Atomic Spectroscopy

Article

Determination of Trace Element Concentrations in Blood Samples and Their Correlation to Breast Cancer

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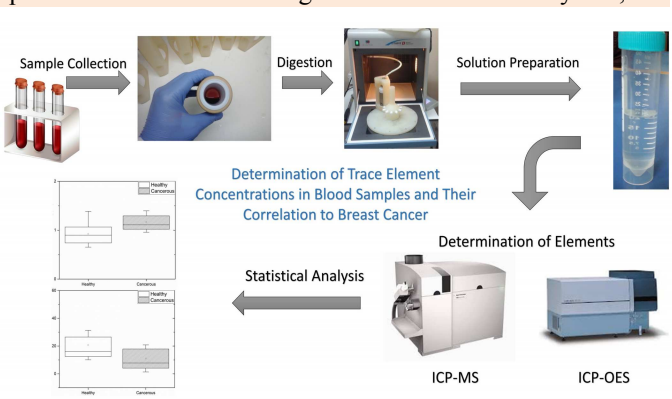
ABSTRACT: The main purpose of this study was to investigate the possible differences in the concentrations of Al, Cu, Ca, Mg, Fe, Ni, and Pb in breast cancer patients and healthy control group. One blood sample each was collected from 40 adult female patients diagnosed with breast cancer and 40 healthy adult female individuals with no medical history of cancer. The concentrations of the elements Al, Cu, Fe, Ni, and Pb were determined by ICP-MS, while Ca and Mg were determined by ICP-OES. Statistical analysis was performed with the Kolmogorov-Smirnov normality test, the Mann-Whitney U test, and the Spearman's rank correlation test. It was observed that the concentrations of Cu and Mg in the breast cancer patients were significantly higher ($p=0.000<0.05$ and $p=0.001<0.05$, respectively) than for the healthy group, while the Al concentration was significantly lower ($p=0.002<0.05$) in the cancer patients. The Mg concentration in the control group and the patients was 33.60±4.51 and 38.10±9.69 mg/kg, respectively. The difference was statistically significant for Cu, Mg, and Al, whereas no difference was observed between the concentrations of Ca and Fe. Furthermore, a positive correlation was observed in breast cancer patients between the concentrations of (Al-Cu), (Al-Ca), (Fe-Mg), and (Cu-Ca).



INTRODUCTION

In cell life, several genetic and epigenetic changes occur that transform normal cells into cancerous cells. Generally, cancerous cells proliferate uncontrollably and, therefore, can invade other regions in the body. According to GLOBOCAN, cancer is the major cause of mortality for the world's population, with about 18.1 million new records and 9.6 million deaths reported in 2018.[1] In the breast, there are lobules bonded to each other *via* milk ducts and join together towards the nipple.[2] Breast cancer is considered the most common type of cancer and the main cause of death worldwide with 2.1 million estimated cases and 626, 679 deaths for 2018.[1] Recently, much attention has been paid to the

inherited mutations of the two breast cancer genes of BRCA1 and BRCA2. These mutations highly augment the risk of breast cancer, especially among families with a history of either breast or ovarian cancer.[3] They can also be an increased risk of developing breast cancer due to exposure to environmental chemicals, radiation, lifestyle, high dietary intake of fat, and alteration in hormonal levels.[4]

It has been well established that trace elements are of great importance for physicochemical, biological, and physiological processes in the human body. However, some of these elements can cause diseases.[5–7] Many studies addressed the relation between different trace elements and various cancers, such as lung cancer, breast cancer, stomach cancer, respiratory tract cancer, urinary system cancer, skin cancer, central nervous system tumors, and prostate cancer. It has also been found that a deficiency or excess of the elements chromium (Cr), iron (Fe), arsenic (As), zinc (Zn), copper (Cu), nickel (Ni), vanadium (V), beryllium (Be), lead (Pb), cadmium (Cd), and cobalt (Co) is associated with different cancer types in humans.[8–10]

In a study by Kuo *et al.* with 94 participants (25 malignant, 43 benign, and 26 control), the Fe levels in patients with malignant tissues were higher compared to the healthy group.[11] In another study performed by Ismail *et al.* with randomly selected 80 female participants (40 healthy, 40 cancerous), the Fe serum levels of the breast cancer patients were higher than for the control group.[12] Another study found that cancer patients suffer from Mg deficiency, while there is less concern for healthy individuals with low Mg.[13] Ragab and colleagues[14] showed that the lead concentrations of the individuals in the breast cancer group were higher than for the healthy group. Topdağı *et al.* [15] reported a significant positive correlation (p=0.045) between the Na/K ratio and electron density (depending on the concentration of the elements) in breast cancer patients. They claimed that the increase in element concentration might be attributed to the presence of breast cancer. In addition, Toker *et al.*[16] showed that the Se and Cr concentrations were significantly lower for the breast cancer patients, while the Na concentrations were significantly higher for the healthy group. All of the above reports indicate that there is a strong correlation between the excess or lack of certain elements in cancer patients, which requires sincere and continuous study.

The elements Al, Cu, Ca, Mg, Fe, Ni, and Pb studied in this report could possibly become parameters for cancer diagnosis or treatment. Blood samples were taken from breast cancer patients and a healthy group for comparison. Elements of Al, Cu, Fe, Ni, and Pb were determined by ICP-MS, and Ca and Mg by ICP-OES. In addition, the correlation between element pairs was also investigated for the breast cancer patients. To the best of our knowledge, no study has been reported in the literature where all of these elements were investigated for possible correlation to causes of breast cancer.

EXPERIMENTAL

All of the analytical steps followed in the present work involving sample collection and element measurement are summarized in Fig. 1.

Sample collection. In this study of 80 female participants, 5.0 mL of blood was collected from each participant and placed into clean tube. The group consisted of 40 patients diagnosed with breast cancer (receiving no treatment) and 40 healthy females with no medical history of cancer.

Sample preparation, digestion, and dilution. Each blood sample was digested by adding 2.0 mL H_2O_2 (30%, Merck) and 6.0 mL HNO_3 (65%, Merck), then placed into a microwave oven (Milestone Start D with an industrial magnetron) to break down the sample matrix prior to analysis. The blank solution was similarly prepared, and all of the acids had identical volume.

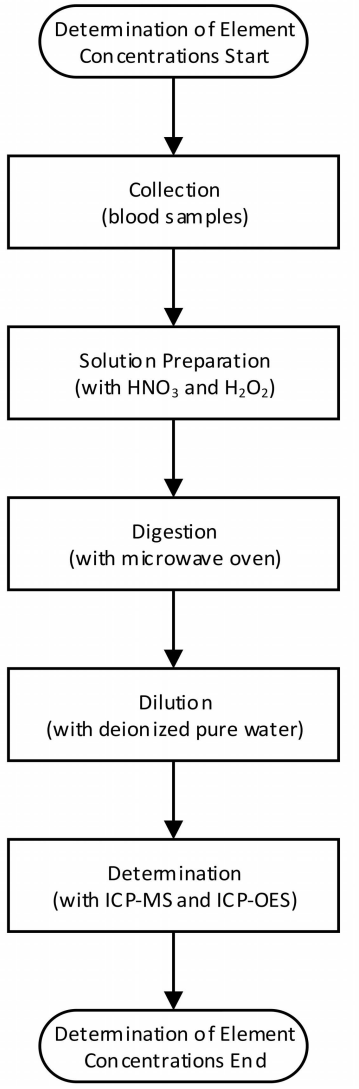


Fig. 1 Procedure for element determination.

Table 1. Operation conditions of ICP-MS [16]

ICP-MS		ICP-OES	
RF Power (W)	1550	**RF Power (W)**	1200
RF Matching (V)	1.80	**Plasma Gas (L min^{-1})**	10
Sample Depth (mm)	8.0	**Auxiliary Gas (L min^{-1})**	0.60
Carrier Gas (L min^{-1})	1.07	**Carrier Gas (L min^{-1})**	0.70
Nebulizer Pump (rps)	0.10	**Exposure Time (s)**	30
S/C TEMP (^{o}C)	2.0	**PPR Speed (rpm)**	20/60

The microwave temperature and time settings were optimized for digestion of the blood samples and are listed as follows: the temperature increases from room temperature to 100 ˚C within 10 min; hold at 100 ˚C for 10 min; increase 100 ˚C to 180 ˚C within 10 min; hold at 180 ˚C for 15 min. Deionized water (Milli-Q® purification system, Millipore Corporation, USA) was used for the diluting, rinsing, and cleaning procedures. After digestion, all solutions were completed to 25 mL volume with deionized water and analyzed by ICP-OES or ICP-MS.

Sample analysis. The samples were analyzed using an Agilent 7700 ICP-MS (Agilent Technologies, USA) and a ICPE-9000 ICP-OES (Shimadzu, Japan). The working parameters of ICP-MS and ICP-OES are listed in Table 1. 80 participants were randomly selected from patients of the Medical Oncology Department at Atatürk Medical Faculty. A signed consent form was obtained from each participant in accordance with the research approval of the Ethics Committee of the Faculty of Medicine, Atatürk University. Of the cancer patients who enrolled in the present study, 35 suffered from invasive ductal carcinoma, and 5 were diagnosed with invasive lobular carcinoma.

Statistical analysis. When the two groups are considered, the test procedures required to explain statistically

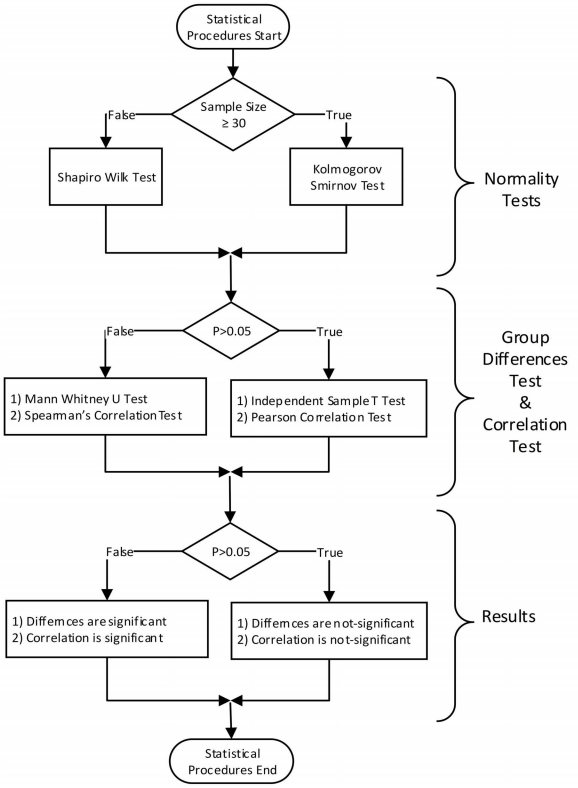


Fig. 2 Statistical procedure flowchart.

the group differences and the correlation tests are shown in Fig. 2. The Kolmogorov-Smirnov test was performed first to explore the normality of the data. Accordingly, the Mann-Whitney U test with a 95% confidence level was applied to compare the means of the corresponding groups. The Spearman's test was carried out to establish a significant correlation between the elements determined in the cancer patients. Finally, the significance of the results was interpreted according to the p values obtained from the group differences and correlation tests.

RESULTS AND DISCUSSION

Some researchers have established that there is a relationship between antioxidative vitamins and trace elements for the prediction of acute myocardial infarction and stroke.[17] Thus, the concentration of trace elements is relevant to the body's antioxidant enzyme activities. Studies show that a small difference in the proper level of trace elements inside the tissue causes particular changes in the metabolism and enforces the probability that a disease could develop.[18,19] It was reported that magnesium deficiency is common with various cancers,[13] hence, the accurate determination of trace elements in the biological systems is very crucial. In this study, the concentrations of Al, Cu, Ca, Mg, Fe, Ni, and Pb were determined by ICP-OES and ICP-MS for both the breast cancer patients and the control group. The accuracy of the applied method was verified using certified reference, material (CRM) NIST 1515 Apple Leaves.

Table 2. Analytical results of CRM NIST 1515 (mg kg^{-1})

Elements	NIST 1515	Measured
Al	286±9	266.34±7.45
Cu	5.64±0.24	5.21±0.06
Ni	0.91±0.12	0.88±0.02
Pb	0.47±0.02	0.48±0.01

The experimental results are in agreed well with the certified values (Table 2).

The generated findings showed that the Al concentrations significantly decreased ($p=0.002<0.05$) in the breast cancer patients (Fig. 3a). The concentration of Cu increased significantly ($p=0.000<0.05$) in the breast cancer patients in comparison to the healthy group (Fig. 3b). In addition, the Mg concentration for the breast cancer patients was significantly higher ($p=0.001<0.05$) than for the healthy group (Fig. 3c). There was no significant difference in the Ca and Fe concentrations ($p=0.810>0.05$, $p=0.419>0.05$, respectively) of the breast cancer patients and the healthy group (Table 3). The correlation coefficients between the elements using the Spearman's test are given in Table 4 with the associated p values. A positive correlation coefficient was observed between the elements Al, Cu, Ca, Fe, and Mg. According to the Spearman's correlation test, there were also positive correlations between the concentrations of the element pairs of Al-Cu, Al-Ca, Fe-Mg, and Cu-Ca in the breast cancer patients. In other words, the concentrations tend to change simultaneously for these pairs of trace element where a positive correlation was evident. With respect to the correlation coefficients (ρ), the two

Table 3. Results of metal concentration in the patient and control samples

Element (ng g^{-1})	Group	Mean±Std. Dev.	Kolmogorov-Smirnov result	Test Procedure	Test Value	p-value	State of H_0 Hypothesis	Decision (Differences)
Al	Control	19.15±11.63	0.005	Mann-Whitney U	462.5	0.002*	Rejected	*Significant*
	Patient	11.10±9.73	0.006					
Cu	Control	0.93±0.21	0.200*		362	0.000*	Rejected	*Significant*
	Patient	1.17±0.31	0.000					
Ca	Control	100.29±24.40	0.048		775	0.810	Not rejected	Not significant
	Patient	99.01±31.19	0.200*					
Mg	Control	33.60±4.51	0.005		466.5	0.001*	Rejected	*Significant*
	Patient	38.10±9.69	0.031					
Fe	Control	352.41±74.54	0.200*		716	0.419	Not rejected	Not significant
	Patient	341.04±115.14	0.000					

* The significant test result means non-normal distributed group in Kolmogorov-Smirnov test and significant difference in Mann-Whitney U test.

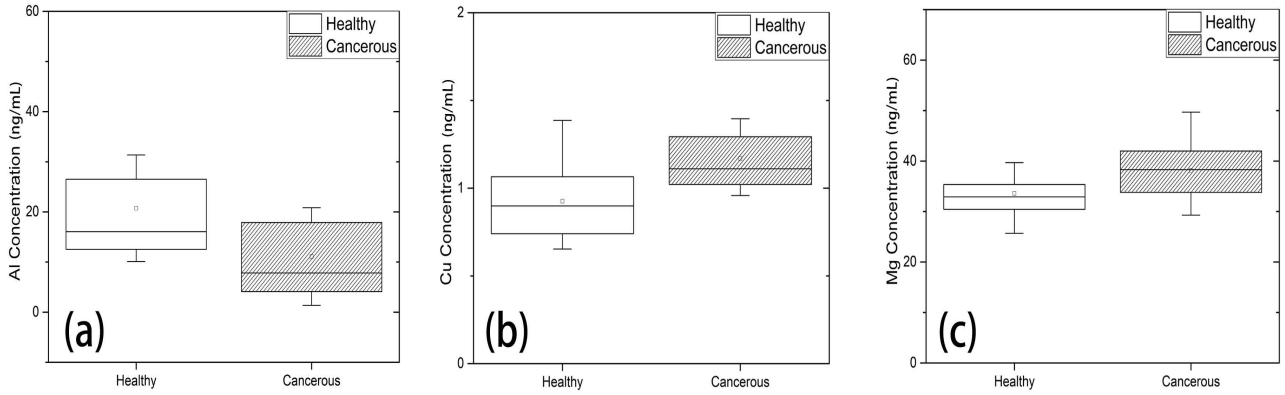


Fig. 3 Boxplot of metal concentration (Al, a; Cu, b; Mg, c) in blood samples of breast cancer patients compared to healthy group.

strongest relations were found between Fe-Mg and Al-Ca ($\rho_{Fe\&Mg}$=0.516 and $\rho_{Al\&Ca}$=0.490).

The relationship between some health problems and element concentrations has been studied for a long time. Ragab and colleagues found relevance with regard to the Al concentration in cancer patients.[14] Some studies suggest that a change in the Cu concentration in breast cancer patients might be an indicator for developing breast cancer.[9,10] This imbalance may also disturb the vital role of Cu as an essential trace element for maintaining the strength of the skin, blood vessels, in addition to its prime role in the production of hemoglobin, myelin, melanin, and the normal functioning of the thyroid gland. In addition, a slow reduction of Cu concentration in a patient may be used as an indicator for serious health problems.[20,21] In this study, significant differences in the concentration of Al, Mg, and Cu between the breast cancer and the healthy group were observed. For breast cancer patients, the Al concentration decreased (p=0.002<0.05), whereas the concentration of Mg and Cu significantly increased compared to the healthy group (p=0.001<0.05 and p=0.000 <0.05, respectively). The Mg level was significantly different in breast cancer patients. Mg influences various functions in the human body such as activating several enzymes, and participates in many metabolic processes such as DNA repair and electrolyte transport across cell membranes.[22] The key role in DNA repair can explain the change in the Mg concentration for breast cancer patients. Therefore, it can be concluded that a significant increase in Mg levels can be used as a marker to indicate the development of breast cancer that is one of the most important findings of this study that should be taken into consideration.

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Table 4. Correlation test results

			Al	Fe	Cu	Ca	Mg
Spearman's rho	Al	Cor. Coe.[a]	1,000				
		Sig. (2-tailed)	.				
		N	40				
	Fe	Cor. Coe.	-,226	1,000			
		Sig. (2-tailed)	0,162	.			
		N	40	40			
	Cu	Cor. Coe.	,410[b]	-,236	1,000		
		Sig. (2-tailed)	,009	,143	.		
		N	40	40	40		
	Ca	Cor. Coe.	,490[b]	,081	,408[b]	1,000	
		Sig. (2-tailed)	,001	,618	,009	.	
		N	40	40	40	40	
	Mg	Cor. Coe.	-,228	,516[b]	-,194	,305	1,000
		Sig. (2-tailed)	,158	,001	,229	,056	.
		N	40	40	40	40	40

[a] Correlation Coefficient; [b] Correlation is significant (2-tailed).

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CONCLUSIONS

This study was performed to highlight the differences in the concentrations of Al, Cu, Mg, Ca, Fe, Ni, and Pb in breast cancer patients *vs.* a healthy control group, while the concentration levels of Ni and Pb could not be evaluated because they were below the detection limits. However, a positive correlation was demonstrated between the concentrations of the element pairs of Al-Cu, Al-Ca, Fe-Mg, and Cu-Ca in the breast cancer patients via the Spearman's correlation test. Significant differences were also found in the concentrations of Al (lower in the breast cancer group), Cu and Mg (higher in the breast cancer group) than for the healthy group. On the basis of the present study, it can be stipulated that when the vital organs fail to function properly, improper concentration levels of trace elements can be found in the circulating blood and should be evaluated, because they might lead to a promising and early prognostic indication of cancer. Doubtless, further studies are still required to establish the relevance of trace element concentrations in cancer diseases. However, it is a first step towards the ability to early cancer diagnosis, thus preventing cancer from progressing by monitoring the trace element concentrations in the body.

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Ethical Approval

Atatürk University Medical Faculty ethics committee; 10.24.2016, session 6, number: 22.

Notes

The authors declare no competing financial interest.

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