DOI: 10.29409/ijcmg.v17i1.353

Cancer Research

Iraqi Journal of Cancer and Medical Genetics

Evaluating the level of trace elements in Iraqi women with early-stage breast cancer

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Abstract

Breast cancer is one of a group of diseases in which cells in the breast tissue change and divide uncontrollably, causing these cells to clump. Invasive cancers can spread to nearby lymph nodes or other organs (metastasize). Elements are found in different forms in nature, and these elements are very necessary for the performance of the body›s various functions; Elements such as iron and zinc are essential components of enzymes where they attract or subtract molecules and facilitate their conversion to specific end products. Few elements donate or accept electrons during redox reactions. This study aimed to follow the change in the level of trace elements (TEs) and the extent to which these changes are related to the risk of breast cancer before taking chemotherapy or removing the tumor, by drawing 5 ml of venous blood from 120 women (60 patients and 60 control), the serum was separated then used to measure Zn, Cu, Fe, Mg, Cr, and whole blood (2ml) to measure Pb, Cd. In addition to studying the receiver operating characteristic curve (ROC) of these elements. Evaluation of trace elements revealed a significant increase in the levels of Fe, Cu, Pb, and Cd, in contrast to a significant decrease in the levels of Zn and Mg in breast cancer patients compared to a healthy control group. In the current study, it was found that the Cu/Zn ratio was significantly increased in serum from breast cancer patients (equal to 2.2) compared to controls (equal to 1.08); this result could be of great value in the diagnosis and evaluation of breast cancer patients, as well as a good indicator of impaired antioxidant defense in patients. In conclusion, ROC curves revealed that trace elements (Fe, Cu, Cd, Pb) can be used as a biomarker for the diagnosis of breast cancer, and further studies are needed to confirm the relationship between trace elements and breast cancer.

Keywords: *Breast cancer, Cu/Zn ratio, Trace elements (TEs), ROC curves, reactive oxygen species (ROS)*

Introduction

Breast cancer is a disease in which abnormal breast cells grow out of control and form tumors. If left unchecked, tumors can spread throughout the body and become fatal [1]. According to the Iraqi cancer registry, it peaked over a decade from 21.75 to 34.6/100,000 female population in 2010 and 2019, respectively, which represent 32.34% and 34% of all female cancer in the same order [2]. Trace elements (TEs) refer to elements that are found in natural and turbulent environments in small quantities, when present in sufficient bioavailable concentrations, and are toxic to the organism [3]. The four main electrolytes, sodium, magnesium, potassium, and calcium, constitute approximately 1.89%, while the rest 0.02% or 8.6 g of an average human adult is made up of 11 typical TEs, however, this small part exerts a tremendous influence on all functions of the body [4]. Certain TEs control important biological processes by binding to molecules at the receptor site on the cell membrane or by altering the structure of the membrane to prevent the entry of certain molecules into the cell and inducing gene expression that results in the formation of a protein involved in life processes, these elements (Zn), copper (Cu), and iron (Fe) play a vital role in biological and metabolic effects, including inhibition or activation of enzymatic reactions and reactive oxygen species (ROS) that are created in inflamed tissues, such as superoxide anion, hydrogen peroxide, and hydroxyl radical, destroy the construction and function of specific tissues by causing protein denaturation and DNA damage, and enhance tumor development, competition between TEs and metal proteins for binding positions, and modifications in the permeability of cellular membranes which effect cancer events [5]. Elements such as iron, zinc, and selenium are essential components of enzymes, where they attract or subtract molecules and facilitate their conversion to specific end products [6].

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Few elements donate or accept electrons during redox reactions, which results in the generation and utilization of metabolic energy, and has an impact on the structural stability and import of certain biological molecules. Iron is involved in the binding, transporting, and release of oxygen in higher animals [7]. This study aimed to follow the change in the level of TEs and the extent to which these changes are related to the risk of breast cancer before taking chemotherapy or removing the tumor for Zn, Cu, Fe, Pb, Mg, Cr, and Cd.

Methods

The study included 120 women, 60 of them were newly diagnosed with breast cancer and 60 apparently healthy individuals as the control group aged 35-60. This study was carried out at the Oncology Teaching Hospital (Baghdad/ Iraq) between January and December 2021. In order to determine the levels of elements in the serum of the studied groups, the completely controlled atomic absorption spectrophotometer was used. The analytical technique was performed as follows: instrumental and gas flow setting for each element studied was performed as shown in Tables 2-4. Aspiration of zero concentration standards was done to set the baseline to read zero absorbance of the spectrophotometer, and this process was repeated frequently to correct baseline drift. Standard working solutions were analyzed sequentially from the most diluted to the most concentrated solutions. The resulting values were used to establish the working calibration curve. A volume of serum from the patient and control subjects was diluted with deionized distilled water and directly aspirated into the flame. The concentration of the metal in serum was autocalculated and determined directly from the spectrophotometer apparatus itself through the use of the appropriate calibration standard curve that was prepared for each element separately. 5- Finally, to obtain optimal results, diluted hydrochloric acid (HCl) was periodically aspirated to clean the nebulizer system, and the burner head was also cleaned before every run.

Determination of Zinc (Zn) and Copper (Cu)

To prepare a standard calibration curve, the following concentrations of the standard working solutions (0, 50, 100, 150, 200) µg/dl of zinc, iron, and copper were prepared from the dilution of the stock solution with deionized distilled water. Frozen samples from the patients and control were allowed to thaw at room temperature and then gently mixed. 0.5 ml of the samples were diluted 10-fold with deionized distilled water and mixed thoroughly. Then the determination of Zn and Cu was carried out, using atomic absorption spectrophotometry, at (wavelength for Zn = 213.9 and Cu=324.7 nm).

Determination of magnesium (Mg)

For determination of magnesium and in order to avoid the effect of serum phosphate on the results, 25 µl of the samples used were diluted 50-fold with lanthanum chloride ($LaCl2.7H2O$).

Standard working solutions of magnesium were prepared by dilution of the stock solution using deionized distilled water to give the following concentrations (0.0, 3, 6, 9, 12, 15, and 18) µg/dL.

The assay was performed on standard working solutions and diluted samples at wavelength 285.2 nm.

Determination of lead (Pb) and cadmium (Cd) in whole blood samples

The whole blood sample (2 ml) was used for quantitative analysis of Pb and Cd. The following steps were taken to perform this analysis. The blood samples were shaken on an electric shaker for an hour. The blood sample was then vigorously mixed with 5 ml of trichloroacetic acid (TCA) (using a wooden stick). The sample was left to stand for 15 minutes to ensure that all cells and proteins were deposited. The sample was then centrifuged at 4000 rpm for 15 minutes to remove cellular debris. The supernatant was taken and placed in a dry and sterile plastic plain tube for examination with atomic absorption spectrometry. The working standard concentration (0.0, 10, 20, 30) µg/dl and (0.0, 0.5, 1.0, 1.5) µg/dL were used to determine a standard calibration curve for Pb and Cd, respectively. Samples, controls, and standards were directly aspirated into an air acetylene flame for lead. Cadmium was determined in the samples by injecting the samples using a micropipette into the graphite tube. The determination of Pb and Cd has been carried out at wavelengths 287 nm and 228.8 nm, respectively. See Table 1

Table 1. Normal values of the selected trace elements

Trace element	Normal values
Zn	µg/dL 80-150
Cu	µg/dL 80-150
Mg	mg/dL 1.2-2.4
Fe	µg/dL 170 - 50
Cr	µg/dL 0.10-0.20
Pb	µg/dL 0-25
Cd	µg/dL 0-0.3

Determination of iron (Fe)

Transferrin-bound ferric ions in the sample are released by guanidinium and reduced to ferrous by ascorbic acid. Ferrous ions react with ferrozine, forming a colored complex that can be measured by spectrophotometry [8].

Determination of Chromium (Cr)

Standard working solutions for chromium (0.0, 0.5, 1, 1.5, 2, 2.5, 3) µg/dL were prepared from the standard stock solution (1000 ppm) by serial dilution with deionized distilled water. The frozen samples were allowed to thaw at room temperature and gently mixed. 20 µl of the sample was injected into the graphite tube of the GFAAS to perform the analysis. The determination of Cr has been carried out at wavelengths of 357.9 nm. Metal concentration was autocalculated and determined from the appropriate calibration standard curve that was prepared for Cr.

Results

The mean ± SD values for the TEs of breast cancer patients and control groups were calculated using the SPSS program and the collective results are presented in Table 2:

Table 2. Serum levels of elements (Fe, Zn, Cu, Cr, Cd, Mg, and Pb) in breast cancer patients compared to the control group

Parameters	Mean level ± SD of groups		Area under curve	P-value
	Patients	**Control**		
Zn (µg/dl)	70.55 ± 8.87	104.52 ± 12.62	0.006	0.012*
Fe (µg/dl)	179.72 ± 16.0	109.82 ± 25.51	0.977	0.004*
Cu (µg/dl)	155.37 ± 10.39	113.70 ± 12.0	0.997	0.001*
Pb (µg/dl)	25.22 ± 3.72	14.43 ± 2.99	0.992	0.001*
Cd (µg/dl)	0.30 ± 0.09	0.16 ± 0.06	0.910	0.001*
Cr (µg/dl)	0.16 ± 0.06	0.15 ± 0.04	0.495	0.231 NS
Mg (mg/dl)	1.33 ± 0.30	1.77 ± 1.30	0.240	0.013*

* Significant at ($P < 0.01$), non-significant at ($P > 0.05$).

Receiver operating characteristic curve:

Receiver operating characteristic (ROC) curves are frequently used to show the connection between clinical sensitivity and specificity for every possible cutoff point for a test or a combination of tests. In addition, the area under the ROC curve gives an idea of the benefit of using the test(s) in question. The curve ROC study was carried out to explore whether TEs could be used to diagnose breast cancer. As shown in Table 3. Fig. (1,2,3,4) shows the receiver operating characteristic curve (ROC) for (Fe, Cu, Cd and Pb), respectively.

Table 3. The values of the area under the curve AUC, sensitivity, specificity, and cut-off value

	AUC	**Sensitivity**	**Specificity**	**Cut-off point**
Fe	0.977	100	92	145
Cu	0.997	98	93	132
Cd	0.910	87	77	0.2
Pb	0.992	98	95	19

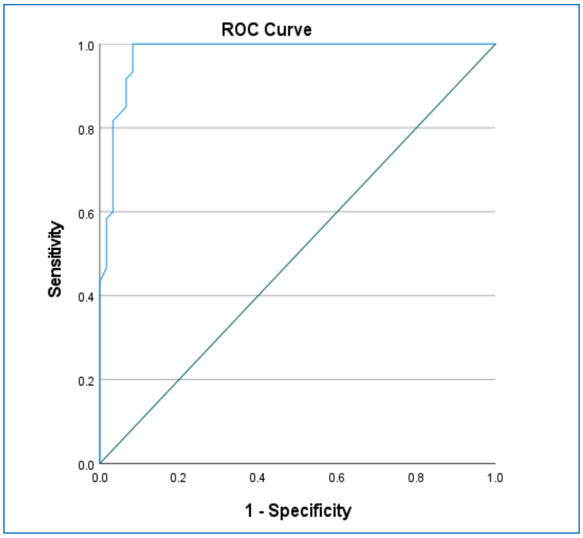

Figure 1. Receiver operating characteristic curve for (Fe)

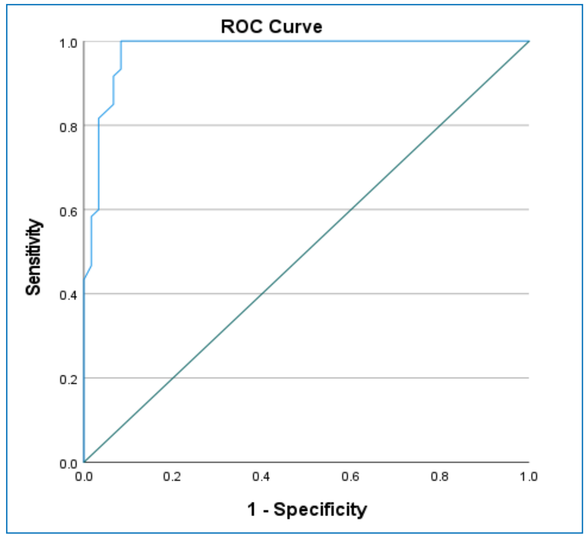

Figure 2. Receiver operating characteristic curve for (Cu)

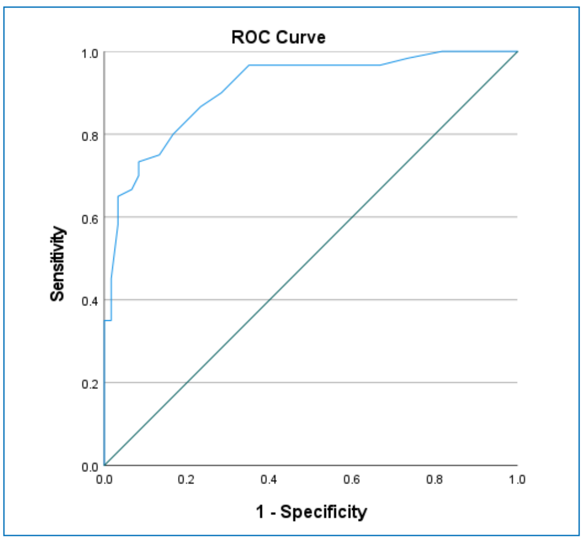

Figure3. Receiver operating characteristic curve for (Cd)

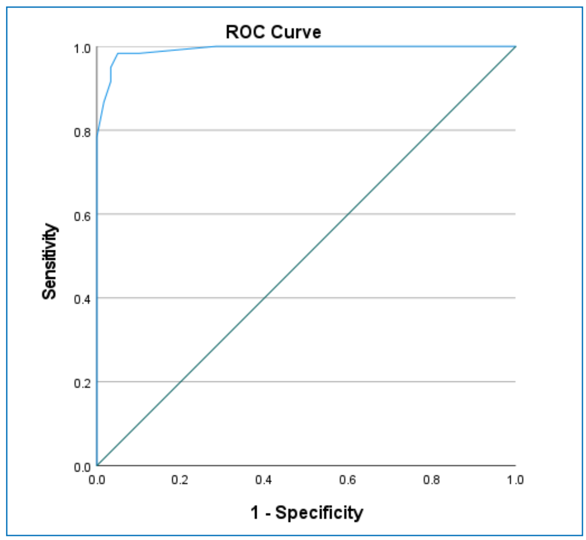

Figure 4. Receiver operating characteristic curve for (Pb)

Discussion

In this research, the levels of seven TEs in human serum were calculated using an atomic absorption spectrophotometer in female patients suffering from breast cancer and healthy people as controls in Iraq. In this study, the patient group had an increase in the values of Cu, Fe, Pb, and Cd, while there was a significant decrease in Zn and Mg ($P<0.01$), as for chromium, it did not give any statistically significant differences ($P>0.05$). The Cu/Zn ratio in serum samples has been used in some studies as one of the prognostic assessment methods in cancer patients. In the current study, it was found that the Cu/Zn ratio was significantly increased in the serum of breast cancer patients (equal to 2.2) compared to controls (equal to 1.08). Data suggest that the Cu/Zn ratio is a good indicator of impaired antioxidant defense in the patient, which are structural ions of superoxide dismutase (SOD) [9]. Zinc is an essential element and a cofactor for more than 300 enzymes and is required for the growth and maintenance of the human body [10]. As an activator of many enzymes involved in the synthesis of DNA and RNA, in addition, zinc can directly prevent the development of DNA gaps and gene mutations and therefore reduce the risk of cancer, therefore, a reduction in serum zinc level can lead to various types of cancers (specify) by decreasing protective effects and increasing antioxidant effects [11].

Iron has been shown to promote breast carcinogenesis in animal models through the generation of oxidative stress and interaction with estrogen. Iron can enhance the production of oxygen radicals that are harmful to DNA, leading to carcinogenic mutations [12], [13]. Cancer cells have a higher demand for copper compared to non-dividing cells, which is an Achilles' heel that can be exploited through the use of oral copper chelation to suppress tumor growth and metastasis in animal cancer models and human patients [14]. Biological processes in which copper has been linked to cancer include mitochondrial respiration, collagen crosslinking, immune system modulation, antioxidant defense, mitogenic signaling, and autophagy [15]. The International Agency for Research on Cancer (IARC) has classified lead as probably carcinogenic to humans, based on associations with cancers of the stomach, brain, kidney, and lungs. Lead might facilitate the carcinogenetic effects of other exposures by impairing DNA repair, Silbergeld suggests that lead may be a facilitative or permissive carcinogen which means that lead may permit or augment the genotoxic effects of other exposures [16]. Cadmium and the bivalent cationic metals activate the estrogen receptor-α, cadmium functions as an estrogen, and the potential role of cadmium in breast cancer [17]. Cd inhibits DNA synthesis, mismatch repair, and enzyme function [18]. It has been confirmed that exposure to Cr(VI) increased lung, bladder, and pancreatic cancer mortality among tanners and found that Cr(VI) increased mortality due to myeloid leukemia and tumors of the endocrine glands. Great progress has been made in identifying the correlation between exposure to Cr(VI) and some respiratory cancers [19]. In postmenopausal women, serum magnesium (Mg) plays an important role in skeletal health, cell proliferation, and cancer. Mg is essential for DNA duplication and repair, and Mg deficiency favors DNA mutations leading to carcinogenesis [20]. A direct negative association and an indirect association were observed by influencing the level of CRP between dietary magnesium intake and breast cancer risk [21].

In this research the area under the curve (AUC) for (Fe, Cu, Cd, and Pb) was found to be (0.977, 0.997, 0.910, and 0.992) respectively, Sensitivity: (100, 98, 87, and 98) and Specificity: (92, 93, 77 and 95) respectively and the cutoff value is 145, 132, 0.2 and 19 µg/dl respectively. These results indicate that Fe, Cu, Cd, and Pb can be trusted as good markers for the diagnosis and monitoring of breast cancer, whenever the AUC result greater than 0.8 to 0.99 is considered an excellent discrimination. For the rest of the measurements, they did not give good ROC curves because the AUC between 0.6-0.5 is poor discrimination.

Conclusions

It was found that TEs have an essential and decisive effect in causing some diseases. This study provides evidence of a significant difference in Fe, Zn, Cu, Mg, Cd, and Pb levels in breast cancer patients compared to healthy people with significantly higher levels ($P < 0.01$). Based on these findings, it is possible to conclude that high levels of Fe, Cu, Cd, and Pb and low levels of Zn and Mg may play a critical and protective role in the occurrence and progression of the disease. The Cu/Zn ratio was calculated and found to increase significantly in patients with breast cancer compared to controls. This result suggests that it could be of great value in the diagnosis and evaluation of breast cancer patients, as well as as a good indicator of impaired antioxidant defense in patients. The ROC results showed that elements (Fe, Cu, Cd, Pb) can be adopted as a biomarker in the diagnosis of breast cancer according to the values of sensitivity, specificity, and AUC.

Acknowledgments

The authors express sincere gratitude to the Iraqi Ministry of Health / Department of Medical City / Oncology Teaching Hospital for contributing to the collection of samples and to Ghazi Hariri Hospital / Poison Center.

Ethical Statement

The ethics clearance was obtained from the Ministry of Health of the Iraqi Government number 1609 at 23 Oct. 2022. Study participants were informed about the study in Arabic, including the purpose of the study. Only those who agreed and signed the informed consent were included. PI ensured participants confidentiality and anonymity, as well as no risk or harm involved.

Author contribution

Each of the researchers (Professor Falah S. Al-Fartusie, Professor Assistant Dheaa Sh. Zgeer) proposed the research idea and developed the necessary work plan. The researcher (Assistant teacher Shahad A. Jarallah) collected samples, analyzed them, interpreted the statistics, and wrote the research under the supervision and follow-up of (Professor Falah S. Al-Fartusie, Professor Assistant Dheaa Sh. Zgeer).

Conflicts of interest

The authors declare no conflict of interest.

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