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# THE QUANTITATIVE LEVELS OF SERUM BRCA1, TOTAL PROTEIN, ALBUMIN, AND SERUM PROTEIN ELECTROPHORESIS PATTERN AS A NOVEL BIOMARKERS OF BREAST CANCER CORRELATING IN THE FAMILIAL BREAST CANCER PATIENTS IN IRAQ.

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## ABSTRACT

**Back Ground:** Breast cancer BC is the most women common malignant tumor worldwide and in Iraq. Early detection of cancer can lead to superior long-term survival. BRCA1 is a tumor suppressor gene inactivated by mutation in familial breast cancer FBC. The protein that is expressed significantly in tissues or serum specimens from a genetically–inherited cancer compared with a sporadic cancer SBC or to healthy individuals controls HCs, should be considered as a biological marker. **Objectives:** To designate serum BRCA1, total protein STP, albumin, serum protein electrophoresis SPE pattern as a novel biomarkers of BC. **Patients and Methods:** 64 BC patients subjected after mastectomy age ranged 28-68 years at onset and 25 age match HCs. Serum BRCA1-ELISA method, total protein, albumin, and SPE were performed. **Results:** The groups were: FBC 37(41.6%), SBC 27(30.3%) and HCs 25(28.1%) with age mean: 44.78 ± 10.3, 47.85 ± 9.8 and 44.00 ± 10.7 respectively. The median serum BRCA1 was significantly correlated with BC compared with HCs, also correlated with FBC compared with SBC. Mean serum: total protein, alph-1 and alph-2 were correlated significantly with BC patients compared with HCs, also correlated with FBC compared with SBC group. Mean serum albumin, beta, and gamma were failed to be significantly different among the three groups. **Conclusions:** Serum BRCA1, alpha-1 and

alph-2 can be used as a tests in the early detection, diagnosis, prognosis and therapy responses of BC in Iraqi women, serum BRCA1 can be used to discriminate FBC from SBC.

**KEYWORDS**: Breast, Cancer, Serum BRCA1, 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 rate2, 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 mortality[5] at this approach, many diseases are correlated with quantitative changes of proteins 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] BRCA1 is a tumor suppressor protein that is potentially useful as TM for screening and identification of high risk individuals and their families.[1]

## 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 protein Bio Rad. Serum protein electrophoresis by Helena, BioSciences Europe.

## 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 ROC method is used to evaluate the performance of a quantitative test in differentiating between a disease status (or an outcome) and a second comparison group.

## RESULTS

The optimum cut-off value of serum BRCA1 protein-ELISA test was ≥ 1.27 ng/ml, which calculated from ROC curve and achieved a high sensitivity and specificity for the detection of BC with: sensitivity (68.8%), specificity (88.0%), high accuracy (74.2%), PPV( 98.1%), and NPV(96.2%) on a clinical ground.

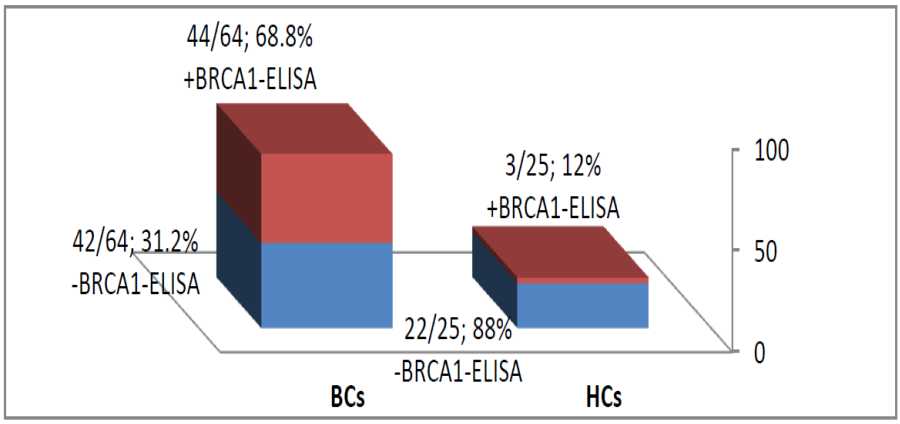



**Figure (1): The frequency of the serum positive BRCA1-ELISA results between breast cancer patients and healthy controls group, P = 0.000***.**

**Table (1): The correlations of the serum positive BRCA1-ELISA results between breast cancer patients 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 the serum proteins between the BC patients and healthy controls group.**

| Study group | | | |
|---|---|---|---|
| | **Healthy controls** | **Cases (BC)** | **P** |
| Serum total protein | | | 0.039 |
| Range | (6.4-8.4) | (6.6-9) | |
| Mean | 7.2 | 7.5 | |
| SD | 0.5 | 0.6 | |
| SE | 0.1 | 0.07 | |
| N | 25 | 64 | |
| Serum Albumin | | | 0.41[NS] |
| Range | (2.8-4.3) | (2.6-4.4) | |
| Mean | 3.8 | 3.7 | |
| SD | 0.3 | 0.3 | |
| SE | 0.07 | 0.04 | |
| N | 25 | 64 | |
| Serum Alpha-1 | | | <0.001 |
| Range | (0.2-0.66) | (0.27-0.8) | |
| Mean | 0.27 | 0.44 | |
| SD | 0.09 | 0.13 | |
| SE | 0.019 | 0.016 | |
| N | 25 | 64 | |
| Serum Alpha-2 | | | <0.001 |
| Range | (0.39-1.4) | (0.5-1.97) | |
| Mean | 0.75 | 1.04 | |
| SD | 0.19 | 0.33 | |
| SE | 0.038 | 0.042 | |
| N | 25 | 64 | |
| Serum beta | | | 0.84[NS] |
| Range | (0.7-1.4) | (0.43-2.1) | |
| Mean | 1.05 | 1.04 | |
| SD | 0.14 | 0.38 | |
| SE | 0.028 | 0.048 | |
| N | 25 | 64 | |

| | | | |
|---|---|---|---|
| Serum gamma | | | 0.35[NS] |
| Range | (0.98-2) | (0.72-2) | |
| Mean | 1.35 | 1.3 | |
| SD | 0.26 | 0.26 | |
| SE | 0.051 | 0.033 | |
| N | 25 | 64 | |

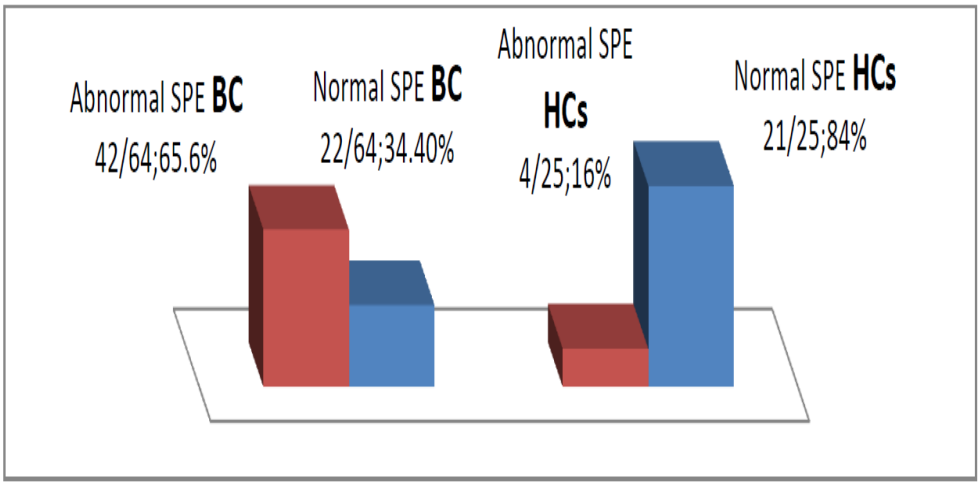
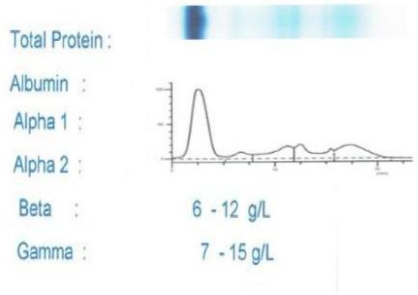
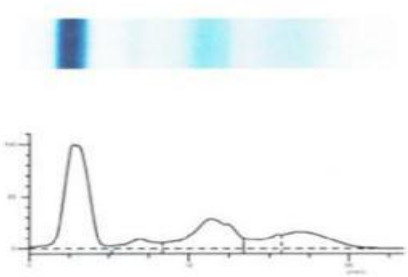
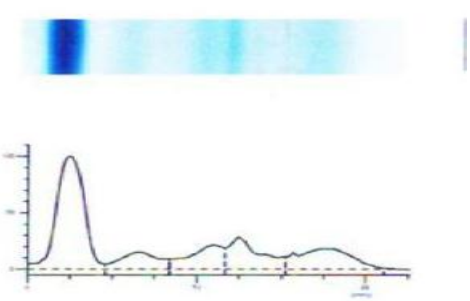



**Figure (2): The frequency of the abnormal SPE pattern between breast cancer patients and healthy controls group, p = 0.000***.**

In general the most SPE pattern abnormalities were seen with: reducing albumin, increasing: mostly alpha-1, alpha-2, beta, and gamma, Figure (3).

| | |
|---|---|
| Total protein 6 - 8 g/dl<br>Albumin 3.5-5.0 g/dl<br>Alpha-1 0.1-0.4 g/dl<br>Alpha-2 0.6-1.2 g/dl<br>Beta 0.6-1.2 g/dl<br>Gamma 0.7-1.5 g/dl<br>a1: The Normal value of serum protein electrophoresis |  <br>a2: normal pattern |
|  <br>b1: increased alpha-2; 1.66 g/dl, reduced beta; 0.48 g/dl |  <br>b2: increased alpha-1 |

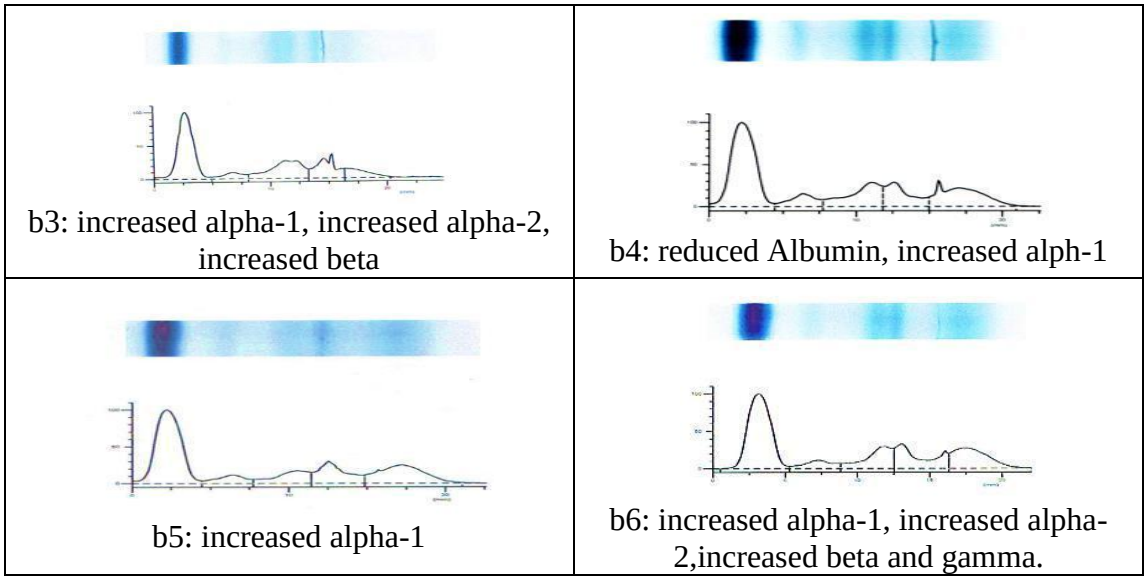



**Figure 3: The diagram of serum protein electrophoresis patterns showing the existing of the some abnormalities of the five legend fractions: a; 1-2: normal pattern. b; 1-6: abnormal patterns with breast cancer patients.**

**Table 3: The frequency of the serum positive BRCA1 results among the three groups. FBC, SBC, and HCs in the study.**

| Features of the subjects | HCs N % | SBC N % | FBC N % | Total N % | chi-square X 2 | P-Value |
|---|---|---|---|---|---|---|
| Positive Serum BRCA1 | 3 12 | 13 48.1 | 31 83.8 | 47 66 | 31.186 | 0.000 *** |
| Negative Serum BRCA1 | 22 88 | 14 51.9 | 6 16.2 | 42 34 | | |
| Total | 25 100 | 27 100.0 | 37 100.0 | 89 100 | | |

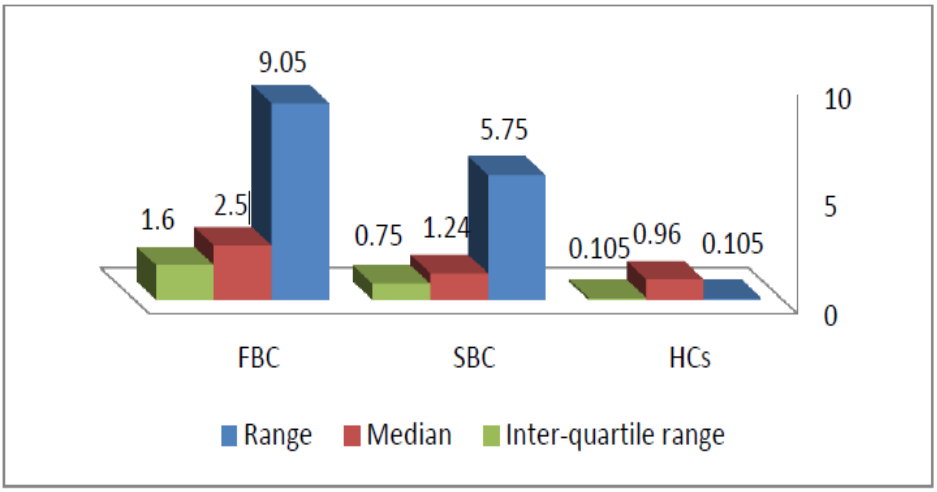



**Figure 4: The range, median and IQR of serum BRCA1-ELISA (ng/ml) among the three groups: FBC, SBC and HCs, P < 0 .001*****

**Table 4: The correlations of serum protens among the three groups: FBC , SBC and HCs.**

| | HCs | SBC | FBC | P |
|---|---|---|---|---|
| Serum total protein | | | | 0.12[NS] |
| Range | (6.4-8.4) | (6.6-9) | (6.6-8.6) | |
| Mean | 7.2 | 7.5 | 7.5 | |
| SD | 0.5 | 0.7 | 0.5 | |
| SE | 0.1 | 0.13 | 0.08 | |
| N | 25 | 27 | 37 | |
| P (Bonferroni t-test) for difference in mean between: | | | | |
| Sporadic BC x Healthy controls=0.22[NS] | | | | |
| Familial BC x Healthy controls=0.19[NS] | | | | |
| Familial BC x Sporadic BC =1[NS] | | | | |
| Serum albumin | | | | 0.71[NS] |
| Range | (2.8-4.3) | (3.2-4.1) | (2.6-4.4) | |
| Mean | 3.8 | 3.7 | 3.7 | |
| SD | 0.3 | 0.3 | 0.4 | |
| SE | 0.07 | 0.06 | 0.06 | |
| N | 25 | 27 | 37 | |
| P (Bonferroni t-test) for difference in mean between: | | | | |
| Sporadic BC x Healthy controls=1[NS] | | | | |
| Familial BC x Healthy controls=1[NS] | | | | |
| Familial BC x Sporadic BC =1[NS] | | | | |
| Serum Alpha-1 | | | | <0.001 |
| Range | (0.2-0.66) | (0.27-0.8) | (0.3-0.79) | |
| Mean | 0.27 | 0.42 | 0.46 | |
| SD | 0.09 | 0.13 | 0.12 | |
| SE | 0.019 | 0.024 | 0.02 | |
| N | 25 | 27 | 37 | |
| P (Bonferroni t-test) for difference in mean between: | | | | |
| Sporadic BC x Healthy controls<0.001 | | | | |
| Familial BC x Healthy controls<0.001 | | | | |
| Familial BC x Sporadic BC =0.47[NS] | | | | |
| Serum alpha-2 | | | | <0.001 |
| Range | (0.39-1.4) | (0.55-1.9) | (0.5-1.97) | |
| Mean | 0.75 | 1.06 | 1.03 | |
| SD | 0.19 | 0.38 | 0.3 | |
| SE | 0.038 | 0.074 | 0.049 | |
| N | 25 | 27 | 37 | |
| P (Bonferroni t-test) for difference in mean between: | | | | |
| Sporadic BC x Healthy controls=0.001 | | | | |
| Familial BC x Healthy controls=0.002 | | | | |
| Familial BC x Sporadic BC =1[NS] | | | | |
| Serum beta | | | | 0.86[NS] |
| Range | (0.7-1.4) | (0.43-2.1) | (0.47-2) | |

| | | | | |
|---|---|---|---|---|
| Mean | 1.05 | 1.01 | 1.05 | |
| SD | 0.14 | 0.38 | 0.39 | |
| SE | 0.028 | 0.073 | 0.064 | |
| N | 25 | 27 | 37 | |
| P (Bonferroni t-test) for difference in mean between: | | | | |
| Sporadic BC x Healthy controls=1[NS] | | | | |
| Familial BC x Healthy controls=1[NS] | | | | |
| Familial BC x Sporadic BC =1[NS] | | | | |
| Serum gamma | | | | 0.54[NS] |
| Range | (0.98-2) | (0.8-2) | (0.72-1.8) | |
| Mean | 1.35 | 1.32 | 1.28 | |
| SD | 0.26 | 0.31 | 0.23 | |
| SE | 0.051 | 0.059 | 0.038 | |
| N | 25 | 27 | 37 | |
| P (Bonferroni t-test) for difference in mean between: | | | | |
| Sporadic BC x Healthy controls=1[NS] | | | | |
| Familial BC x Healthy controls=0.82[NS] | | | | |
| Familial BC x Sporadic BC =1[NS] | | | | |

**Table 5: ROC area for selected measurements when used as test to diagnose BC differentiating it from HCs.**

| **ROC area** | | |
|---|---|---|
| Serum Alpha-1 | 0.913 | <0.001 |
| BRCA1-Elisa (ng/ml) | 0.844 | <0.001 |
| Serum Alpha-2 | 0.801 | <0.001 |
| Serum zinc (ug/dl) | 0.674 | 0.011 |
| HER2-Elisa (ng/ml) | 0.649 | 0.029 |
| Serum total protein | 0.622 | 0.08[NS] |
| Serum Beta | 0.585 | 0.21[NS] |
| Serum Gamma | 0.551 | 0.46[NS] |
| Serum Albumin | 0.453 | 0.49[NS] |

**Table 6: ROC area for selected measurements when used as test to differentiate BC cases with a FBC from those with a SBC.**

| | **ROC area** | **P** |
|---|---|---|
| BRCA1-Elisa (ng/ml) | 0.778 | <0.001 |
| Serum Alpha-1 | 0.621 | 0.1[NS] |
| Serum Beta | 0.537 | 0.62[NS] |
| Serum total protein | 0.525 | 0.73[NS] |
| Serum Albumin | 0.509 | 0.9[NS] |
| Serum Alpha-2 | 0.503 | 0.97[NS] |
| Serum Gamma | 0.497 | 0.97[NS] |

## DISCUSSIONS

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 result with a result from a study by (Qing Zhu et al in 2015)[8] 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.[9] The current study, was agreed with 10, 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 level of BC group was increased significantly about twofold times more than that for HCs, p< 0.001*** which it was dependent in 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 4, 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.[11] 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]

Similarly, proteins play a chief role in cell configuration and function. It is a proved fact that serum protein levels may undergo alterations during the course of BC[7], with many quantitative change levels of protein and protein fractions.[7] The patients in this study were post-mastectomies and the mean serum total protein STP was equal in the both of the two BC groups and significantly higher than that for the HCs. The current study disagree with(Jsiem RH, et al. 2012)[12] that record decrease serum level in the estimation of serum and / or tissue in bladder cancer. The current study agree with several studies that recorded an increase of STP in the brain tumor[13] and with a Jordanian study which recorded an increased STP in BC[14], comparing the current study with the results of a study by(Tahzeeb F., et al., January 2013)[15] which show a non-significant increase of STP comparing BCs with HCs, and with[16] which reported a non-significant difference in the STP between a pre-treatment and post-treatment between those BC patients whom receiving a radiation therapy with those who did not receive.

The current study explanation was, increasing of serum total protein in BC patients comparing with HCs is owing to the changing in albumin and globulins levels duo to the oxidative stress that associated with cancer.[17] In addition, plasma circulates through the tissues, collects the released proteins from their resourceful sites due to certain physical actions rather than from the cancer action, including; tissue transformation, trauma and cell death. Additionally, breast surgery induced distinct changes in the concentrations and dynamics of serum proteins in invasive BC patients compared with HCs women and non-invasive tumor patients.[18] In this study, the OCV for serum Albumin was > 3.7 g/dl, this result was within the range (3 - 4.15) g/dl which was recorded in a study on a systematic search using the Medline database.[19]

In the current study serum Alb level was normal in both of the two BC groups comparing with HCs and were similar to the results of (Chauhan P. et al., 2016)[20], the albumin level was reported to be within a normal reference range during the different courses of chemotherapy. Comparing the results of the current study with the results by[15] which show lowered serum albumin and higher serum gamma globulins and non-gamma globulins in BC patients when compared with HCs with a non-significant regaining of this protein after chemotherapy and surgery. Other study show that a low serum albumin level was observed during the chemotherapy treatment and the baseline was a powerful prognostic variable, and with[16] who reported a non-significant serum Alb between a pre-treatment and post-treatment as well as between patients who received a radiation therapy with those did not receive. The explanation in this study goes with[18] which found that the patients with post mastectomy group without any disease had a higher serum Alb level than that of patients with any amount of breast carcinomas. So, serum total protein elevation is due to the changing in the serum globulin level which can be elucidated clearly by serum protein electrophoresis SPE test.

The most consistent and significant abnormalities observed on SPE pattern were those of; Alph-1, Alph-2, beta and gamma globulin. The mean serum alph-1 and alph-2 in the BC group was significantly higher than HCs ($p<0.001$). There are distinct protein patterns associated with a BC serum profile relative to a profile for a normal healthy status, women will be treated for BC would likely to revert to a normal profile and women with a progression of disease or recurrence would continue to have a cancer profile.[21] In regarded to alpha-1 fraction which contains many proteins: α1-antitrypsin, alpha1-antichymotrypsin, Orosomucoid (acid glycoprotein), Serum amyloid A and alpha1-lipoprotein[22], alpha-1 abnormalities are usually due to alpha-1 antitrypsin changes. Increases are found in acute inflammatory disorders (an acute phase reactant). Various authors have found significantly elevated serum levels of BC serum.[23] which are related to an invasive growth of the tumors, notably, AAT has been histologically detected in sections of paraffin-embedded biopsy specimens of BC patient. These findings suggest a local production of AAT by the cells with a more aggressive tumor behavior.[24] Alpha 1-antichymotrypsin (α1AC) is an early-stage acute-phase plasma protein has been found to be overexpressed in BC25, also, has identified the quantitative and qualitative changes in Alpha-1-Acid Glycoprotein AGP among patients with malignant and nonmalignant BC.[25]

In regarded to Alpha-2 globulin which contains mainly haptoglobin HP 23 Haptoglobin levels increase in stress, infection, inflammation and tissue necrosis and locally in tumor tissue have been observed in diverse types of malignancies including breast and ovarian cancer.[26,27]

In regarded to serum beta; transferrin and beta-lipoprotein (LDL) comprises the beta-1, increased beta-1 protein due to the increased level of free transferrin is typical of iron deficiency anemia, pregnancy, and estrogen therapy.[28] Serum lipoprotein metabolism was regulated and controlled by the specific apolipoprotein (Apo-) constituents of the various lipoprotein classes such as ApoAI, ApoCI, ApoH (beta2 glycoprotein). Several classes of apolipoprotein in serum have been discovered as putative BC biomarkers using proteomic techniques. Some studies have been identified increased ApoH in sera of the BC patients compared to that of HCs subjects. Beta-2 comprises C3 (Complement protein 3). It is raised in the acute phase response, C3a-desArg was previously observed to be higher in BC sera compared to healthy controls in several studies.[29]

Immunoglobulins (IgG, A, M) are a heterogonous group of proteins contained in the gamma, beta, and alpha-2. The IgG was found to be reduced in prostate and BC patients. Serum IgA was found to be raised in patients with cancer of epithelial secretory organs, such breast, prostate, and uterine cervix.[30]

In conclusion, both familial and sporadic breast cancer patients as present alone or in combination were associated with disturbances in the serum BRCA1which reflected the burden and/or the prognosis of the breast cancer disease, the responding to the therapy or the recurrences, also the disturbance in some of serum proteins reflected the a decreasing in the immunity responses of the patients.

## CONCLUSIONS

Serum BRCA1-ELISA can serve as a good quantitative screening test as it showed significant high concentration in the breast cancer patients than the healthy control and/or cut off value of the test, so it could be considered as a method to diagnose breast cancer differentiating it from healthy controls (ROC area B = 0.844) as well as when used as a test to discriminate breast cancer cases with a familial history from those with sporadic breast cancer (ROC area = 0.778), with good validations that yielded a high: sensitive 68.8%, specific 88%, ppv 98.1% and npv 96.2%. ELISA was a reliable tool to assess the

BRCA1status and could be considered as a novel reliable biomarker, and is a relatively inexpensive, easy to use and interpret method, as a step forward in the early detection of breast cancer in Iraqi women. 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 proteins: serum BRCA1-ELISA, serum alpha-1 globulin.

**Recommendations**

Adopt measuring serum BRCA1-ELISA and serum protein in addition to protein electrophoresis (serum alpha-1 and alpha-2 globulin ) as a screening test for breast cancer patients in the routine work specifically in those who declare a family history of the disease.

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