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Evaluation of Activated Leukocyte Cell Adhesion Molecule as a Biomarker for Breast Cancer in Egyptian Patients

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Abstract: Breast cancer is an important public health issue. It is the most common cancer affecting women worldwide. The ability to detect human malignancy via a simple blood test has long been a major objective in medical screening. In this study, serum activated leukocyte cell adhesion molecule (ALCAM) levels were evaluated in 41 primary breast cancer patients and 20 healthy women and its diagnostic value was quantified and compared with those of carbohydrate antigen 15-3 (CA15-3) and carcinoembryonic antigen (CEA). Also, its prognostic value was examined. Serum ALCAM levels were also evaluated before and after surgical treatment. Serum levels of ALCAM and CA 15-3 were significantly higher in breast cancer patients than healthy controls (P=0.002, P=0.043 respectively), but the difference in serum CEA levels did not reach statistical significance. Serum ALCAM levels had significant area under the curve (AUC) (P=0.002), but serum levels of CA 15-3 and CEA had non-significant AUCs and various combinations between them did not result in any improvement. A significant association was found between serum levels of ALCAM and CEA with age and menopausal status in breast cancer patients. Non-significant difference was shown in serum levels of ALCAM, CA 15-3 and CEA before and after surgical treatment. In conclusion, this study suggests that serum ALCAM may represent a diagnostic biomarker for early detection of breast cancer.

Key words: ALCAM · Breast cancer · CA15-3 · CEA · Biomarker

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide, accounting for 23% of the total new cancer cases and 14% of the total cancer deaths in 2008 [1]. In Egypt, breast cancer is the most common malignancy among Egyptian females, accounting to about 37.6% of all malignancies [2]. Cell adhesion molecules (CAMs) are cell surface receptors that mediate cell-cell and cell-substrate interactions. These molecules can be grouped into four families: integrins, cadherins, selectins and the immunoglobulin (Ig)-like CAMs (Ig-CAMs) superfamily [3]. Alterations in cellular adhesion and communication can contribute to uncontrolled cell growth [4]. Activated leukocyte CAM (ALCAM) is a glycoprotein cell surface Ig superfamily member involved in cell-cell interactions through homophilic and heterophilic (ALCAM-Cluster of differentiation [CD]6) binding [5,6]. ALCAM has 5 extracellular Ig domains (2 NH_2-terminal, membrane-distal variable-(V)-type and 3 membrane-proximal constant-(C_2)-type Ig folds) [D1–D5], transmembrane region and a short cytoplasmic tail [6, 7]. The N-terminal domain (D1) regulates affinity, whereas membrane proximal domains D4 and D5 control affinity [6, 8]. The cytoplasmic tail contains 32 amino acid residues [9]. The molecular weight of the native protein is 65 kDa and N-glycosylation at 8 putative sites results in a mature ALCAM species of 110

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kDa [10]. ALCAM is expressed in activated lymphocytes, neuronal cells, hepatocytes, pancreatic cells and selected epithelia (i.e. in mammary ducts and acini), as well as in embryonic cells, i.e. bone marrow, endothelial and yolk sac cells [11,12]. ALCAM may act as a cell surface sensor to register local growth saturation and to regulate cellular signaling and dynamic responses [13]. ALCAM-CD6 interaction is required for optimal activation of T-cells suggesting a possible ALCAM involvement in the immunologic response to tumor cells [14]. ALCAM may favor interactions between tumor and endothelial cells [13].

In this study, evaluation of serum ALCAM levels was estimated in healthy controls and patients suffering from breast cancer and its diagnostic value was quantified, aiming to investigate if ALCAM, either alone, or in combination with the classical breast cancer biomarkers (carbohydrate antigen 15-3 [CA15-3] and carcinoembryonic antigen [CEA]) represent a strategy for breast cancer diagnosis with high sensitivity and specificity in serum, in an attempt to find a simple diagnostic blood test for early detection of breast cancer. The association between serum ALCAM levels with various clinicopathologic parameters was also examined. The study is also aiming to evaluate serum ALCAM levels in breast cancer patients before and after surgical treatment.

MATERIALS AND METHODS

Subjects: This study was carried out on forty one Egyptian women with histopathologically proven primary breast cancer, they were admitted to National Cancer Institute, Cairo University, from January 2011 to June 2011 and twenty healthy Egyptian women matched in age and socioeconomic status. They were divided into two groups:

Group 1: 20 healthy women were considered as a normal control group (age, mean ± standard deviation [SD], 49.950±11.095 years; 12 premenopausal, 8 postmenopausal).

Group 2: 41 women breast cancer patients before taking any type of treatment (age, mean ± SD, 50.150±10.468 years; 19 premenopausal, 22 postmenopausal). 15 from them were followed up after surgical treatment (9 modified radical mastectomy, 2 simple mastectomy, 4 breast conserving surgery).

Table 1: The clinicopathological characteristics of breast cancer patients.

Clinicopathological characteristics	Breast cancer patients n (%)
Age (years)	
≤50	18 (44%)
>50	23 (56%)
Menopausal status	
Pre	19 (46%)
Post	22 (54%)
Histological type	
invasive duct carcinoma	31 (75%)
invasive lobular carcinoma	4 (10%)
Metaplastic carcinoma	2 (5%)
mixed invasive duct and lobular carcinoma	4 (10%)
Tumor size	
T1	3 (7%)
T2	20 (49%)
T3	6 (15%)
Unknown	12 (29%)
Histological grade	
Grade II	27 (66%)
Grade III	6 (15%)
Unknown	8 (19%)
Estrogen receptor (ER) status	
Positive	23 (56%)
Negative	14 (34%)
Unknown	4 (10%)
Progesterone receptor (PR) status	
Positive	23 (56%)
Negative	14 (34%)
Unknown	4 (10%)
Human epidermal growth factor receptor-2 (HER-2)/neu status	
Positive	6 (15%)
Negative	27 (66%)
Unknown	8 (19%)
Lymph node status	
Positive	27 (66%)
Negative	10 (24%)
Unknown	4 (10%)

n = Number of patients.
T = Tumor size

Exclusion Criteria:

- Subjects that had a history of any serious or chronic diseases.
- Subjects that had a history of any type of cancer.

An informed phrasal consent was obtained from each subject and the study was approved by the local committee of Ethics of the Scientific Research of the Faculty of Medicine. The clinicopathological data of breast cancer patients are shown in Table 1.

Specimen Collection: Venous blood samples were collected into vacutainer tubes containing clot activator after 12 hours overnight fasting and were left to clot at room temperature, then were centrifuged at 3000 rpm for 10 minutes to remove serum, which was stored at -20ºC until further analysis.

Measurements: Serum ALCAM was determined using enzyme-linked immunosorbent assay (ELISA) technique by using RayBio® human ALCAM ELISA kit (RayBiotech Inc. USA). Serum CA 15-3 was determined using immunoradiometric assay (IRMA) technique by using MUC-1 gene associated antigen (CA 15-3) IRMA kit (Immunotech, France). Serum CEA was determined using IRMA technique by using IRMA-coat® CEA kit (DiaSorin Inc. USA). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using colorimetric method according to Sherwin [15]. Serum urea was determined using urease-colorimetric method (modified urease-berthlot method) according to Tietz [16]. Serum creatinine was determined using buffered kinetic Jaffé reaction without deproteinization method according to Tietz [17].

Statistical Analysis: Data were presented as mean ± SD. Independent-samples t-test was used to compare variables between breast cancer patients and healthy controls and to examine the association between serum levels of ALCAM, CA 15-3 and CEA with various patients and tumor characteristics. Spearman's rank correlation coefficient was used to assess the correlations among biomarkers. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic usefulness of the markers. For each ROC curve, the area under the curve (AUC) was calculated. The ROC curve analysis was first conducted on individual markers and then in combination, to explore the potential that a marker panel can lead to improved performance. Paired-samples t-test was used to compare serum levels of biomarkers in breast cancer patients before and after surgical treatment. $P<0.05$ was considered to be statistically significant. Statistical analysis was performed using statistical package for the social sciences (SPSS) version 15 software, while the presentations were performed using Microsoft Excel 2007.

RESULTS

In the present study serum levels of ALCAM, CA 15-3, CEA, liver functions (AST, ALT) and renal functions (urea, creatinine) were evaluated in breast cancer patients. The results of patients were compared with those of healthy controls. The diagnostic value of serum levels of ALCAM, CA 15-3 and CEA was evaluated. The association between serum levels of ALCAM, CA 15-3 and CEA with various clinicopathologic parameters (age, menopausal status, tumor size, histological grade, ER status, PR status, HER-2/neu status, lymph node status) was examined. Serum levels of ALCAM, CA 15-3 and CEA were also evaluated and compared in patient group before and after surgical treatment. Serum ALCAM levels were significantly higher in breast cancer patients than healthy controls (patients mean ± SD, 97.00±10.65 µg/L; controls mean ± SD, 86.41±9.81 µg/L; $P \leq 0.002$). There were a significantly higher serum CA 15-3 levels in breast cancer

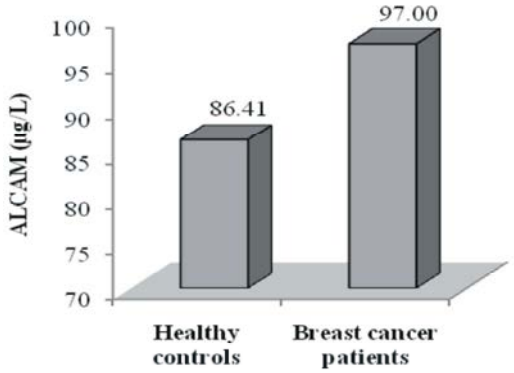

Fig. 1: Serum ALCAM levels (mean) in healthy controls and breast cancer patients.

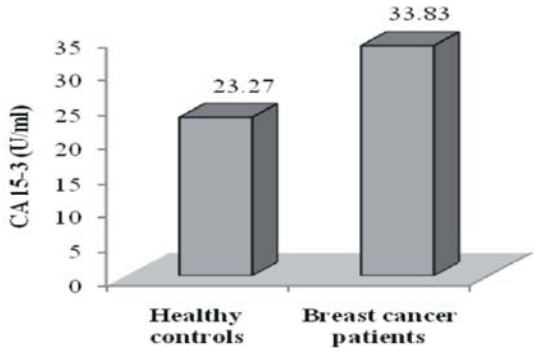

Fig. 2: Serum CA 15-3 levels (mean) in healthy controls and breast cancer patients.

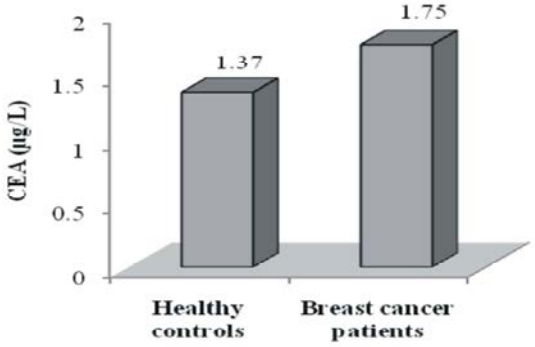

Fig. 3: Serum CEA levels (mean) in healthy controls and breast cancer patients.

patients as compared with those of healthy controls (patients mean ± SD, 33.83±19.21 U/ml; controls mean ± SD, 23.27±12.68 U/ml; P= 0.04), but the difference in serum CEA levels did not reach statistical significance (patients mean ± SD, 1.75±1.44 µg/L; controls mean ± SD, 1.37±0.80 µg/L; P=0.29) (Figs. 1-3). There were no significant differences between breast cancer patients and healthy controls with respect to serum levels of AST, ALT, urea and creatinine. Table 2 illustrates the correlation between serum levels of ALCAM, CA 15-3 and CEA in healthy controls and breast cancer patients. No statistical correlation was shown between serum levels of ALCAM, CA 15-3 and CEA in the examined groups.

Figure (4) illustrates the ROC curves of serum levels of ALCAM, CA 15-3 and CEA. Results of the ROC curves analysis of serum levels of ALCAM, CA 15-3 and CEA and various combinations between them are given in Table 3. Serum ALCAM levels had significant AUC (P=0.002), but serum levels of CA 15-3 and CEA had non-significant AUCs. Combining serum levels of ALCAM and CA 15-3, serum levels of ALCAM and CEA and serum levels of ALCAM, CA 15-3 and CEA had significant AUCs (P=0.005, P=0.003, P=0.004 respectively), but Combining serum levels of CA 15-3 and CEA had non-significant AUC. At specificity of 70%, serum ALCAM levels yielded a sensitivity of 77%, compared with 59% for serum CA15-3 levels and 29% for serum CEA levels. At specificity of 80%, serum ALCAM levels yielded a sensitivity of 65%, compared with 47% for serum CA15-3 levels and 18% for serum CEA levels. Likewise, at 90% specificity, serum ALCAM levels displayed higher sensitivity than serum levels of CA15-3 and CEA. Various combinations between them did not yield any improvement in the sensitivity compared with serum ALCAM levels.

Table 4 illustrates the association between serum levels of ALCAM, CA 15-3 and CEA with various patients and tumor characteristics such as age, menopausal status, tumor size, histological grade, ER status, PR status, HER-2/neu status and lymph node status in breast cancer patients. No statistical association was shown between serum levels of ALCAM, CA 15-3 and CEA with various clinicopathologic parameters in breast cancer patients except that, there was a significant association between serum levels of ALCAM and CEA with age and menopausal status. Breast cancer patients with age >50 years displayed significantly higher serum levels of ALCAM and CEA than breast cancer patients with age ≤50 years (P=0.001, P=0.016, respectively). Also, postmenopausal breast cancer patients displayed significantly higher serum levels of ALCAM and CEA than premenopausal breast cancer patients (P=0.002, P=0.015 respectively). Table 5 illustrates serum levels of ALCAM, CA 15-3 and CEA in breast cancer patients before and at one month after surgical treatment. Non-significant difference was shown in any of them before and after surgical treatment.

Table 2: Correlation between serum levels of ALCAM, CA 15-3 and CEA in healthy controls and breast cancer patients.

	Healthy control			Breast cancer patients		
Item	ALCAM	CA 15-3	CEA	ALCAM	CA 15-3	CEA
ALCAM	1	-0.198	0.243	1	0.260	0.212
P	-	0.447	0.347	-	0.232	0.333
CA 15-3	-	1	0.414	-	1	-0.183
P	-	-	0.098	-	-	0.404
CEA	-	-	1	-	-	1

P value < 0.05 is statistically significant.

Table 3: ROC curves analysis of serum levels of ALCAM, CA 15-3 and CEA and various combinations between them.

			Sensitivity		
Item	AUC	P	90% Specificity	80% Specificity	70% Specificity
ALCAM	0.79	0.002	0.41	0.65	0.77
CA 15-3	0.67	0.07	0.24	0.47	0.59
CEA	0.55	0.58	0.18	0.18	0.29
Combining ALCAM and CA 15-3	0.76	0.005	0.18	0.59	0.65
Combining ALCAM and CEA	0.78	0.003	0.41	0.59	0.77
Combining ALCAM, CA 15-3 and CEA	0.77	0.004	0.18	0.59	0.71
Combining CA 15-3 and CEA	0.67	0.07	0.24	0.47	0.59

P value < 0.05 is statistically significant.

Table 4: Association between serum levels of ALCAM, CA 15-3 and CEA with various clinicopathologic parameters in breast cancer patients.

Item	n	ALCAM (µg/L) Mean ± SD	CA 15-3 (U/ml) Mean ± SD	CEA (µg/L) Mean ± SD
Age (years)				
≤50	18	88.44±18.97	31.63±16.81	1.14±0.67
>50	23	106.52±12.99	35.47±22.38	2.12±1.70
P		0.001	0.56	0.016
Menopausal status				
Pre	19	88.08±17.20	28.78±16.23	1.13±0.68
Post	22	105.24±16.27	36.35±20.94	2.13±1.68
P		0.002	0.23	0.015
Tumor size				
T1+T2	23	97.88±10.83	34.57±17.43	1.36±0.80
T3	6	98.75±11.41	33.82±24.68	2.31±2.35
P		0.90	0.95	0.11
Histological grade				
Grade II	27	98.03±11.10	32. 70±18.20	1.62±1.36
Grade III	6	95.50±10.42	31. 25±22.79	0.98±0.74
P		0.68	0.89	0.14
ER status				
Positive	23	98.00±11.18	28.90±15.22	1.42±0.90
Negative	14	96.31±8.54	34.55±21.79	1.43±1.67
P		0.69	0.41	0.97
PR status				
Positive	23	97.43±10.36	30.45±17.30	1.36±0.84
Negative	14	97.44±10.58	32.44±19.97	1.52±1.72
P		0.998	0.77	0.76
HER-2/neu status				
Positive	6	95.63±9.97	35.87±21.70	1.21±0.62
Negative	27	97.42±10.24	31.19±19.19	1.57±1.40
P		0.76	0.67	0.35
Lymph node status				
Positive	27	97.29±10.04	32.80±18.45	1.44±0.85
Negative	10	98.07±10.99	27.75±16.97	1.44±1.98
P		0.88	0.45	0.995

P value < 0.05 is statistically significant.

n = Number of patients.

T = Tumor size

Table 5: Serum levels of ALCAM, CA 15-3 and CEA in breast cancer patients before and at one month after surgical treatment.

Item	Breast cancer patients before surgical treatment (Mean ± SD) n=15	Breast cancer patients after surgical treatment (Mean ± SD) n=15	P
ALCAM (µg/L)	92.61±13.72	96.17±20.18	0.53
CA 15-3 (U/ml)	33.73±18.21	31.30±14.34	0.38
CEA (µg/L)	1.24±0.87	1.12±0.79	0.26

P value < 0.05 is statistically significant.

n = Number of patients.

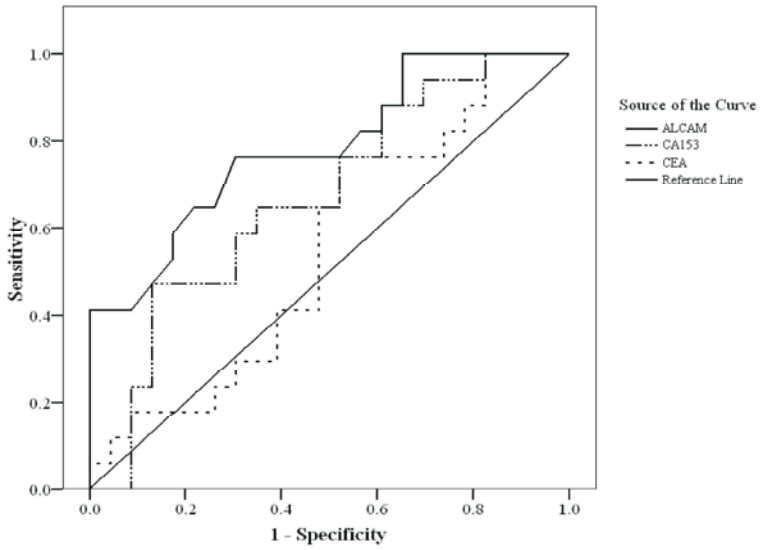

Fig. 4: ROC curves of serum levels of ALCAM, CA 15-3 and CEA.

DISCUSSION

Unfortunately, other than definitive diagnosis by biopsy and histopathology, no diagnostic or screening test is presently suitable for early detection of breast cancer [18]. The ability to detect human malignancy via a simple blood test has long been a major objective in medical screening. CA15-3 and CEA, discovered more than 2 and 4 decades ago, respectively, are the most commonly used tumor markers for breast cancer [19-21]. CA15-3 and CEA levels in serum are recommended for monitoring therapy of advanced breast cancer [18]. However, these cancer biomarkers have proven to be ineffective in detecting the early stages of the disease because of low diagnostic sensitivity and specificity [22, 23]. This study shows that serum ALCAM levels were significantly higher in breast cancer patients than healthy controls. This result is in agreement with previous studies which demonstrated that serum ALCAM levels were significantly elevated in breast cancer patients when compared with healthy controls [24, 25]. In this study, also there were a significantly higher serum CA 15-3 levels in breast cancer patients as compared with those of healthy controls, but the difference in serum CEA levels did not reach statistical significance. According to serum CA 15-3 levels this finding is in agreement with Kulasingam *et al.* [24] who reported that serum CA 15-3 levels were significantly elevated in breast cancer patients when compared with healthy controls. However, according to serum CEA levels the result is inconsistent with Kulasingam *et al.* [24] who found that serum CEA levels were significantly elevated in breast cancer patients when compared with healthy controls. Elevated serum ALCAM levels may be due to shedding of the protein into the serum by a disintegrin and metalloproteinase (ADAM)-17, also known as tumor necrosis factor-α (TNF-α)-converting enzyme (TACE).

ADAM-17 is one of the most widely investigated ADAMs and one of the most important sheddases identified to date [26, 27]. Based on a proteomic approach, Bech-Serra *et al.* [28] showed that ALCAM is an ADAM-17 substrate. Rosso *et al.* [29] and Miccichè *et al.* [30] indicated that surface ALCAM can be actively cleaved by ADAM-17-mediated proteolysis in epithelial ovarian cancer cells and thyroid cancer. Lendeckel *et al.* [31] reported higher levels of ADAM-17 mRNA in 24 breast cancers compared with corresponding normal breast tissue. Also, McGowan *et al.* [32] and Narita *et al.* [33] observed that, at both mRNA and protein levels, ADAM-17 expression was up-regulated in breast cancer compared with normal breast tissue. The proportion of active form to total ADAM-17 increased progressively from normal breast tissue to primary breast cancer to lymph node metastases [32]. In breast cancer [34-36] and ovarian cancer [37], ALCAM cytoplasmic overexpression and low membrane expression were associated with disease progression. The clinical relationship of membrane ALCAM loss with progression may relate to the process of ALCAM shedding by ADAM-17 [30]. Witzel *et al.* [25] illustrated that, his finding that elevated serum ALCAM levels were not significantly correlated with high ALCAM expression in tumor tissue was not

contradictory, where he suggested that ALCAM serum levels may be a sign of receptor activation and active shedding of the protein into the serum. No statistical correlation was shown between serum levels of ALCAM, CA 15-3 and CEA in healthy controls and breast cancer patients. However, Kulasingam *et al.* [24] observed that CEA appeared to be weakly correlated with ALCAM in both cases and controls, whereas CA15-3 was weakly correlated with ALCAM among cases only.

By studying ROC curves of serum levels of ALCAM, CA 15-3 and CEA and various combinations between them, this study shows that serum ALCAM levels had significant AUC, but serum levels of CA 15-3 and CEA had non-significant AUCs and various combinations between them did not result in any improvement in the AUC compared with serum ALCAM levels. These results are in agreement with Kulasingam *et al.* [24] who demonstrated that serum ALCAM levels had significant AUC. However, Kulasingam *et al.* [24] also demonstrated that serum levels of CA 15-3 and CEA had significant AUCs, but ALCAM had the best performance. Also, Kulasingam *et al.* [24] illustrated that combining CA15-3 and ALCAM yielded a ROC curve with higher AUC than ALCAM and combining CA15-3, ALCAM and CEA did not result in any improvement in ROC curves compared with CA15-3 and ALCAM. At specificity of 90%, 80% and 70%, serum ALCAM levels displayed higher sensitivity than serum levels of CA15-3 and CEA. Various combinations between them did not yield any improvement in the sensitivity compared with serum ALCAM levels. These findings are consistent with Kulasingam *et al.* [24] who showed that at 90% and 80% specificity, ALCAM displayed higher sensitivity than CA15-3 and CEA. However, Kulasingam *et al.* [24] also showed that combining CA15-3 and ALCAM yielded a higher sensitivity than ALCAM.

In this study, no statistical association was shown between serum levels of ALCAM, CA 15-3 and CEA with various clinicopathologic parameters in breast cancer patients except that, there was a significant association between serum levels of ALCAM and CEA with age and menopausal status. Breast cancer patients with age >50 years displayed significantly higher serum levels of ALCAM and CEA than breast cancer patients with age ≤50 years. Also, postmenopausal breast cancer patients displayed significantly higher serum levels of ALCAM and CEA than premenopausal breast cancer patients. These results are in concordance with previous studies which observed that no statistical association was shown between serum levels of ALCAM [24,25], CA 15-3 and CEA with various clinicopathologic parameters in breast cancer patients [24], except that, a significant association was obtained for serum levels of ALCAM and CEA with age and menopausal status [24]. But Kulasingam *et al.* [24] also, found that levels of ALCAM were not significantly associated with stage whereas CEA and CA15-3 were significant. Although a statistically significant p-value was not obtained for an association between ALCAM values and tumor grade, a general trend was observed with elevated ALCAM levels corresponding to increased tumor grade [24]. Witzel *et al.* [25] illustrated that high serum ALCAM levels were significantly associated with shorter disease-free survival. When comparing serum levels of ALCAM, CA 15-3 and CEA in breast cancer patients before and at one month days after surgical treatment, non-significant difference was shown in any of them before and after surgical treatment.

In conclusion, this study shows that breast cancer patients have higher serum ALCAM levels than healthy controls and that ALCAM has better diagnostic value than the classical breast cancer biomarkers, CA 15-3 and CEA. The present data provides evidence that serum ALCAM may represent a biomarker for breast cancer patients, which may have potential utility as a diagnostic tool. Further studies with larger number of subjects as well as examining serum ALCAM levels in larger number of samples obtained from patients before and after surgical treatment are needed. Further validation studies that integrate serum ALCAM levels with mammography might reveal potential clinical utility of serum ALCAM for breast cancer. Also, further studies are needed to establish the other clinical usefulness of this biomarker such as predicting response to therapy, surveillance after primary treatment and monitoring response to therapy for breast cancer.

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