer patients compared to benign breast disease and that the patients with advanced breast cancer had higher serum copper levels than patients with early diagnosed breast cancer (30).

The balance between Zn and Cu is clinically more important than the concentration of them in blood serum because alterations in the balance affect several organic systems. Elevated copper and decreased zinc are the most common trace element imbalances (31). It has been demonstrated that there is a strong association between the ratio of Cu/ Zn and oxidative stress (32). Our results indicated that Cu/Zn ratio was significantly higher in breast cancer patients compared to the control groups and are consistent with the study of Adeoti *et al.,* who showed that the concentrations of copper and copper-zinc ratio (Cu/Zn) were significantly higher in the breast cancer patients compared to the controls (12), and Gupta *et al*., who observed that the Cu/Zn ratio was highly significantly increased in women with breast cancer, but not in women with benign breast diseases (33). In contrast to our results, a study by Adnan *et al*. reported a significant reduction in Cu/Zn ratio in breast cancer women in comparison to the control group (34).

Conclusions

It is concluded from this study that the elevation of copper and Cu/Zn ratio with decreased zinc in women with breast cancer may indicate increased oxidative stress which implicated in the etiology of breast cancer and that Cu/Zn ratio can be used as a marker for oxidative stress during the disease process. These parameters should be of value as an addition to the screening panel of biological tumor markers.

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