Serum Levels alteration of α-carotene, Copper, and Zinc in Patients with Breast Cancer

Abtisam F. Al-Shukri¹, Abbas F. Almulla², Ali Abbas Abo Algon³, Haider Ali Alnaji⁴

1) Department of Analysis, Kufa Institute, Al-Furat Al-Awsat Technical University, 31001 Kufa, Al-Najaf, Iraq.
2,4) Medical Laboratory Technology Department, College of Medical Technology, The Islamic University, Najaf, Iraq.
3) Iraqi Education Ministry, Najaf, Iraq

Corresponding Author: Dr. Abbas F. Almulla
Medical Laboratory Technology Department, College of Medical Technology, The Islamic University, Najaf, Iraq. Email: Abbass.chem.almulla1991@gmail.com

Disclosure statement

In relation to the submitted article, none of the authors have any financial or other conflicts of interest.
Abstract

Background: Breast cancer is the most common malignancy in women globally, and 70–80 percent of patients with early-stage, non-metastatic illness can be cured.

Patients and methods: One hindered twenty patients with BC, and sixty healthy controls recruited in this study from the centre of cancers and tumours in Al-Najaf governorate in Iraq from August 2020 to October 2020. Serum biomarkers were measured using the Elisa technique and spectrophotometry.

Results: In the present study, serum levels of the α-carotene, Zinc, and copper were significantly different P< 0.05 in patients with BC than healthy control. Employment, residency, BMI, and genetic factors showed a significant difference P< 0.05 among BC patients and healthy control. Significant differences P < 0.05 have been recorded in age, Zinc, and copper with different menstrual cycle stages in patients with BC. Significant correlation has been identified with zinc and copper r = 0.39, P<0.05, zinc and α-carotene r = -0.35, P <0.05, copper with α-carotene r = -0.41, P<0.05 also α-carotene with BMI r = 0.24, P <0.05.

Conclusion: Serum levels of α-carotene, Zinc, and copper are abnormal in patients with BC, combined with genetic and other environmental factor that may be a risk factor for developing BC. The different status of the menstrual cycle affects the level of Zinc and copper in BC patients, so it may be one of the factors that aid in developing the disease along with hormonal imbalance and the status of the menstrual cycle.

Keywords: Breast cancer, α-carotene, Zinc, Copper.

Introduction
Breast cancer (BC) is a disease in which the breast cell's growth goes out of control; the cancer incidence and mortality still governed with BC in most developed countries worldwide (1). The BC risk factors have widely investigated with significant international research; most identified risk factors are not modifiable (2). Many research have been published around the world looking into various elements of the link between physical exercise and the reduction of BC risk. (3). Physical activity has been shown to have strong impacts on postmenopausal women, women of normal weight who have no family history of BC, and women who are parous (4). Physical activity can lower BC risk through a number of interconnected physiological pathways that include obesity, sex hormones, insulin resistance, adipokines, and chronic inflammation. (5).

The intake of vitamin A and provitamin A-rich food are inversely related to the incidence of certain cancers, including BC; this reported with many epidemiological studies (6). Using cell culture models in several studies conducted to examine the effects of retinoid (all-trans-retinoic acid, mainly), and to a lesser extent of carotenoids, on BC. Generally, all-trans-retinoic acid on different BC cells (7). Many studies have been compared with one review to use retinoid and carotenoids as treatments for BC cells and reports what may be the fundamental cause of these compounds' differential effects on the same cell lines (8). Furthermore, both prospective and retrospective studies have consistently shown that a lower diet of vegetables, fruits, and carotenoids is linked to an increased risk of lung cancer. (9). Besides, reduced serum level of β-carotene associated with the subsequent development of lung cancer due to the protective effects of β-carotene also plays this role even without the requirement to convert it into vitamin A (10, 11). α-Carotene also affects the regulation of the tumor microenvironment, cancer immunity, BC stem cells, and cancer-related microRNAs. (12).
There is significant inhibition of mammary adenocarcinoma initiation associated with α-carotene. This preventive effect was seen in the initiation and post-initiation phases of mammary tumorigenesis (13). Despite the lack of proof in the human clinical studies, laboratory and animal studies provide supporting evidence from using α-carotene as a therapeutic and preventive agent in BC; also, the exact role of α-carotene in cancer cells needs more investigations (14, 15). Studies also suggest anti-cancer effects for alpha-carotene due to inhibiting proliferation and promoting apoptosis (16). Hormone-dependent people with BC can also interfere with α-carotene, as evidenced by numerous experimental investigations, and it can severely reduce epidermal growth factor (EGF)-mediated expression of both MMP-9 and MMP-13 in BC cells, which can be active in up to one-third of BC patients, preventing BC cells from migrating. (17). The purpose of the study is to look into the level of α-carotene in relation to serum levels of zinc and copper in BC patients, as well as the changes in the measured markers levels as the patients' menstrual cycle status varies.

**Materials and methods**

**Participants**

From August to October 2020, female patients recruited from the centre for cancers and tumours in the governorate of Al-Najaf in Iraq. One hundred twenty females diagnosed with BC involved in the present study as patients, and 60 normal women allocated as a healthy control group. The researcher used a mini-interview with people in the current study to record all sociodemographic information about both groups. Both the patients and the controls evaluated. Any disorders that could alter the biomarkers are ruled out through a thorough medical history. Before applying the methodology's details, all of the selected participants were asked a series of questions to ensure that no smokers, drug users, or alcoholics were included in the study. All of the participants lived in various locations both inside and outside of Najaf.
Furthermore, none of the subjects were afflicted with any other illnesses. The study was authorized by the University of Kufa's institutional review board (IRB) (#515, February 2018). The patients' or their close first-degree relatives' written informed permission was obtained in accordance with the IRB’s protocols.

**Blood Sample Collection and clinical measurements**

Venous blood samples (5 ml) have been withdrawn from each participant in the present study in the morning (9-11 AM) while they are fasting. All of the samples of the blood transferred to gel tubes. After freezing, all samples transferred to the laboratory to perform the clinical measurements. The levels of Zinc and copper were determined through routine tests via spectrophotometric reagents kits (Cromatest, Spain). α-carotene concentration in the blood has been determined through the Elisa technique using a specific ELISA kit provided by meslen company from china.

**Statistical Analysis**

The SPSS program version 25.0 was used for data management and statistical analysis. To check for normalcy, the Kolmogorov–Smirnov test was used. (mean standard deviation) is the descriptive information about the results of normally distributed variables. The one-way ANOVA test was used to compare baseline characteristics and results. Following the process, Pearson correlation was used to examine correlations between parameters. If the p value was less than 0.05, the differences were considered significant. The same version of SPSS was used to create the graphs.

**Results**

**Sociodemographic and clinical characteristics**

There is a significant difference in some sociodemographic characteristics: education, employment, residency, BMI, genetic factors among patients with BC, and healthy control (P > 0.05), as shown in table 1. Our results showed no significant difference in age, weight, length and
marital status (P > 0.05). The levels of serum biomarkers zinc (μg/dL), copper (μg/dL), Zn/Cu ratio and α-carotene (μmol/L) were showed a significant difference (P > 0.05) between a group of BC patients and healthy control as illustrated in table 1.

**Table 1: Sociodemographic and clinical data of all participants**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=60)</th>
<th>Patients(n=120)</th>
<th>df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40 (±18)</td>
<td>39 (±21)</td>
<td>1/178</td>
<td>0.476</td>
<td>0.491</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>76.95 (14.258)</td>
<td>74.04 (14.491)</td>
<td>1/178</td>
<td>1.62</td>
<td>0.204</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>160.93 (5.801)</td>
<td>161.08 (6.102)</td>
<td>1/178</td>
<td>0.058</td>
<td>0.810</td>
</tr>
<tr>
<td>Education (ille./lit)</td>
<td>22/38</td>
<td>32/88</td>
<td>1</td>
<td>3.547</td>
<td>0.043</td>
</tr>
<tr>
<td>Employment (Y/N)</td>
<td>38/22</td>
<td>65/55</td>
<td>1</td>
<td>18.25</td>
<td>0.000</td>
</tr>
<tr>
<td>Residency (Ru/Ur)</td>
<td>13/47</td>
<td>34/86</td>
<td>1</td>
<td>24.35</td>
<td>0.000</td>
</tr>
<tr>
<td>Marital state (M/S)</td>
<td>54/6</td>
<td>111/9</td>
<td>1</td>
<td>4.258</td>
<td>0.058</td>
</tr>
<tr>
<td>BMI</td>
<td>29.78 (5.76)</td>
<td>27.18 (5.188)</td>
<td>1/178</td>
<td>5.51</td>
<td>0.021</td>
</tr>
<tr>
<td>Genetics factor(Y/N)</td>
<td>0</td>
<td>75/45</td>
<td>1</td>
<td>111.14</td>
<td>0.000</td>
</tr>
<tr>
<td>Zn μg/dL</td>
<td>90.68 (±37.23)</td>
<td>63.46 (±29.69)</td>
<td>1/178</td>
<td>78.51</td>
<td>0.000</td>
</tr>
<tr>
<td>Cu μg/dL</td>
<td>91.54 (±21.40)</td>
<td>128.68 (±25.54)</td>
<td>1/178</td>
<td>132.05</td>
<td>0.000</td>
</tr>
<tr>
<td>Zn/Cu ratio</td>
<td>0.954 (±0.231)</td>
<td>0.616(±0.162)</td>
<td>1/178</td>
<td>7.89</td>
<td>0.032</td>
</tr>
<tr>
<td>α-carotene μmol/L</td>
<td>0.066 (±0.032)</td>
<td>0.026 (±0.011)</td>
<td>1/178</td>
<td>67.05</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values are expressed as mean (SD). P-value < 0.05: significant difference. Kg: Kilogram, cm: Centimeter, illet: Illiterate, Lit: Literate, Y: Yes, N:No, Ru: Rural, Ur: Urban, M: Married, S: Single, BMI: Body Mass Index, Zn:Zinc, Cu: Copper.

**Comparison of some parameters in different stages of MC**

The comparison results showed a significant difference P < 0.05 in some of the parameters among different menstrual cycle phases (MC) in patients with BC table 2. Table 2 compared the age, weight, Zn, Cu and α-carotene in patients with BC and with the different state of MC. The results showed that BC patients significantly differ in serum zinc and copper during the MC's other form. There was no significant difference (P > 0.05) in length, weight, α-carotene and BMI among MC stages in BC patients.
Table 2: The results of comparison of some parameters in different stages of MC

<table>
<thead>
<tr>
<th>Variables</th>
<th>normal MC (n=26) A</th>
<th>Menopause (n=48) B</th>
<th>Peri-menopause (n=29) C</th>
<th>Post-menopause (n=17) D</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28 (±6) B,D</td>
<td>40 (±7) A,D,C</td>
<td>39 (±5) B,D</td>
<td>54 (±4) A,B,C</td>
<td>19.9</td>
<td>0.000</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>75.93 (14.362)</td>
<td>72.40 (13.591) C</td>
<td>78.02 (16.465) B</td>
<td>75.70 (12.869)</td>
<td>1.4</td>
<td>0.227</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>161.39 (5.621)</td>
<td>160.89 (5.760)</td>
<td>162.71 (6.260)</td>
<td>158.22 (5.944)</td>
<td>3.2</td>
<td>0.023</td>
</tr>
<tr>
<td>BMI</td>
<td>28.00 (4.83)</td>
<td>28.21 (6.07)</td>
<td>28.99 (6.58)</td>
<td>28.18 (4.37)</td>
<td>0.2</td>
<td>0.862</td>
</tr>
<tr>
<td>Zn μg/dL</td>
<td>85.43 (±11.98) C</td>
<td>69.78 (±9.56) C</td>
<td>49.77 (±12.71) A,B</td>
<td>65.12 (±24.78)</td>
<td>5.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Cu μg/dL</td>
<td>116.1 (±17.87) D</td>
<td>115.6 (±14.39) D</td>
<td>125.4 (±19.98) A,B</td>
<td>122.16 (7.483) A,B</td>
<td>4.4</td>
<td>0.005</td>
</tr>
<tr>
<td>Zn/Cu ratio</td>
<td>0.525(±0.062)</td>
<td>0.584(±0.031)</td>
<td>0.544(±0.072)</td>
<td>0.516(±0.211)</td>
<td>2.2</td>
<td>0.071</td>
</tr>
<tr>
<td>α-carotene μmol/L</td>
<td>0.019 (±0.015)</td>
<td>0.022 (±0.011)</td>
<td>0.018 (±0.013)</td>
<td>0.020 (±0.009)</td>
<td>0.9</td>
<td>0.409</td>
</tr>
</tbody>
</table>

A: Significant difference (p<0.05) between normal mc with other stages of MC.
B: Significant difference (p<0.05) between menopause and other stages of MC.
C: Significant difference (p<0.05) between peri-menopause and other stages of MC.
D: Significant difference (p<0.05) between post-menopause and other stages of MC.
MC: Menstrual cycle, μg/dL: Microgram/Deciliter, BMI: Body Mass Index, Kg: Kilogram, cm: centimeter, μmol/L: Micromole/Litter.

Comparison of the α-carotene among BC patients with and without genetic factor:

Figure 1 shows the serum level of α-carotene in BC patients, which have a genetic factor for disease and the group without genetic factor. This comparison study showed a significant difference (P <0.05) among both groups of BC patients, namely the patients with BC. The genetic one of the risk factors for them showed less serum α-carotene than patients who do not have this genetic factor.
Figure 1: Comparison study of α-carotene with and without genetic factor

Correlation between the serum levels of Copper and Zinc in BC patients

A correlation study among serum levels of Zinc and copper showed a strong positive relationship $r = 0.390$, $P < 0.05$, namely the serum level of Zinc is highly affected by the serum level of copper in BC patients illustrated with figure 2.

Figure 2: Correlation study of Zinc and Copper in BC
**Correlation between the serum levels of α-carotene and Zinc in BC patients**

Figure 3 shows a significant relationship $r = -0.350$, $P < 0.05$ between serum levels of α-carotene and Zinc in BC patients. This relationship is reversible, whereas when the serum level of the α-carotene increases, serum zinc level decreases in BC patients.

![Simple scatter of Zinc by α-carotene](image)

**Figure 3: Correlation study of zinc and α-carotene in BC**

**Correlation between the serum levels of α-carotene and copper in BC patients**

The correlation study on the serum level of copper and serum level of α-carotene showed a significant negative correlation $r = -0.41$, $P < 0.05$ namely, as the level of the α-carotene increased in the serum, the level of the copper is diminished in patients with BC as shown in figure 4.
Figure 4: Correlation study of copper and $\alpha$-carotene in BC

Correlation between the serum levels of $\alpha$-carotene and BMI in BC patients:

In the study the correlation between the BMI and the serum level of $\alpha$-carotene, there is a significant positive correlation $r = 0.24$, $P < 0.05$ among these to markers, that's mean with the high BMI value, the serum level of $\alpha$-carotene increased as shown in figure 5:

Figure 5: Correlation study of BMI and $\alpha$-carotene in BC
Discussion

In the presented case-control study, serum levels of α-carotene, copper, and Zinc have investigated in patients with BC during stages of the disease and several phases related to the menstrual cycle. Our results showed decreased Zinc and α-carotene while elevated levels of serum copper in a group of patients compared with the healthy control group. The zinc\copper ratio was also significantly different p<0.05 among the study groups, as shown in Table 1. These findings are in line with earlier researches (18, 19). Table 1 also showed a significant difference between patients and the control group p<0.05 in the employment state, BMI, residency, genetic factor. Employment people always have more movement than unemployed people because this movement is considered a part of daily exercise and may be protective against the risk of BC(18).

A meta-analysis of twelve prospective studies of circulating carotenoids and BC risk was undertaken by Aune et al. They proposed that blood-carotene, α-carotene, lutein, and total carotenoids play a protective effect. (20). Smoking reported as playing a role in BC initiation (18, 21). Because randomized controlled trials of high-dose α-carotene supplementation in smokers resulted in unexpectedly higher lung cancer rates, the carotenoids have a variety of biological impacts. (22). Additional functions, including stimulating cell communication and immune system enhancement, have also been reported for these carotenoids (23). Smoking-induced oxidative stress (24), as well as decreased plasma antioxidants such as carotenoids and other antioxidants, may be risk factors for breast tissue initiation abnormalities (25).

People with high BMI suffering from increased oxidative stress and elevated inflammatory biomarkers in response to the overweight, and it may be a risk factor for developing BC (20). Greater plasma carotenoid concentrations may assist obese people with higher levels of oxidative stress and inflammation.(26). However, Eliassen et al. found inverse relationships of numerous
plasma carotenoids only among thinner women, and a nonsignificant positive association for all subtypes and total carotenoids among overweight women, contradicting this theory. (27). Wang, Ying, and colleagues also discovered a link between plasma α-carotene, lycopene, and BMI (18). However, in leaner or overweight women, the inverse relationship between -carotene and BC was nonlinear. Overall, one study's findings show that plasma α-carotene may be a reliable predictor of BC risk. They cannot, however, rule out the possibility of confounding by other dietary or nondietary factors. (18).

As shown in table 2, there is a significant difference p<0.05 in some measured biomarkers as well as some sociodemographic characteristics during different stages of the menstrual cycle in patients with BC, while the levels of Zn are significantly different p<0.05 among menopause and peri-menopause patients compared to regular menstrual cycle patients, and the level of the Cu is significantly different p<0.05 among post-menopause and menopause in compared with regular menstrual cycle patients. C. Michos et., al; reported the same findings and also, they found a fluctuation in the serum level of both Zinc and copper associated with different stages of menstrual cycles (28). Trace elements like Zn and Cu are required in trace amounts to maintain a variety of physiological processes (28). Because their free form (particularly Cu) is poisonous, they are transported and stored tightly attached to proteins. Cu that is unattached to DNA, lipids, or other biomolecules is redox active and can cause serious oxidative damage. As a result, the concentration of free Cu ions in vivo is nearly nil (29), (30).

Excess Cu, on the other hand, is not good for you. Wilson disease symptoms are caused by it, and it can cause increased oxidative damage, among other things. Cu appears to play a function in the regulation of female sex hormones, according to numerous research (31). Copper appears to boost the release of luteinizing hormone (LH) and even the release of follicle-stimulating hormone (FSH)
in vivo (FSH). Copper and GnRH complexes are more potent than native GnRH at releasing FSH and LH. In the anterior pituitary's gonadotropic cells, Cu complexes with GnRH bind to GnRH receptors and influence intracellular signaling (31). Copper ions have been demonstrated to enhance basal and GnRH-stimulated LH production in the pituitary cells of immature female rats in vitro (32).

Zinc is required for sexual development, ovulation, and the menstrual cycle in females (33, 34). Reactive oxygen species (ROS) are important in the processes of oocyte maturation, ovulation, luteolysis, and follicular atresia, and Zn may play a role in these because of its antioxidant qualities (33, 35). More than 80 metalloenzymes involved in DNA transcription and protein synthesis require Zn as a cofactor. Zinc is thought to be required for reproduction because DNA transcription is an important step of egg development. Many observational studies imply that mild Zn deficiency is linked to dwarfism and delayed sexual maturation in adults, as well as decreased growth and mental development in newborns and children (36).

**Conclusion**

In conclusion, our results showed a significantly decreased level of the α-carotene and serum zinc in patients with BC compared to healthy control; also, there is a significant increase in serum levels of copper. Patients in different stages of menstrual cycles show a fluctuation in the serum levels of Zinc and copper. Serum levels of Zinc and copper showed a significant positive correlation in BC patients. α-carotene reversibly correlates with serum levels of Zinc and copper, while it is positively associated with BMI. A combination of genetic and other environmental factors may contribute to BC. The decreased level of the α-carotene and the variance in the levels of copper
and Zinc affect the stages of menstrual cycles and indirectly induce hormone imbalance, which may be a risk factor for BC.

References


