Evaluation of Zinc, Copper, and Lead Levels in The Blood of Breast Cancer Women in Baghdad City

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Abstract
The current study was conducted on 100 females who were divided into two main groups; 60 with breast cancer and 40 healthy controls. Blood samples were collected from both premenopausal and postmenopausal breast cancer and healthy women. The samples were appropriately processed for the analysis of trace elements (zinc, copper, and lead) by using flame atomic absorption spectrophotometry (FAAS). The results showed a highly significant decrease (p < 0.01) in the mean serum level of zinc of in both pre- and postmenopausal breast cancer women (71.7 ± 5.1 and 70.4 ± 5.4 µg/dL, respectively) compared with healthy controls (89.7 ± 10.2 and 97.5 ± 13.2 µg/dL, respectively). Also, a highly significant elevation (p < 0.01) in the mean serum level of copper (157.2 ± 13.9 µg/dL and 157.4 ± 11.9 µg/dL, respectively) was found in pre- and postmenopausal breast cancer women as compared to healthy controls (122.2 ± 15.5 µg/dL and 112.2 ± 15.8 µg/dL, respectively). Furthermore, a highly significant elevation (p < 0.01) in the mean blood level of lead (20.7 ± 2.5 µg/dL and 19.9 ± 1.7 µg/dL, respectively) was found in pre- and postmenopausal breast cancer women compared to healthy controls (15.1 ± 2.0 µg/dL and 14.6 ± 2.3 µg/dL, respectively). It is concluded that the disturbance in the homeostasis of some trace elements (zinc, copper, and lead) may lead to the development and even progression of breast cancer in both pre- and postmenopausal women.

Keywords: Breast cancer, Trace elements, Heavy metals, Flam atomic absorption spectrophotometry, Risk factors.
Breast cancer (BC) is an abnormal breast tissue development in an uncontrolled manner, with the ability to invade normal tissues locally. The primary tumour begins in the breast, but when it becomes invasive, it may possibly metastasize to the lymph nodes at the same region. This tumour could happen in woman and men, but it is more common in women because of their breast growth and exposure to estrogens for lifelong [1]. Breast tumours are highly heterogeneous in their biology, morphology, and responsiveness to treatment [2]. The risk factors for breast cancer involve age, gender (female), previous breast cancer, benign breast disease, and hereditary factors, e.g. family history of breast cancer or other cancers types and women who have \textit{BRCA1} or \textit{BRCA2} mutated genes [3]. Moreover, menarche at early age, menopause at late age, first full-term pregnancy at late age, the use of hormonal therapy, e.g. oral contraceptives, obesity, lack of exercise, diet, smoking, drinking of alcohol, low physical activity, and exposure to high doses of radiation throughout early life, are considered as risk factors for breast cancer [4]. Trace elements are known as micronutritive elements existing in minute quantities of body mass. These elements are divided into essential elements that have numerous functional roles in human body and non-essential elements which have negative effects on living organisms, even at very low concentrations [5]. Heavy metals are defined as moderately dense metals, which have potential toxic effects, mainly in environmental contexts [6]. Heavy metals are found naturally in the earth crust and become concentrated as a result of human activities. Human can be exposed to heavy metals through food, inhalation, and manual handling [7]. An additional source for heavy metals is through skin contact, e.g. contact with soil [8]. The toxic heavy metals are difficult to metabolize and thus can accumulate in the human body [9]. These metals can bind to and interfere with the function of essential cellular components [10]. Heavy metals exert the activity of estrogen, thus causing in increased number of breast cells in the human breast cancer [11]. Hence, the aim of this study is to evaluate the levels of some trace elements (Zn, Cu and, Pb) in the serum of pre- and postmenopausal breast cancer women and healthy controls to show any correlation between these elements and breast cancer.

2. Materials and Methods

This study was conducted at two main medical facilities in Baghdad: The Main Training Center for Early Detection of Breast Tumors / Oncology Teaching Hospital and the Poisoning Consultation Center/ Specialized Surgeries Hospital, during the period from November / 2016 to March / 2017. A total of 100 women were recorded in my study, divided into two groups. The first group included 60 female patients aged 30-60 years. The second group involved 40 healthy women who have normal breast tissue and without previous history of any systemic disease.
2.1 Sample Collection
Blood samples of about 5 mL were collected from each woman. About 2.5 mL of blood was dispensed into plastic tube containing ethylenediaminetetraacetic acid (EDTA) to estimate lead (Pb). The remaining blood was dispensed into plane tube, allowed to clot, and then centrifuged at 3000 rpm for 10 minutes. The serum was transferred into another labelled tube and stored frozen at -20 °C until estimation of trace elements (Zn and Cu).

2.2 Measurement of Trace Elements
Frozen serum was allowed to thaw at room temperature. The assessment of Zn, Cu, and Pb was performed by using the Atomic Absorption Spectrophotometer (FAAS) [12]. The principle of this instrument is based on the separation of the element from its chemical bonds by the flame heat to form unexcited (ground) state atoms. The absorbed amount of radiant energy at the specific wavelength is proportional to the trace element concentration in the sample. The analyte concentration is usually determined by a calibration curve obtained via standards of known concentrations [13].

2.2.1 Preparation of Standards
The preparation of standards and calibration curve should be accurate. The standard solutions should be prepared using high purity compounds and solvents [14]. Working calibration solutions of Zn, Cu, and Pb were prepared from stock solutions, starting from zero concentration and upwards, of at least three concentrations or more, as will be mentioned later. Aspiration of blank was performed to set the baseline to read zero absorbance; this step was replicated frequently to correct baseline drift.

2.2.2 Calculations
The absorbance of standard solutions with a range of concentrations was measured and plotted on a graph to obtain a calibration curve, as shown in Figure 1. The unknown concentration of the element in the sample was then calculated once the absorbance was known.

2.2.3 Measurement of Zinc and Copper
Five concentrations of the working standard (0.0, 50, 100, 150 and 200 µg/dL) of zinc and copper were made for calibration. Serum (0.5) mL was taken from a thawed sample, dispensed into a clean 10 ml tube, and diluted to 5 ml with deionized water (1:10). The diluted serum was mixed well and directly aspirated into the flame atomic absorption spectrophotometer.

2.2.4 Measurement of Lead
Whole blood (2.5) ml was mixed well with 2.5 ml of trichloroacetic acid (TCA) by a wooden stick and centrifuged at 3000 rpm for 10 minutes to remove cellular debris. The supernatant was transferred to a clean plane tube and aspirated directly into the FAAS. The concentration of the working standard (0.0, 0.5, 1.0, and 1.5 µg/dL) was used to plot a standard curve. The optimum working conditions and instrumental setting for the measurements of zinc, copper, and lead are mentioned in the Table 1.

Table 1- Optimum working conditions & instrumental setting for each selected element using Atomic Absorption Spectrophotometer

| Element | Wave length (nm) | Slit width (nm) | Air flow (L/min) | Acetylene flow (L/min) |
|---------|-----------------|----------------|------------------|-----------------------|
| Zn      | 213.9           | 0.7            | 20.75            | 2.75                  |
| Cu      | 324.7           | 0.7            | 20.75            | 2.75                  |
| Pb      | 283.2           | 0.7            | 20.75            | 2.75                  |

2.3 Statistical Analysis
The data were entered on the Statistical Package of Social Science Software program version 18 (SPSS) to be statistically analyzed. All the data were expressed as mean and standard deviation (SD) of the mean. The comparison between groups (study and control groups) was
performed with independent sample t-test for quantitative variables. Pearson correlation coefficients were calculated to test for the significance of the associations between different variables.

3. Results

3.1 Results of serum zinc

The mean of serum zinc concentration in premenopausal and postmenopausal women of the study and control groups was measured and presented in Table 2.

| Groups   | Pre-M (Mean± SD.) µg/dL | Post-M (Mean± SD.) µg/dL | t-test | P-Value | Sig.       |
|----------|-------------------------|--------------------------|--------|---------|------------|
| Control (n=40) | 89.7 ± 10.2                | 97.5 ± 13.2              | 7.742  | 0.000   | P<0.01(HS) |
| Study (n=60)  | 71.7 ± 5.1                 | 70.4 ± 5.4               | 6.902  | 0.000   | P<0.01(HS) |
| t-test       | 6.745                     | 8.585                    |        |         |             |
| P-Value      | 0.000                     | 0.000                    |        |         |             |
| Sig.         | P<0.01(HS)                | P<0.01(HS)               |        |         |             |

Pre-M= Premenopausal, Post-M= Postmenopausal, Sig=Significance, n= sample number, P-value= Probability value, SD= Standard Deviation.

Table 2 presents the distribution of study and control groups according to serum zinc level. The results in the table show that the mean of serum zinc concentration in the premenopausal and postmenopausal study groups (71.7 ± 5.1 and 70.4 ± 5.4 µg/dL, respectively) was highly significantly (p< 0.01) lower than that of the healthy control women (89.7 ± 10.2 and 97.5 ± 13.2 µg/dL, respectively). It also showed a highly significant increase of the mean when premenopause were compared to post menopause patients (p< 0.01). Also, the comparison of mean values between healthy control subjects showed that the premenopause controls had highly significantly lower level than that of postmenopausal controls (p< 0.01).

3.2 Results of serum copper

The mean of serum copper concentration in the premenopausal and postmenopausal study and control groups was measured and shown in Table 3.

| Groups   | Pre-M (Mean± SD.) µg/dL | Post-M (Mean± SD.) µg/dL | t-test | P-Value | Sig.       |
|----------|-------------------------|--------------------------|--------|---------|------------|
| Control (n=40) | 122.2 ± 15.5                | 112.2 ± 15.8             | 10.46  | 0.000   | P<0.01(HS) |
| Study (n=60)  | 157.2 ± 13.9               | 157.4 ± 11.9             | 7.891  | 0.000   | P<0.01(HS) |
| t-test       | 7.025                     | 11.112                   |        |         |             |
| P-Value      | 0.000                     | 0.000                    |        |         |             |
| Sig.         | P<0.01(HS)                | P<0.01(HS)               |        |         |             |

Pre-M = Premenopausal, Post-M = Postmenopausal, Sig=Significance, n= sample number, P-value = Probability value, SD= Standard Deviation.

The results showed that the mean serum copper concentration in the premenopausal and postmenopausal study groups (157.2 ± 13.9 and 157.4 ±11.9 µg/dL, respectively) was significantly higher (p< 0.01) than that of healthy control women (122.2 ± 15.5 and 112.2 ± 15.8 µg/dL, respectively). It also showed a highly significant decrease when premenopause patients were compared to postmenopause patients (p< 0.01). The comparison of mean values between healthy control subjects showed that the premenopause controls had highly significantly higher level than that of postmenopausal controls (p< 0.01).

3.3 Results of blood lead
The concentration of blood lead in the premenopausal and postmenopausal study and control groups was estimated and revealed in Table 4. **Table 4-Comparison of Mean Blood Lead Concentration (µg/dL) among Premenopause and Postmenopause women of Study and Control Groups.**

| Groups      | Pre-M (Mean± SD.) µg/dL | Post-M (Mean± SD.) µg/dL | t-test | P-Value | Sig.       |
|-------------|-------------------------|--------------------------|--------|---------|-----------|
| Control (n=40) | 15.1 ± 2.0             | 14.6 ± 2.3               | 7.494  | 0.000   | *P<0.01(HS)* |
| Study (n=60)   | 20.7 ± 2.5              | 19.9 ± 1.7               | 6.710  | 0.000   | *P<0.01(HS)* |
| t-test        | 8.639                   | 7.452                    |        |         |            |
| P-Value       | 0.000                   | 0.000                    |        |         |            |
| Sig.          | *P<0.01(HS)*            | *P<0.01(HS)*             |        |         |            |

Pre-M= Premenopausal, Post-M= Postmenopausal, Sig=Significance, n= sample number, P-value= Probability value, SD= Standard Deviation

The data demonstrated in Table 4 revealed that the mean of blood lead level in the (premenopausal and postmenopausal study groups (20.7 + 2.5 µg/dL, 19.9 + 1.7 µg/dL, respectively) was significantly higher than that in healthy control groups (15.1 + 2.0 µg/dL, 14.6 + 2.3 µg/dL, respectively) (*p< 0.01*). Also, the mean of blood lead level in premenopausal (study & control) groups (20.7 + 2.5 µg/dL, 15.1 + 2.0 µg/dL, respectively) was significantly higher when compared to the mean in the postmenopausal (study & control) groups (19.9 + 1.7 µg/dL, 14.6 + 2.3 µg/dL, respectively) (*p< 0.01*).

**4. Discussion**

The results listed in Table 2 were compatible with those of a study conducted by Singhal et al. in India. They showed that the level of zinc is significantly lower in breast cancer women as compared to controls [15]. Also, a highly significant decrease in serum zinc in the pre- and postmenopausal breast cancer women was observed by Mohammed [16], which is in agreement with the present study. Zinc is an essential trace element and deficiency of zinc is associated with numerous diseases, including breast cancer. There is some suggestion for an inverse association between zinc and breast cancer [17]. The reason for reduced levels of serum zinc in breast cancer women may be related to the possibility that zinc interferes with copper absorption through the intestine. Therefore, any absorbed copper could replace zinc because copper has a high affinity to metallothionein protein [16]. Furthermore, zinc deficiency may be due to its function in antioxidant defense. Whether this deficiency leads to the disease or happens in defense against the cancerous process is still unclear. However, it is suggested that a deficiency of zinc prompts chromosomal damage [18]. Furthermore, additional factors could be responsible for the hypozincemia in breast cancer women, such as the increased loss of this element via the urine and its increased uptake by cancer cells that utilize it for proliferation [19]. Conversely, El-Deeb et al. found significantly higher serum zinc in the breast cancer groups as compared to the control group. The reason may be related to the variances in sample size and diet habits of subjects enrolled in both studies [20].

Moreover, the results presented in Table 3 are compatible with those of Pavithra et al, who found a statistically significant elevation in serum copper of patients with breast cancer when compared to controls [21]. Also these results are in agreement with those found by Abdul-Mounther, who reported significantly increased serum levels of copper in postmenopausal breast cancer women when compared to controls [16]. Copper is considered as an essential trace element that is necessary for many biological functions, the copper role in biological systems come from its presence in the structure of several enzymes known as cuproenzymes that are essential for normal physiological functions, for example, cytochrome oxidase, lysyl oxidase, superoxide dismutase, ceruloplasmin, and metallothioneins. Elevation of serum copper level has a potential role in breast carcinogenesis due to the generation of the reactive
oxygen species (ROS) through the activation of several organic peroxides. These free radicals induce mutations through damaging DNA [22]. Moreover, the molecular processes of angiogenesis needs copper as an essential co-factor [23]. Furthermore, the low zinc status could raise the levels of serum copper [24]. Low zinc levels may reduce the synthesis of metallothionein, which is an intestinal protein that binds to copper with high affinity and inhibits its absorption [25]. These findings disagree with those of Abdel-Salam et al., who reported that there was no significant difference in the mean of serum copper among controls and breast cancer patients [26]. Furthermore, this study showed significantly higher levels of blood lead in breast cancer women when compared with the healthy controls, which is in agreement with Olusegun et al., who reported the same results [27]. Lead is a toxic metal to several body organs, due to interfering with the DNA binding properties of the zinc-finger regions of transcription factors that could probably cause various consequences [28]. Moreover, lead has genotoxic properties and may lead to the inhibition of DNA repair [29]. The probable reason that explains the elevation of lead levels in the blood of breast cancer women may be their reduced serum zinc levels. The absorption of lead throughout the gastrointestinal tract and the sensitivity to its effects are affected by the adequacy of essential elements. Thus, the deficiency of zinc results in the competition of lead and zinc on the same transport protein (metallothionein) in the intestine, that lead to increasing intestinal absorption of lead in human body [30].

5. Conclusions
The present study suggests that the variation in trace elements metabolism may be related to the development and even progress of breast cancer among women in Baghdad city. This study recommends higher emphasis on the notion that the trace elements should be checked routinely in women with breast cancer. This could be helpful in improving the general health and reducing disease progression. Also, blood specimens could be replaced by hair, nail, and breast tissue specimens for the evaluation of Zn, Cu, and Pb in breast cancer women, especially in cases of chronic exposure to these elements. Moreover, the evaluation of additional elements, such as chromium, selenium, and arsenic in the serum of breast cancer women is recommended.

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8. Conflict of interest: The author declares no conflict of interest.

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