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Study of the Association of Some Tumor Markers and Serum Protein Electrophoresis Patterns with Breast Cancer in Iraqi Population

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Abstract

Breast cancer is the most commonly diagnosed cancer and a leading cause of cancer mortality among females worldwide. The recruitment of less invasive serum protein electrophoresis and serum breast cancer tumor markers as monitoring factors for patient response to therapy is still controversial.

Hence, the present study aimed to designate serum protein electrophoresis pattern of carcinoembryonic antigen (CEA), carcinoma antigens (CA125 and CA 15-3) as novel biomarkers in monitoring breast cancer patients under and post radiotherapy or chemotherapy.

Samples included 23 breast cancer women patients after mastectomy and under or post chemotherapy and radiotherapy. Tumor markers CEA, CA 15-3, and CA 125 were measured by using electrochemiluminescence method used in Cobas ®. Total serum protein was performed by using commercially available kits suitable with semiautomated chemistry analyzer. Quantitative estimation of serum proteins was accomplished by agarose serum protein electrophoresis.

Serum total proteins showed a non-significant increase in under and post therapy compared with healthy control. A significant decrease in serum albumin level (p<0.0001) was observed in under and post therapy patients. Additionally, serum gamma globulins and alpha-2 globulins levels showed significant increase in under and post therapy patients. Alpha-1, alpha2, gamma globulin fractions, CA 125 and CA-15-3 showed the highest sensitivity and specificity.

Keywords: Breast cancer, Serum protein electrophoresis, Carcinoembryonic antigen, Carcinoma Antigen 15.3, Carcinoma antigen 125.

دراسة ارتباط بعض مؤشرات الأورام وأنماط الترحيل الكهربائي لبروتين المصل لدى المصابين بسرطان الثدي في المجتمع العراقي

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الخلاصه

سرطان الثدي هو من أكثر أنواع السرطانات شيوعًا وسببًا رئيسيًا للوفيات الناتجة عن السرطان بين الإناث في جميع أنحاء العالم. لا يزال استخدام الرحلان الكهربائي لبروتينات المصل و دلالات ورم سرطان الثدي كعوامل مراقبة لاستجابة المريض للعلاج أمرًا مثيرًا للجدل. تهدف الدراسة الحالية إلى تحديد نمط الترحيل الكهربائي لبروتينات المصل وتحديد مستضداتمؤشرات الاورام واستخدامها كمعلمات حيوية جديدة في متابعة مرضى سرطان الثدي تحت العلاج الإشعاعي أو العلاج الكيميائي وبعده.

تضمنت الدراسة23 مريضة بسرطان الثدي بعد استئصال الثدي في مرحلة ضمن أو بعد العلاج الكيميائي والعلاج الإشعاعي. تم قياس مؤشرات الاورام (المستضد السرطاني المضغي، المستضد السرطاني 125 و المستضد السرطاني 15−3) بطريقة التألق الكهروكيميائي. تم تقدير مستويات بروتينات المصل الكلية بطريقة شبة الية وباستخدام العدة المتوفرة تجاريا وتم التقدير الكمي لبروتينات المصل باستخدام الترحيل الكهربائي بهلام الاغاروز.

أظهرت مستويات البروتينات الكلية في الدم زيادة غير معنوية عند المرضى ممن هم في فترة تحت اوما بعد العلاج بالمقارنة مع مجموعة السيطرة. كما لوحظ انخفاض معنوي في مستوى الألبومين في الدم عند المرضى تحت العلاج وبعدة. بالاضافة الى ذلك، فقد اظهرت مستويات الغلوبيولين كاما و الغلوبيولين الالفا 2 زيادة معنوية عند المرضى. واظهرت الاجزاء الفا−1، الفا−2، غاما، المستضد السرطاني 125 والمستضد السرطاني 15−3 اعلى حساسية ونوعية.

1. Introduction:

Globally, the burden of breast cancer (BCa) among women is significant where it is the cancer with the highest incidence and mortality rates. BCa accounts for 2.3 million of diagnosed cancer cases, representing around 11.7% of all cancers in the world [1], which constitutes about one third of the registered cancer cases among the Iraqi population [2].

BCa evaluation often includes a biopsy and assessment of tumor estrogen receptor (ER), progesterone receptor (PgR), tumor HER2 status and computerized tomography (CT) staging and a whole-body bone scan.

Growing evidence suggests that serum tumor markers, a less invasive and cost-effective method, may provide a tool for supporting the diagnosis of BCa and may also monitor the response of the disease to therapy [3]. Nevertheless, the feasibility of using tumor markers is still of much debate. In fact, irrational utilization of tumor markers is full of risks of mistreatment (under-treatment or over-treatment) and its consequences. Therefore, there is an urgent need for designing a highly sensitive and specific tumor markers panel for monitoring breast cancer patients during and post radiotherapy or chemotherapy.

The most commonly used breast cancer tumor markers are carcinoma antigen 15.3 (CA15.3), carcinoembryonic antigen (CEA) and cancer antigen 27.29 (CA27.29). All three are recommended for use by the American Society of Clinical Oncology (ASCO) [3]. CA 15-3 is a protein encoded by the *MUC1* gene. It is produced by a variety of cells, in particular glandular epithelial cells in alveoli and mammary ducts in normal mammary tissues. In malignant breast cancer highly elevated MUC1 expression results in 10-fold increase of this protein [4]. CEA is a group of glycoproteins that are usually produced during fetal development, and their production ceases prior to birth. This group's elevation is utilized as a tumor marker to monitor the progress of treatment of various carcinomas, to detect recurrences and for the staging of tumors [5].

However, a recent study by Zhang *et al.* has mentioned a sharp elevation in CEA and CA153 levels in post adjuvant chemotherapy patients and noticed a linear relationship with their

levels before adjuvant chemotherapy and excluded the effects of chemotherapy on tumor markers levels, suggesting a potential feasibility for breast cancer monitoring [6]. Another study by Uygur and Gumus assessed the utility of CEA and CA15-3 for metastasis prediction and established the cut off values for both [7]. The present study aimed to designate serum protein electrophoresis SPE pattern, CEA, CA 125 and CA 15-3 as novel biomarkers in monitoring breast cancer patients under and post radiotherapy or chemotherapy.

2. Materials and Methods

2.1 Subjects

The study enrolled 23 BCa women patients treated at Al-Amal Hospital (Baghdad/ Iraq) from 2020 to 2021. And 25 apparently healthy control HC women with no evidence of BCa and/or ovarian cancer were selected as case-control group. The patients were diagnosed with BCa and under post mastectomy radiotherapy. Informed consent was obtained from all enrolled patients. The study was performed in accordance with the ethical standards of the Declaration of Helsinki 1975 and was approved by the ethical review boards of Al-Amal hospital.

2.2 Blood Samples

Briefly, 5 ml of whole blood samples were collected by venipuncture, and then transferred to plain tubes and centrifuged at 3000 rpm for 10 minutes after an hour of collection to obtain serums. Serum samples were kept frozen at ¯20°C for further experiments for tumor markers and serum protein electrophoresis.

2.3 Tumor Markers

Tumor markers CEA, CA 15-3, and CA 125 were quantitatively measured by using electrochemiluminescence assay (ECLIA) on Roche cobas®, Germany.This method is based on the use of a ruthenium-complex and tripropylamine (TPA). The chemiluminescence reaction for the detection of the reaction complex is initiated by applying a voltage to the sample solution resulting in a precisely controlled reaction [8]. Results were considered positive or negative if they were above or below the cut-off values provided by the manufacturer.

2.4 Total Serum Protein and Serum Protein Electrophoresis

Total serum protein edtimation tests were performed by using commercially available kits by the addition of the Biuret reagent, and the reagent copper content bindwith peptide bondsthen a purple coloured complex forms. The amount of complex formed is directly proportional to the amount of total protein in the sample which is then measured by semiautomated chemistry analyzer. Quantitative estimation of serum proteins albumin, alpha-1, alpha-2, beta, and gamma globulin was accomplished by agarose serum protein electrophoresis after which the proteins were separated according to the charge by using tris-barbital buffer and acid blue stain to allow visualization and quantitative estimation of serum proteins [9].

2.5 Statistical Analysis

Statistical analysis of results was carried out by using IBMSPSS version 24 computer software. Continuous variables are reported as means ± standard deviations (SD). *P*-value less than 0.05 was considered statistically significant. Quantitative variables between healthy controls and Bca patients were tested using student's t-test. The receiver operating characteristic (ROC) method was used to evaluate the specificity and sensitivity of quantitative tests to differentiate between the diseased cases and healthy controls .

3. Results

All patients in this study were post mastectomy and under chemotherapy. The mean serum total proteins levels in BCa patients and HC women were 7.145 g/dl and 7.131 g/dl respectively. This result showed a non-significant increase in under and post therapy compared to HC.

The mean serum values in BCa patients for CEA, CA-125, CA 15-3 were 1.887 ±.974, 50.1733 and 28.71± 20.41 accordingly. For the HC women group, the CEA, CA-125, CA 15-3 mean values were 1.85 ±0.89, 8.51 ±8.14 and 12.18±3.38 accordingly. The correlation was significant for CA15-3 and CA 125 ($p > 0.05$) and non-significant for CEA between BCa group and HC women group (Table 1).

Table 1: The correlations of the serum tumor markers between the BCa and HC groups.

Tumor Marker	HC Group	BCa Group	*P-value*
CEA			0.357 (NS)
Mean	1.8510	1.887	
SD	.9	5.6	
N	25	23	
CA-125			0.045
Mean	8.51	50.17	
SD	8.14	93.9	
N	25	23	
CA15-3			<0.001
Mean	12.18	28.71	
SD	3.38	20.41	
N	25	23	

There was a significant correlation between CA-15 and CA-125 in BCa group, while the correlation between CEA and CA125, CEA and CA15-3 was insignificant (Table 2). Whereas, in HC women group, the correlation was significant between CA 125 and CEA, CA125 and CA15-3 (Table 3).

Table 2: The correlation of tumor markers CAE, CA-125 and CA15-3 in BCa group.

		CEA	CA15	CA125
CEA	Pearson Correlation	1	-.024	-.217
	Sig. (2-tailed)		.925	.387
	N	23	23	23
CA15-3	Pearson Correlation	-.024	1	.859**
	Sig. (2-tailed)	.925		.000
	N	23	23	23
CA125	Pearson Correlation	-.217	.859**	1
	Sig. (2-tailed)	.387	.000	
	N	23	23	23

** Correlation is significant at the 0.01 level (2-tailed).

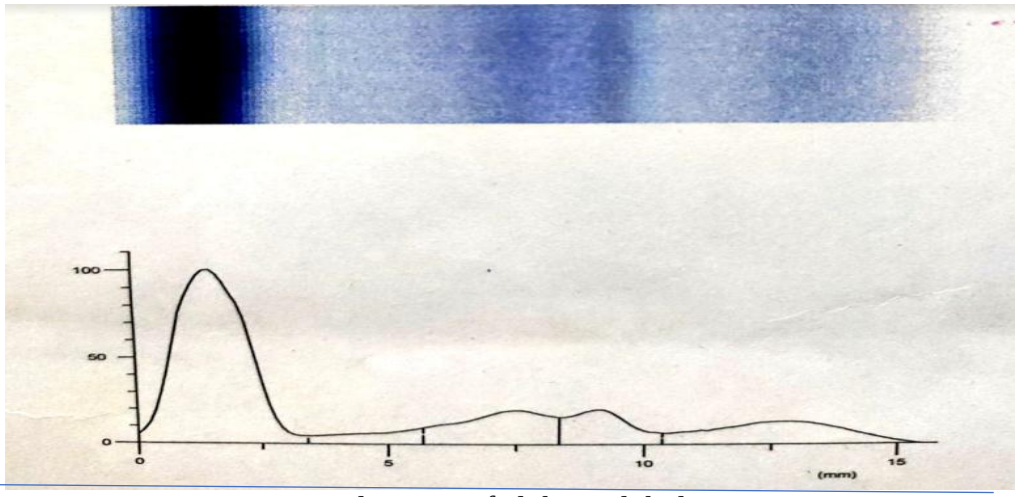
Table 3: The correlation between tumor markers: CAE, CA125 and CA15-3 in HC women group.

		CEA	CA15-3	CA125
CEA	Pearson Correlation	1	.019	.536*
	Sig. (2-tailed)		.936	.012
	N	25	25	25
CA15-3	Pearson Correlation	.019	1	-.494*
	Sig. (2-tailed)	.936		.023
	N	25	25	25
CA125	Pearson Correlation	.536*	-.494*	1
	Sig. (2-tailed)	.012	.023	
	N	25	25	25

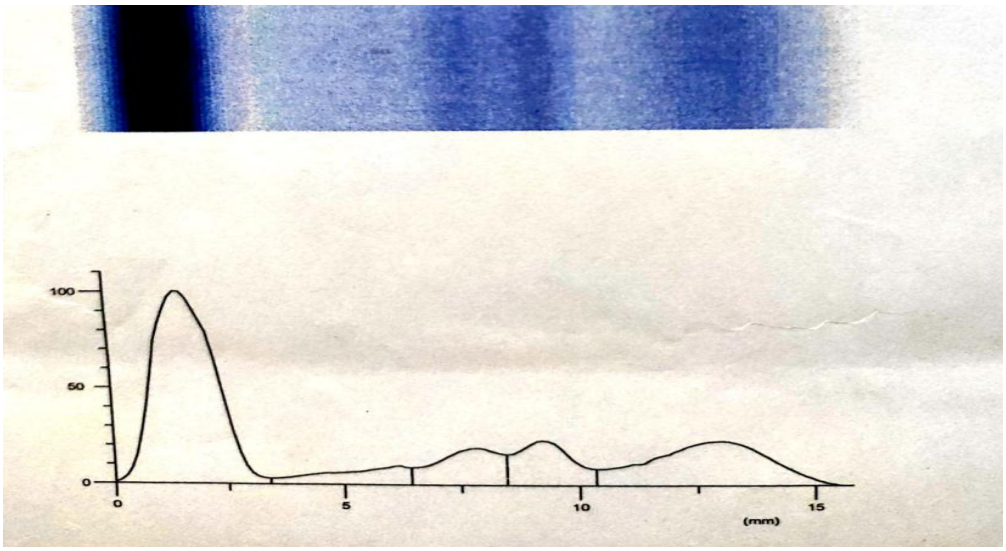
Table 4: ROC area for serum protein fractions and selected tumor markers when used as a test to diagnose BCa differentiating it from HC.

Area Under the Curve

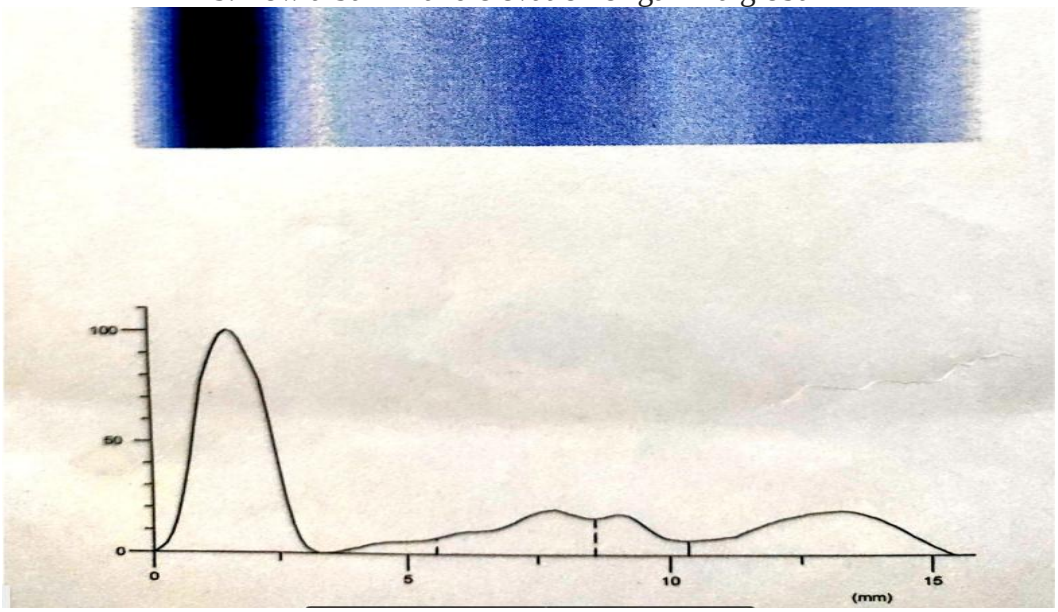
Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
CEA	.447	.112	.630	.228	.666
CA15-3	.832	.088	.002	.660	1.000
CA125	.813	.080	.004	.655	.970
TOTALPROTEIN	.543	.112	.693	.324	.763
ALBUMIN	.029	.031	.000	.000	.089
ALPHA1	.978	.023	.000	.934	1.000
ALPHA2	.942	.053	.000	.839	1.000
BETA	.329	.101	.120	.131	.527
GAMMA	.800	.081	.006	.641	.960



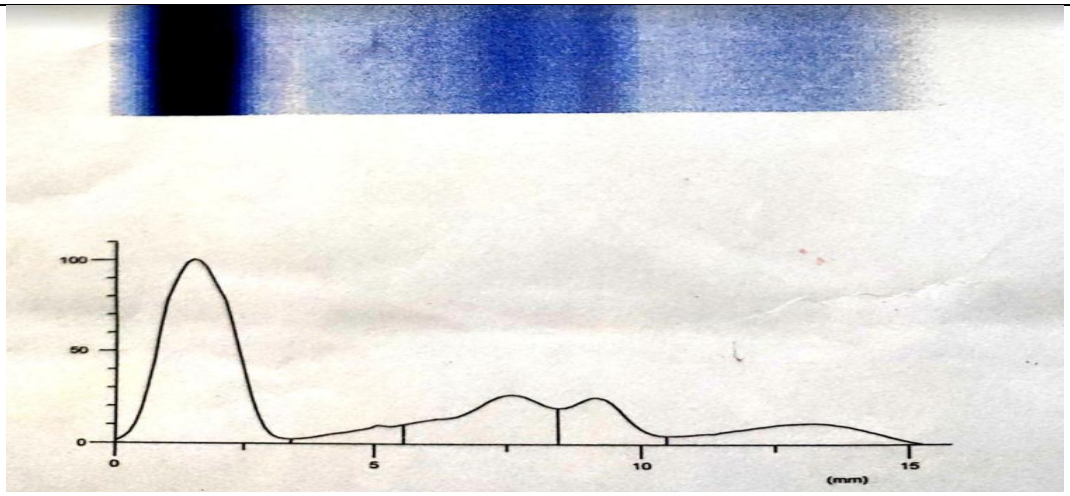
a. Elevation of alpha 2 globulin



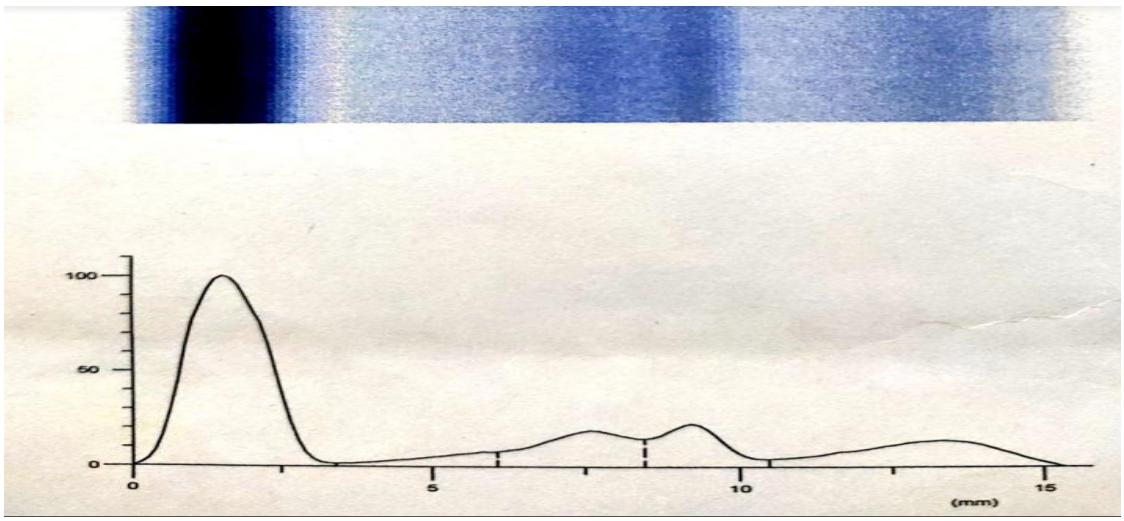
b. Low albumin and elevation of gamma globulin



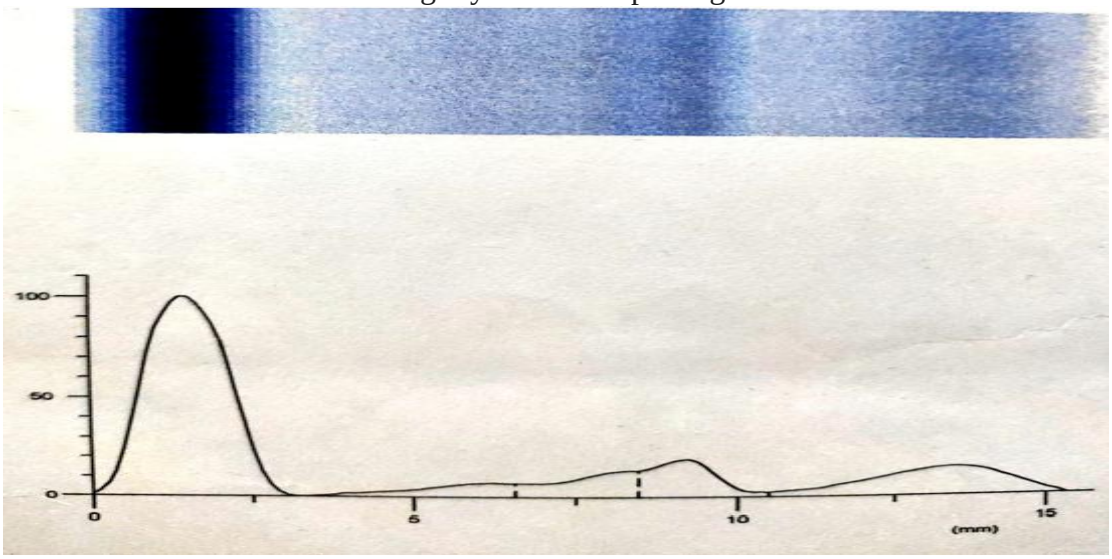
c. Low albumin, elevated alpha2 and gamma globulin.



d. Low albumin, elevation of alpha 2 globulin.



e. Slightly elevated alpha 2 globulin.



F. Normal pattern.

Figure 1: Serum protein electrophoresis patterns showing the existing of some abnormalities of the five fractions: a-e: abnormal patterns in breast cancer patients, f. normal pattern.

Discussion

Most recently, serums CEA, CA125, and CA15-3 have been routinely used for breast cancer diagnosis and for determining the success of mastectomy or the efficacy of chemotherapy. It has been proposed that the detection of elevated levels of these tumor markers after surgery would indicate either incomplete elimination of the tumor, recurrence or the occurrence of metastases [10]. However, due to their low sensitivity and /or specificity, their clinical value is still controversial. Thus, designing a tumor marker panel consisting of a combination of serum tests is an urgent need.

Multiple tumor markers are routinely ordered for cancer diagnosis and monitored due to the heterogeneity in cell composition of each tumor [11]. The most important criterion for ideal tumor markers are specificity and sensitivity to detect small tumors to allow early diagnosis or help in monitoring. The current study utilized the ROC curve to measure specificity and sensitivity of the included parameters. Alpha-1, alpha2, gamma globulin fractions, CA 125 and CA-15-3 showed the highest sensitivity and specificity (Table 4).

CA15-3 is routinely used for therapy monitoring and predicting recurrences in breast cancer patients [7]. The current study observed a significant elevation (p>0.001) in CA15-3 in BCa group when compared to HC women and a high sensitivity and specificity. However, some previous studies reported a transient elevation that was followed by a decrease in CA15-3 during and post chemotherapy [12]. In the current study, the patients were clinicopathologically heterogeneous and were treated with different intervals and chemotherapeutic agents. Our results agreed with a previous study by Yang *et. al*. which reported elevated CA15-3 levels and a correlated relationship with hormonal status, number and location of metastatic lections in BCa patients after mastectomy and chemo-radio therapy [13]. However, further investigation is still required to establish a reliable conclusion.

Previous studies have suggested that CEA could be useful in the postsurgical follow-up of breast cancer patients for an early detection of recurrence and for monitoring treatment response. The current study observed a non-significant difference in CEA levels between healthy control and BCa group and a low sensitivity of CEA. This result agrees with a study by Shinkins *et al.* [14] which also reported a low sensitivity of CEA in detecting colorectal cancer.

In the current study, alpa-2 globulin levels elevated in BCa patients which may be attributed to haptoglobin elevation [15] [16]. This assumption is further evidenced by a study by Chen *etal.* [17] who reported a significant role of haptoglobin in breast cancer oncogenicity and resistance to chemotherapy by regulating cell cycle progression and apoptosis in breast cancer cells. This result may support the presumptive feasibility of haptoglobin as prognosticator for breast cancer.

In the current study, a significant elevation of gamma-globulin was observed in BCa patients. This elevation has also been reported in a variety of other cancer types including cervical [18] and nervous system lymphoma [19]. This elevation in gamma-globulin may be attributed to increased immunoglobulins, the main component of the γ-globulin fraction.
However, growing evidence has revealed a high level of Igs expression in malignant cells. Cancer-derived Ig showed pro-tumorigenic effects *via* multiple mechanisms, including enhancing the malignant behaviors of cancer cells, mediating tumor immune escape, inducing inflammation and activating the aggregation of platelets [20]. Thus, cancer-derived Ig may provide a promising potential as a diagnostic and therapeutic target in cancer patients.

As a major blood protein, albumin has versatile roles as an antioxidant, detoxifier and transporter of drugs [21] and important nutrients. Serum albumin levels have been widely documented as markers for evaluating patient's nutritional status and as a prognosticator for cancer survival rate. In fact, many studies have established a correlation between serum albumin levels and various cancer cases. A study by Saito *et al.* [22] mentioned a correlation between gastric cancer outcomes and decreased pre- and post-operative albumin levels. Another study by Jing *et al.* [23] also concluded the same correlation with urethral cancer. In the current study, serum albumin levels decreased in 56% of under therapy BCA patients with a mean value 3.93 ± 0.36. These results agree with the results of studies by Hwang *et al.* [24] and Xiaoan *et al*. [25] who noticed a positive correlation between breast cancer prognosis and preoperative albumin levels. The results of the current study however disagree with the results of the study conducted by Ibrahim *et al*. [2] in which no significant changes in serum albumin were reported. However, the underlying mechanism for albumin decrease may be explained by malnutrition [26], [27] and systematic inflammatory response to tumors which suppresses albumin synthesis by the liver. The most common consequences of malnutrition can be summarized by decreased life quality, low treatment response and increased treatment-related toxicity.

Conclusion:

Serums CA 125, CA15-3 gamma globulin and alpha-2 globulin can be used as monitoring parameters for breast cancer patients' response to chemotherapy and radiotherapy. These biomarkers introduced a high sensitivity and specificity in differentiating under and post chemotherapy therapy BCa patients from healthy women group.

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