Original Research Article

Determination of Red blood cells Trace Elements as Risk Factors for Breast Cancer by X-Ray Fluorescence

Abstract

Introduction: Breast cancer is a heterogeneous disease that cause women death. Several factors influence the development of breast cancer. Trace elements are associated with breast cancer risk factors. This study investigated six trace elements in red blood cells of women newly diagnosed with breast cancer, compared to healthy women. **Methods:** 38 Sudanese women participated in this study; 19 of them were breast cancer patients, who were compared with 19 healthy women of the same age to determine the concentrations of K, Zn, Cu, Fe, Ca, and Pb in red blood cells by X-ray fluorescence. **Results:** K concentration was significantly increased in breast cancer patients compared to the control group (P= < 0.001). Zn and Cu were slightly decreased in patients compared to the control group. In contrast, insignificant increase was observed for Fe, Ca and Pb in breast cancer patients compared to the control group. Correlations between Fe and Cu, K and Pb were found in breast cancer patients. **Conclusion:** High K concentration in red blood cells is associated with breast cancer risk in Sudanese women. K can be used as a biomarker for breast cancer prognosis and development.

Keywords: Red Blood cells, Trace elements, Risk factors, Breast cancer

1. Introduction

Breast cancer is a common disease in women and causes deaths worldwide [1]. Due to the heterogeneity of the disease, several factors affected the development of breast cancer. Risk factors include genetic, and environmental [2], exposure to ionizing radiation, dietary habits, alcohol consumption, body mass index [3], and ethnicity [4] that influence the incidence of breast cancer. Trace elements are micronutrients that are essential for biological processes. These include metabolism of genetic material for growth and differentiation, programmed cell death and necrosis, protection from oxidative damage, and anti-inflammatory and anti-carcinogenic effects [5]. Trace elements have been associated with the pathogenesis of breast cancer [6].

Several studies have found that trace elements levels change in patients with breast cancer, but their role has not been fully elucidated, and they may be associated with the risk of this disease [7]. The determination of elements in red blood cells (RBCs) is helpful for the status of essential elements, with important intracellular functions, such as potassium (K), calcium (Ca), iron (Fe), copper (Cu), and zinc (Zn). In addition, the analysis of erythrocyte elements is crucial for the evaluation of exposure to toxic metals such as arsenic (As), cadmium (Cd), and lead (Pb), which preferentially accumulate in erythrocytes [8]. Deficiency or excess of these essential elements affects numerous metabolic processes [9]. For example, Cu plays a crucial role in the production of hemoglobin; it acts as a coenzyme required for the binding of iron to its transport protein -transfer -, so iron deficiency anemia may occur due to copper deficiency [10]. Zinc plays an essential role in cellular metabolism [11].

X-ray fluorescence (XRF) is a representative multi-element technique for the analysis of trace elements [12]. The X-ray fluorescence technique is based on the excitation of atoms in a material by an X-ray beam of suitable energy, and subsequent detection of the emitted characteristic radiation, which is proportional to the concentration of particles in the material [13,14]. XRF is a rapid and straightforward analytical technique, for many samples with high sensitivity and low detection limits, allowing the determination of trace element concentrations [15,16]. The aim of this study was to determine some trace elements including; copper (Cu), zinc (Zn), iron (Fe), potassium (K), calcium (Ca) and lead (Pb) in the red blood cells of newly diagnosed breast cancer patients compared to a healthy control group.

2.Materials and Methods

2.1. Study population:

A total of 38 Sudanese women participated in this study. 19 of them, were newly diagnosed breast cancer patients who visited the Radiation and Isotope Center – Khartoum for initiation of therapy, and were randomly selected, and compared with 19 healthy women.

2.2. Sample collection:

Venous blood samples were collected from both groups in EDTA vacutainer tubes, and then centrifuged at 3000 g for 10 minutes. The supernatant plasma was aspirated into Eppendorf tubes using a Pasteur pipette. Red blood cells were washed with phosphate buffer saline, and stored in cryotubes at -80°C until analysis.

2.3. Preparation of samples for analysis

Freeze-drying was used to prepare samples for red blood cell analysis. Samples were frozen at -20°C for 2-4 days, and the Lyotrap was run until the temperature reached -50°C. Then, the samples were placed in the condensation chamber for 48 hours until the sublimation process was completed (freeze dryer. Lyotrap, series, K12173-5. United Kingdom). The samples obtained in powder form were also distributed and mixed homogeneously. Then, each sample was accurately weighed and the weighed amount was transferred to a special cup, and then placed in the sample holder to perform the elemental analysis.

2.4. X-ray fluorescence measurements

The dried blood samples were analyzed using the X-ray fluorescence instrument in the Laboratory for Nuclear Applications, located in the Department of Physics, College of Khartoum, and jointly operated by the College of Khartoum, and the Sudan Atomic Energy Commission. (Canberra model number 7013-04755 and serial number 10-3499), and a separate multichannel analyzer (MCA) (Series 35-Canberra). The energy resolution of the detector was 190 eV at 5.8 KeV of Mg. The X-ray spectra were analyzed using a computer program called Analysis of X-ray Spectra by Iterative Least-Square fitting (AXIL). This is a Fortran program developed by Van Espen for deconvolving complex X-ray spectra with a Si (Li) detector (XRF manual). Each sample was analyzed for 1000 seconds. Analysis of 38 blood samples or the International Atomic Energy Agency (IAEA) standard reference material - animal blood (A-13) - was performed using an energy dispersive XRF (EDX) analysis system.

2.5. Statistical analysis:

Data was analyzed using SPSS Statistics version 22.0 software. T-test was used to compare mean element concentrations, and a Pearson correlation was performed. The P-value < 0.05 was considered statistically significant.

3. Results:

The median age of the patients was 38 years, the age range was (26-53 years), with different tumor stages. No significant difference was found between the patients and the control group in trace element levels, except that potassium levels were significantly increased in the breast cancer patients compared with the control group ($P < 0.001$). An insignificant decrease was observed for Zn and Cu in the patients, compared with the control group. In contrast, Fe, Pb, and Ca levels were not significantly increased in breast cancer patients compared with the control group (see Table 1). Figure 1 shows the mean K values in the study groups.

A strong positive correlation was observed between Fe and Cu (r=0.6, P=0.002). An inverse correlation was observed between K and Pb (r=0.8, P= < 0.001), as explained in Figure 2 and 3, respectively.

Table1: Comparison of red blood cells levels of trace elements between breast cancer patients and control group

Elements	Patients	Control	P. Value
	Mean ± STD (Ppm)	Mean ±STD (Ppm)	
Cu	3.0 ± 0.9	3.8±2.8	0.2
Zn	3.5 ± 1.1	4.3±2.8	0.2
Fe	2426 ± 685	1776±1441	0.07
Pb	0.9 ± 1.5	0.57 ± 1.5	0.3
Ca	49.8 ± 27.3	38.3 ± 36.3	0.2
K	6087 ± 2184	2625 ± 1311	< 0.001

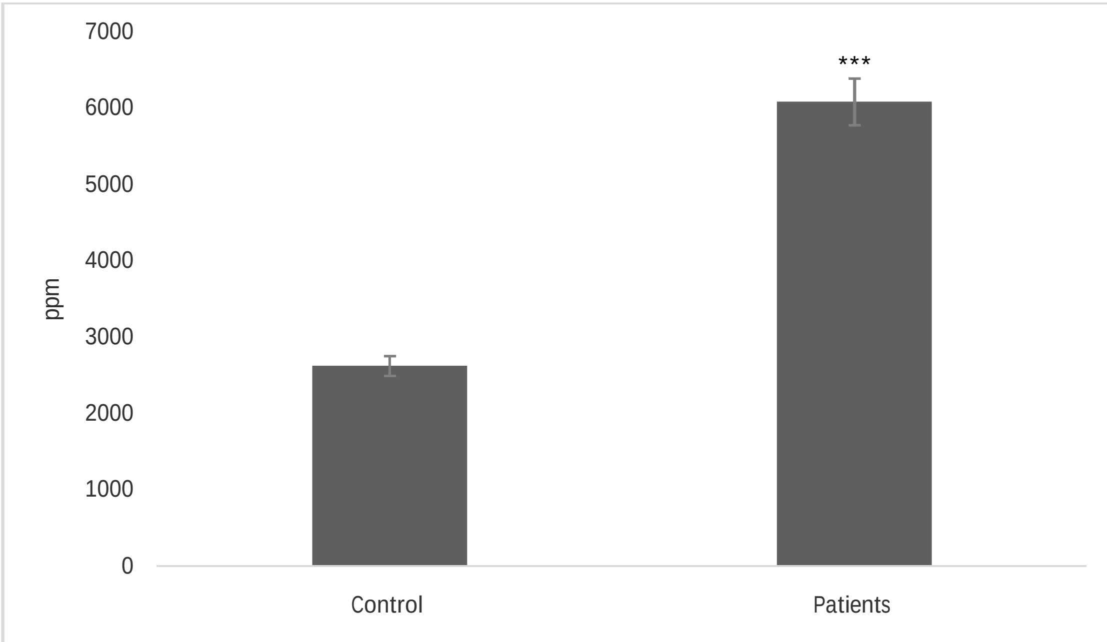

Figure1: Mean of K concentrations for study groups (P =< 0.001)

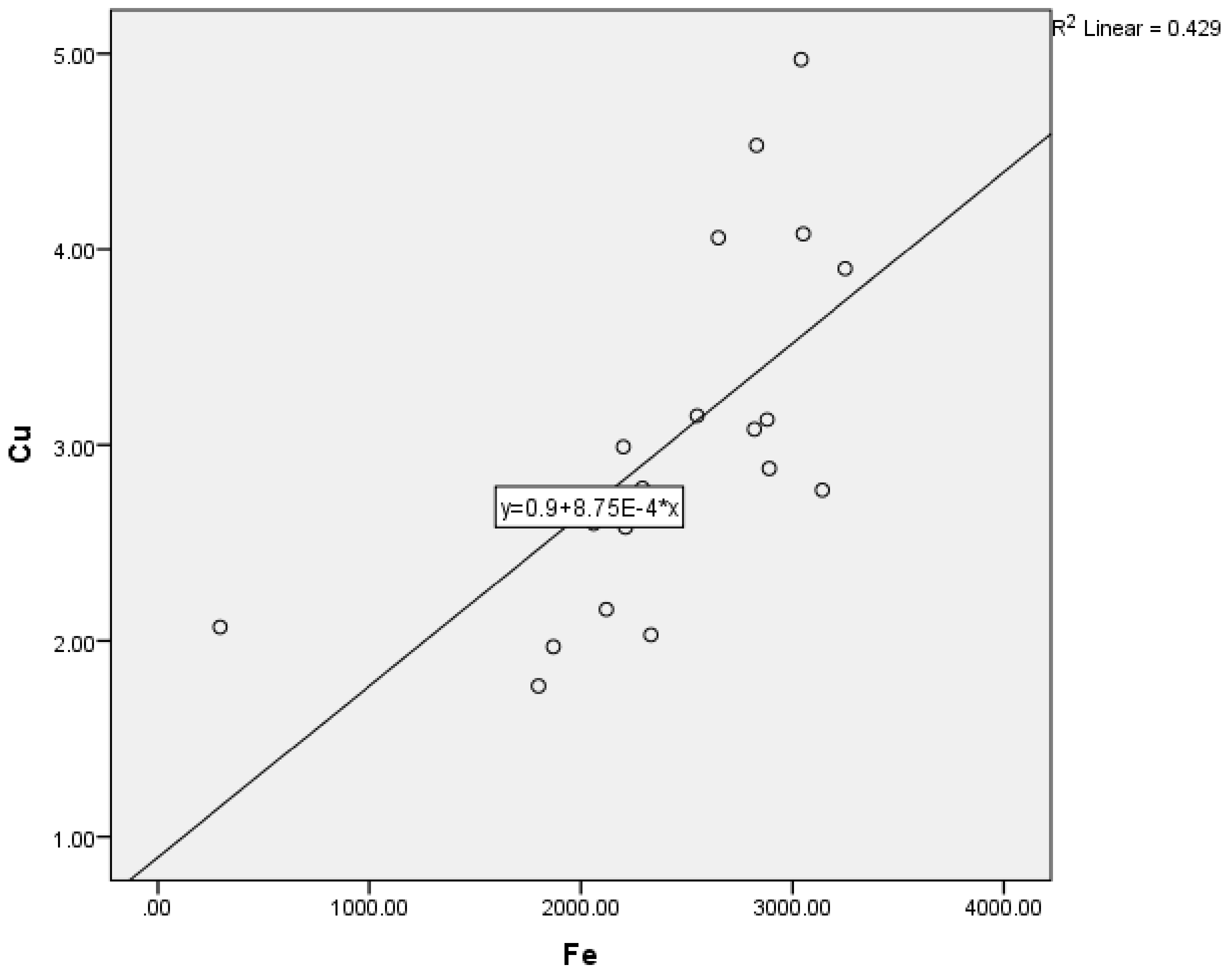

Figure 2: Correlation between Fe and Cu in breast cancer patients (r=0.6, P = 0.002)

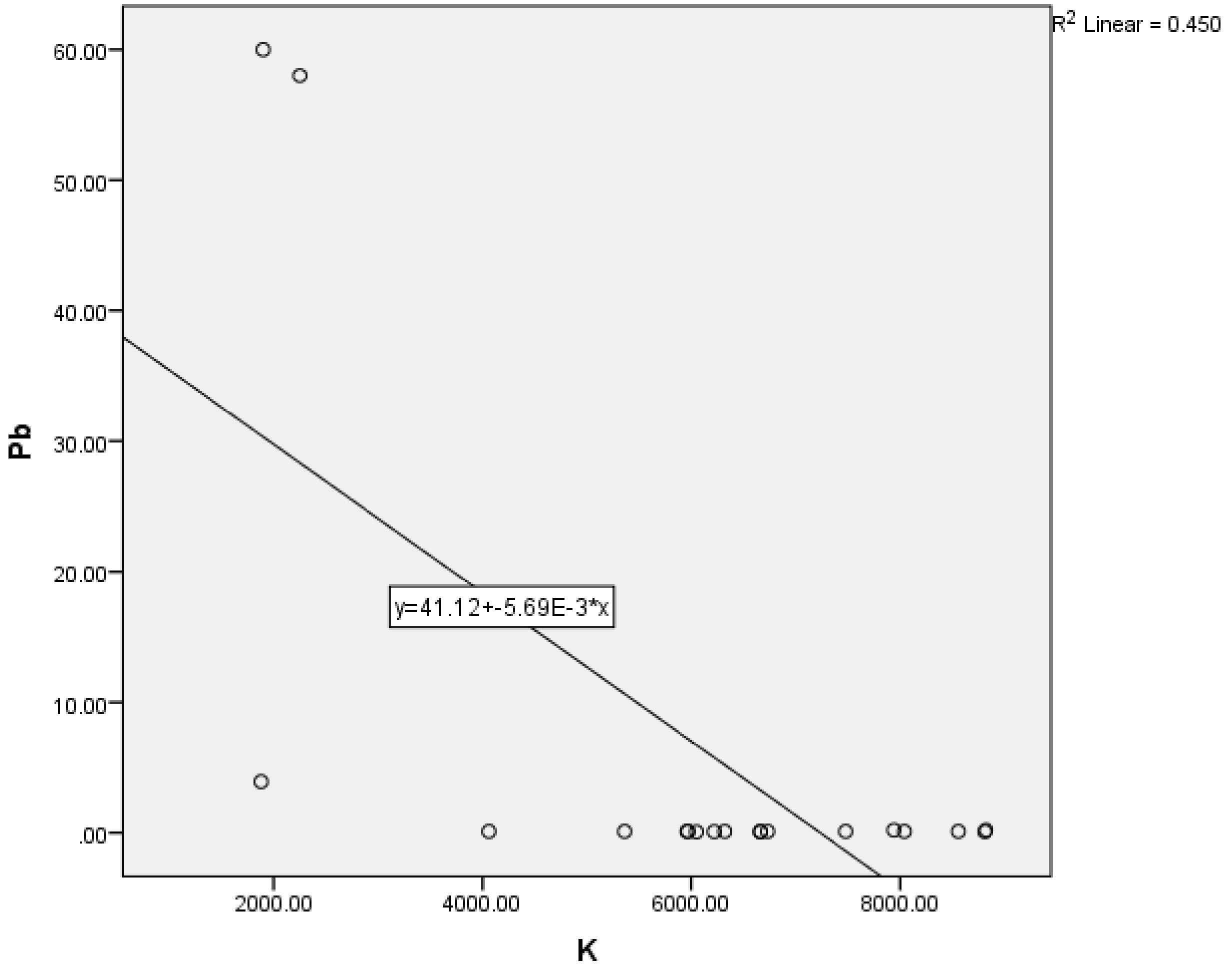

Figure 3: Correlation between K and Pb in breast cancer patients (r=0.8, P= < 0.001)

4. Discussion

In this study, the concentration of six trace elements in the erythrocytes of Sudanese breast cancer patients and control subjects, was measured by XRF. The results showed that K concentration was significantly increased in breast cancer patients compared to healthy women. High blood potassium level (hyperkalemia) occurs in various diseases, including cancer. However, a high K level in cancer is an indicator of tumor cell lysis [17]. Pseudo-hyperkalemia results from the rapid proliferation of tumor cells, which leads to the release of large amounts of potassium. This condition must be differentiated from high K(hyperkalemia) caused by renal disease [18]. Tumor cell lysis syndrome is characterized by elevated potassium, high phosphate, and low calcium levels. The most common tumor associated with this syndrome is lymphoma [19], and certain cancer patients may be affected by the development of tumor lysis syndrome [20]. High K concentration leads to abnormal metabolism, which may lead to complications during cancer therapy [21]. It also leads to other diseases such as renal insufficiency and cardiac arrhythmias, so patients should limit potassium intake in this case [22]. Previous studies on Zn and Cu content in red blood cells, showed that both Zn and Cu content in red blood cells of breast cancer patients were lower than those in the control group [23,24], confirming this result. Another study on serum zinc content, also showed no significant difference between breast cancer patients and control subjects [25,26]. Another study showed no significant difference in serum levels of Zn, Ca, and Pb between breast cancer patients and the healthy group of Chinese women [27], which is in line with our study findings. Previous studies conducted in Canada, [28] and India, [29] reported no significant differences in Cu levels between the two groups, which is also consistent with our study on Sudanese women. The relationship between iron and copper is based on the protein (ceruloplasmin), that binds copper, and acts as an iron carrier in the body. When copper absorption is low due to low consumption, ceruloplasmin levels increase [30], Fe and Cu levels correlate in malignant breast tissue [31], which is consistent with our results showing a strong positive correlation between Fe and Cu in breast cancer patients. Several studies showed opposite results for increased Cu and Zn levels in breast cancer patients compared to the control group [32, 33]. Pb can alter B cell proliferation, and differentiation by interacting with B cells to enhance their proliferation, and differentiation, and activate the proliferation of lymphocytes and leukocytes [34]. Thus, exposure to toxic metals such as Pb could lead to specific health problems [35]. Another previous study confirmed, our findings and reported that, there were no differences in the concentrations of Pb, Ca, and Zn between breast cancer patients and control subjects in China [36]. In this study, a relationship between breast cancer and trace elements was found, with emphasis on K, because erythrocytes were the best indicator of K status. In breast cancer patients, the correlation of K and Pb showed inverse relationships. Further studies are needed to confirm the importance of K monitoring in breast cancer patients.

5. Conclusion

High K levels in red blood cells were associated with the risk of breast cancer in Sudanese women. K can be used as a biomarker for breast cancer prognosis and development.

Ethical Approval :

The Ethics Committee of the Ministry of Health, Khartoum, Sudan approved this study.

Consent

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

References:

1. Rezaei M, Hashemi M, Hashemi SM, Mashhadi MA, Taheri M. APOBEC3 Deletion is Associated with Breast Cancer Risk in a Sample of Southeast Iranian Population. Int J Mol Cell Med. 2015; 4(2):103-8.
2. Hashemi M, Yousefi J, Hashemi SM, Amininia S, Ebrahimi M, Taheri M, et al. Association between Programmed Cell Death 6 Interacting Protein Insertion/Deletion Polymorphism and the Risk of Breast Cancer in a Sample of Iranian Population. Dis Markers. 2015:854621.
3. Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol. 2001; 2(3):133-40.
4. Hashemi SM, Arbabi N, Hashemi M, Mashhadi MA, Allahyari A, Sadeghi M. Association between VDR Gene Polymorphisms (rs 1544410, rs 7975232, rs 2228570, rs 731236 and rs 11568820), and Susceptibility to Breast Cancer in a Sample of Southeastern Iranian Population. Int J Cancer Manag. 2017; 10(3).
5. John E, Laskow TC, Buchser WJ, Pitt BR, Basse PH, Butterfield LH, *et al*. Zinc in innate and adaptive tumor immunity. J Transl Med 2010; 8:118
6. Suzana S, Cham BG, Ahmad Rohi G, Mohd Rizal R, Fairulnizal MN, Normah H, *et al*. Relationship between selenium and breast cancer: A case-control study in the Klang Valley. Singapore Med J 2009; 50:265-9.
7. Ding X, Jiang M, Jing H, Sheng W, Wang X, Han J, et al. Analysis of serum levels of 15 trace elements in breast cancer patients in Shandong, China. Environ Sci Pollut Res Int. 2015; 22(10):7930-5.

8. Shamberger RJ. Calcium, magnesium, and other elements in the red blood cells and hair of normals and patients with premenstrual syndrome. Biol Trace Elem Res. 2003; 94(2):123-9.
9. Susan J. Fairweather-Taita Kevin Cashman. Menirals and Trace Elements. Nutrition for the Primary Care Provider. World Rev Nutr Diet. 2015; 111: 45–52.
10. Araya M, Pizarro F, Olivares M, Arredondo M, Gonzalez M et al. Understanding copper homeostasis in humans and copper effects on health. Biol Res. 2006; 39: 183-187.
11. Classen HG, Gröber U, Löw D, Schmidt J, Stracke H. Zinc deficiency: Symptoms, causes, diagnosis and therapy. Med Monatsschr Pharm. 2011; 34: 87-95.
12. Silva MP, Tomal A, Perez CA, Ribeiro-Silva A, Poletti ME: Determination of Ca, Fe, Cu and Zn and their correlations in breast cancer and normal adjacent tissues. X-Ray Spectrom. 2009, 38 (2): 103-111.
13. Poletti ME, Goncalves OD, Perez CA, Magalhaes SD: A preliminary study of the distribution of trace elements in healthy and neoplastic breast tissues with synchrotron radiation X-ray fluorescence. Radiat Phys Chem 2004, 71(3–4):975–976.
14. Janssens K: X-ray based methods of analysis. Non-Destructive Micro Analysis of Cultural Heritage Materials. 2004, 42: 129-226.
15. Janssens K: X-ray based methods of analysis. Non-Destructive Micro Analysisof Cultural Heritage Materials 2004, 42:129–226.
16. Potts PJ, Ellis AT, Kregsamer P, Streli C, Vanhoof C, West M, Wobrauschek P: Atomic spectrometry update-X-ray fluorescence spectrometry. J Anal At Spectrom. 2006; 21(10):1076–1107.
17. Abu-Alfa AK, Younes A. Tumor lysis syndrome and acute kidney injury: evaluation, prevention, and management. Am J Kidney Dis. 2010; 55(3): 1–13.
18. Sevastos N, Theodossiades G, Archimandritis AJ. Pseudohyperka- lemia in serum: a new insight into an old phenomenon. Clin Med Res.2008;6(1):30-32.
19. Niederhuber, John E.; Armitage, James O.; Doroshow, James H.; Kastan, Michael B.; Tepper, Joel E. (2014). Aebeloff's Clinical Oncology, Fifth Edition. Philadelphia: Elsevier Saunders.
20. Coiffier, Bertrand; Riouffol, Catherine. "Management of tumor lysis syndrome in adults". Expert Review of Anticancer Therapy. 2007; **7** (2): 233–239.
21. Davidson MB, Thakkar S, Hix JK, Bhandarkar MD, Wong A, Schreiber MJ. "Pathophysiology, clinical consequences, and treatment of tumor lysis syndrome". Am. J. Med. 2004; 116 (8): 546–54.
22. Bellinger G, Santoro D, Savica V. Emerging drugs for hyperphosphatemia. Expert Opin Emerg Drugs. 2007; 12:355–65.
23. Maynar, M., Grijota, F.J., Siquier-Coll, J. et al. Erythrocyte concentrations of chromium, copper, manganese, molybdenum, selenium and zinc in subjects with different physical training levels. J Int Soc Sports Nutr 2020(35).
24. C. M. Tinoco-Veras, M. S. Bezerra Sousa, B. Borges da Silva, S. M. Franciscato Cozzolino, et al. Analysis of plasma and erythrocyte zinc levels in premenopausal women with breast cancer. Nutr Hosp. 2011; 26(2):293-297.
25. Xiujuan Wu1, Jing Tang2 & Mingjun Xie. Serum and hair zinc levels in breast cancer: a meta-analysis. Scientific RepoRts. 2015; 5:12249.

26. Rihwa Choi, Min-Ji Kim, Insuk Sohn, Serim Kim, Isaac Kim, Jai Min Ryu. Serum Trace Elements and Their Associations with breast Cancer Subgroups in Korean Breast Cancer Patients. Nutrients. 2019:11,37.
27. Ding X1, Jiang M, Jing H, Sheng W, Wang X, Han J, Wang L. Analysis of serum levels of 15 trace elements in breast cancer patients in Shandong, China. Environ Sci Pollut Res Int. 2015; 22(10):7930-5.
28. Kotsopoulos J., Sukiennicki G., Muszynska M., Gackowski D., Kaklewski K., Durda K., Jaworska K., Huzarski T., Gronwald J., Byrski T., et al. Plasma micronutrients, trace elements, and breast cancer in brca1 mutation carriers: An exploratory study. Cancer Causes Control. 2012; 23:1065–1074.
29. Siddiqui M.K., Jyoti, Singh S., Mehrotra P.K., Singh K., Sarangi R. Comparison of some trace elements concentration in blood, tumor free breast and tumor tissues of women with benign and malignant breast lesions: An indian study. Environ. Int. 2006; 32:630–637.
30. Sharp, P. The molecular basis of copper and iron interactions. Proc Nutr Soc. 2004; 63(4), 563- 569.
31. Lawrence DA. Heavy metal modulation of lymphocyte activities--II. Lead, an in vitro mediator of B-cell activation. Int J Immunopharmacol. 1981; 3(2):153–161.
32. Choi R, Kim MJ, Sohn, Kim S, Kim I, Ryu JM, et al. Trace elements; copper, zinc and selenium, in breast cancer afflicted female patients in LAUTECH Osogbo, Nigeria. Indian J Cancer. 2015; 52(1):106-9.
33. Adeoti ML, Oguntola AS, Akanni EO, Agodirin OS, Oyeyemi GM. Trace elements; copper, zinc, and selenium, in breast cancer afflicted female patients in LAUTECH Osogbo, Nigeria. Indian J Cancer .2015; 52:106–9.
34. Silva, M. P, Tomal, A, Pérez, C. A, Ribeiro-Silva. A Poletti, M. E. Determination of Ca, Fe, Cu and Zn and their correlations in breast cancer and normal adjacent tissues. X-Ray Spectrometry .2009; 38(2):103-111.
35. De Guise S, Bernier J, Lapierre P, Dufresne MM, Dubreuil P, Fournier M. Immune function of bovine leukocytes after in vitro exposure to selected heavy metals. Am J Vet Res. 2000; 61(3):339–344.
36. Xiao Ding, Meng Jiang, Luhua Wang. Analysis of serum levels of 15 trace elements in breast cancer patients in Shandong, China. Environmental Science and Pollution Research.2015; (22), 7930–7935.