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# THE QUANTITATIVE SERUM BRCA1AND SERUM ZINC AS A NOVEL BIOMARKERS WITH THE CORRELATIONS BETWEEN THEM IN THE FAMILIAL BREAST CANCER PATIENTS IN IRAQ.

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## ABSTRACT

**Background:** Breast cancer is the most common malignant tumor in women worldwide and in Iraq. Many studies show BRCA1 and zinc are the substances that expressed significantly in tissues or serum specimens from a genetically–inherited cancer compared with a sporadic cancer or to healthy individuals, which in turn should be considered as a biological markers. **Objectives:** To describe serum BRCA1 and zinc levels as a novel biomarkers and correlating between them within familial patients comparing with sporadic breast cancer beside healthy controls group. **Patients and Methods:** 64 BC patients and 25 HCs. The patients were subjected after mastectomy age ranged (28-68 years) at the time of diagnosis. Serum BRCA1-ELISA method and serum zinc were performed. **Results:** The three groups were: familial breast cancer FBC 37 (41.6%) age mean 44.78 ± 10.3, sporadic SBC 27(30.3%) age mean 47.85 ± 9.8 and healthy controls HCs 25 (28.1%) age mean 44.00 ± 10.7. The median serum BRCA1 was significantly correlated with breast cancers compared with healthy controls and correlated more precisely with familial compared with sporadic breast cancer, while mean serum zinc was inversely correlated with breast cancer patients compared with healthy controls. Serum BRCA1 had a significant an indirect linear correlation with serum zinc. **Conclusions: S**erum BRCA1 and zinc can be used as a tests in the early detection, diagnosis, prognosis and therapy responses as a novel biomarkers of breast cancer in Iraqi women, moreover, serum BRCA1 can be used as a test to discriminate familial from sporadic breast cancer.

**KEYWORDS:** Breast, Cancer, Serum BRCA1, Serum zinc, familial breast cancer.

## INTRODUCTION

Breast cancer is the most common malignant tumor in women worldwide and it constitutes about one third of the registered cancer cases among the Iraqi population. Many data demonstrate that we will be able to assess both early disease detection and progression from the blood.[1] So an urgent need to search for cancer biomarkers. Early detection is one of the most vital strategies to improve BC survival rate[2], which it is for a asymptomatic BC identified in a large screening project for a relative 5 and 10 years were 88% and 79% respectively and it is lower among women with a more advanced stage at diagnosis.[2,3] and as it well known, BC is not preventable[4], therefore, earlier detection is the keystone for reduction of mortality5, at this approach, many diseases are correlated with quantitative changes of substances in the fluids, and plasma carries important information which potentially could help to improve early disease detection, prognosis, and response to therapeutic treatments.[6,7] Zinc had been widely reported as an essential trace element and it functions as an antioxidant that play a role in the maintenance of genomic stability[8], and there was an inverse link between dietary and serum Zn levels with the risk of breast cancer developing.[9]

## MATERIALS AND METHODS

### subjects

This prospective cross sectional study was conducted on 64 patients diagnosed with BC at the Main Referral Training Center for Early Detection of Breast Tumors (Oncology Teaching Hospital/Medical City) and referred after mastectomy to the Iraqi National Cancer Research Center (Medical College/ Baghdad University), during the period from April 2014 to July 2015. The patients were sub divided into two groups: those with positive family history (Group 1 (FBC) = 37) and those without, i.e., Sporadic BC (Group 2 (SBC) = 27). Within the former group positive family history was reported in the first, second and third degree relatives in 21, eight and eight patients respectively. The 27 sporadic BC group who did not record any family history of breast or ovarian cancer was used for comparison analysis. Twenty five apparently healthy individuals with no BC nor family history of breast and/or ovarian were selected as case-control group.

**Blood samples**

Five mls. of venous blood samples were aspirated to obtain the serum for ELISA and other biochemical testes. The hemolysed samples were discharged.

## MATERIALS

Serum BRCA1-ELISA by E-EL-H0601 kit from Elabscience Biotechnology Co. Serum zinc kit from BioSystems.

## STATISTICAL ANALYSIS

An expert statistical advice was sought for. Statistical analyses were done using IBMSPSS version 23 computer software (Statistical Package for Social Sciences) in association with Microsoft Excel 2016. Continuous variables are reported as means ± standard deviations (SD) and categorical variables are presented as percentages or numbers. A p value less than 0.05 was considered statistically significant. Quantitative and qualitative variables were tested using Student's t-test and the chi-square test respectively. An estimate was considered statistically significant if its P value was less than an alpha level of significance of 0.05. The linear correlation analysis LCCA (r) method is used to evaluate the strength of the associations between two parameters.

## RESULTS

**Table (1): The correlations of serum BRCA1-ELISA between BC patients group and healthy controls group.**

| Study group | | | |
|---|---|---|---|
| | **Healthy controls** | **Cases (BC )** | **P** |
| BRCA1-Elisa (ng/ml) | | | <0.001 |
| Range | (0.45-1.5) | (0.45-9.5) | |
| Median | 0.96 | 1.77 | |
| Inter-quartile range | (0.895-1) | (1.2-2.6) | |
| N | 25 | 64 | |
| Mean Rank | 23 | 53.6 | |

**Table (2): The correlations of serum zinc between BC patients and healthy controls group.**

| Study group | | | |
|---|---|---|---|
| | **Healthy controls** | **Cases (BC)** | **P** |
| Serum zinc (ug/dl) | | | <0.001 |
| Range | (67-142) | (52-130) | |
| Mean | 103 | 87 | |
| SD | 23.9 | 15.2 | |

| | | | |
|---|---|---|---|
| SE | 4.77 | 1.9 | |
| N | 25 | 64 | |

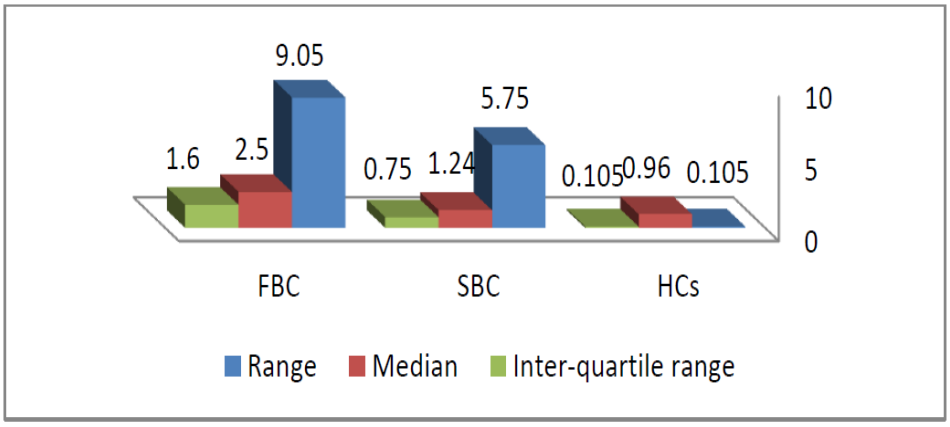


**Figure (1): The range, median and IQR of serum BRCA1-ELISA (ng/ml) among the three groups; FBC, SBC and HCs, P < 0 .001*****

**Table (3): The correlations of serum zinc among the three study groups: FBC, SBC and HCs.**

| **HCs** | **SBC** | **FBC** | **P** |
|---|---|---|---|
| Serum zinc (ug/dl) | | <0.001 | |
| Range | (67-142) | (70-130) | (52-115) |
| Mean | 103 | 91.8 | 83.5 |
| SD | 23.9 | 13.8 | 15.4 |
| SE | 4.77 | 2.66 | 2.54 |
| N | 25 | 27 | 37 |
| P (Bonferroni t-test) for difference in mean between: | | | |
| Sporadic BC x Healthy controls=0.08[NS] | | | |
| Familial BC x Healthy controls<0.001 | | | |
| Familial BC x Sporadic BC =0.21[NS] | | | |

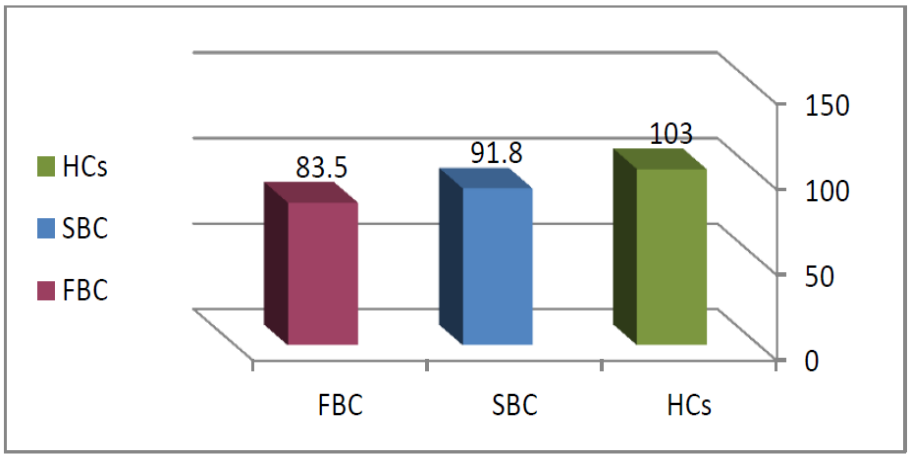


**Figure (2): The mean serum zinc among the study groups: FBC, SBC and HCs, P <0.001*****

**Table (4): The linear correlation analysis LCCA (r) of serum BRCA1-ELISA**

| | Linear correlation coefficient LCCA (r) | P |
|---|---|---|
| Serum zinc (ug/dl) | -0.428 | P<0.001 |

## DISCUSSION

BRCA1 is a tumor suppressor protein that is potentially useful as TM for screening and identification of high risk individuals and their families.[1] According to the serum cut-off value COV of BRCA1-ELISA (1.27 ng/ml) in this study; the BC group had an extremely significantly high percentage of serum positive BRCA1-ELISA: 68.8% P<0.000 compared with HCs 3.4%. Compared this study results with a result from a study by (Qing Zhu et al in 2015)[10] who used the same analytical method in which they found serum positive BRCA1-ELISA 19.1% for BC patients compared with (0.7%) HCs (p <0.001). The present study differs from that study in that there was a different patients group, also, in this study the optimum COV of serum BRCA1was determined by using the ROC curve which offered a reliable method to determine the positive reaction with a high sensitivity (68.8%) and high specificity (88%), while in that study the optimum COV designated as (mean+3SD). The ROC curve method showed the tradeoff between sensitivity and specificity and it was the better method to detect the performance of a developed test which classified subjects into two categories such as diseased and non-diseased which provided a comprehensive and visually attractive way to summarize the accuracy of predictions, while the other method involved some drawbacks as a diagnostic test subjected to chance variation under certain varying conditions.[11] The current study, was agreed with[12], which explained the accumulation of the BRCA1 in the cytoplasm due to the protein-protein interaction leading it to leakage to the blood, which explained the higher percentage of the serum positive BBRCA1- ELISA.

In the current study, median serum BRCA1-ELISA of BC group was increased significantly about twofold times more than that for HCs, p< 0.001*** which was dependent to the tumor status, necrotic tumor cell, proliferation volume, proteolytic activities and releasing extent from the tumor cells of BC patients.[1]

As shown in Fig 1, the range, Median and IQR of serum BRCA1-ELISA in the three groups were sensibly increased both in FBC and SBC patients compared with HCs, additionally, the serum BRCA1-ELISA of FBC was increased significantly than that for SBC (P<0.001***). The explanation was; the FBC group had more aggressive disease than SBC with higher

tumor stages and grades as it seen in the questioner formula that associated of this study, also the activities of the tumor cells were greater in the FBC than SBC1. As the tumor stage progresses the tumor will elaborate higher levels of the oncoproteins; therefor, represent a good correlation between the tumor size and the level of the oncoprotein expression in the tumor tissue itself[1], correspondingly, serum BRCA1 level increased in the same patients. Additionally, it could be predicted a prior because there were distinct protein patterns associated with a BC serum profile comparative to a profile for a normal healthy status, the women who effectively treated for BC would likely revert to a normal profile while the women with progression of disease or recurrence would continue to have a cancer profile, or a subset thereof which indicated a successful therapy.[13]

Additionally, serum level of tumor markers reflected the success of surgery or the efficacy of chemo-therapy and detecting raised levels of a tumor marker after surgery would indicate either imperfect elimination of the tumor, recurrence, or the existence of metastases.[1] The measurement of serum TMs during chemotherapy also gave a sign of the usefulness of the drug used and a guide for the choice of the most effective drug for each discrete case.[1]

In this study, there was a significant decrease of mean serum zinc in BC patients 87(ug/dl) compared with HCs 103(ug/dl) ($p<0.001$) according to the OCV of the current study (95.5ug/ml). This result is similar to the results obtained by previous studies which suggested that serum Zn level in BC patients decreases significantly as reported by (Alam et al in 2012) 9 and (Pavithra et al in 2015)[14], and with a meta-analysis containing random effects model with 12 studies which recorded a decreased in mean serum zinc level in BC group, in contrast to six studies which recorded no significant differences and with one study which recorded a significant increase in mean serum zinc in BC patients compared with HCs (Gumulec J. ,2014 June 18).[15] Many studies showed a correlation between low serum Zn levels and the risk for developing BC, others, revealed the erythrocytes Zn levels was significantly low in women with BC compared with normal women as it more correctly that didn't attributed to dietary Zn deficiency.[9]

In the current study, almost patients had a Zinc supplement with their diet which declared in the questioner formula, however, serum zinc was significantly low that reflect a defective zinc metabolism in such patients, there was a high level of regulation of zinc importer (Zip) and transporter (ZnT) proteins families which enable the cellular zinc homeostasis (Chasapis CT, et al., 2014 Dec)[16] and several of these proteins appear to be disturbed in BC cells. In

the point of view, zinc acts as an antioxidant, it was a vital for functions of many transcriptional factors and proteins that recognize certain DNA sequences and regulate gene transcription which affected inheritably. In addition, low levels of zinc are a vital precondition for precancerous changes and zinc is important microelements regulate the physiological roles of various organs and related in the production of pathological changes in these organs.

Another complex issue was appeared when dealing with the regulation of serum Zn levels compared to its levels in the corresponding tumor tissues of cancer patients, breast biopsies from cancer patients have significantly higher Zn levels compared with normal breast tissues[9], on the other hand, the majority of tumors, zinc level decrease in both serum and tissue to certain extent and it is uncertain, whether low serum level is a result of tumor, chronic stress or of both of these effects, that, leads to relocation of zinc between body compartments, in addition, chronic inflammation is a common mark of cancer which might be an important mechanism of serum zinc level decrease[15], on the other hand, examination of BC biopsies as well as cultured cells revealed abnormal expression of multiple proteins that play a role in zinc homeostasis.[9] Moreover, abnormal functioning of both ZnTs and ZIPs as well as the imbalances in their activities could play a role in Zn dyshomeostasis and cancer development. High levels of zinc supplementation had a positive effect on reducing oxidative stress and improving immune responses in cancer patients. However, some studies have indicated that zinc serves as a co-factor for cancer cell fission and replication.[17]

## CONCLUSION

The present study is promising for early detection of the breast cancer and for monitoring prognosis, response to the therapy and recurrence by measuring the serum BRCA1-ELISA, also, showed that serum zinc correlated inversely with those who had a high serum BRCA1-ELISA level and was lower significantly in familial breast cancer than with sporadic breast cancer, in turn it was lower than that of healthy individuals, which represented a good indicator for the disease aggressiveness in such patients.

### Recommendations

1. Adopt measuring serum BRCA1-ELISA in addition to serum zinc as a screening test for breast cancer patients in the routine work specifically in those who declare a family history of the disease.

2. Consider serum zinc measurement as a complementary biochemical test which could be helpful in the early detection of the BC.
3. Provide breast cancer patients with regular zinc supplements throughout life.

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