Activated Leukocyte Cell Adhesion Molecule (ALCAM) in Saudi Breast Cancer Patients as Prognostic and Predictive Indicator

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ABSTRACT

BACKGROUND: Activated leukocyte cell adhesion molecules (ALCAMs) play an essential role in tumor metastasis and are higher in some patients with breast cancer.

AIM: This study aimed to evaluate ALCAM as an early diagnostic biomarker for breast cancer and how it compares with other markers.

SUBJECTS AND METHODS: One-hundred and sixty-one women were selected for this study. They were divided into three groups: Group 1 consisted of 42 healthy individuals (control) while a patients groups divided into two groups according to tumour grade, Group II, Include 58 breast cancer patient's grade II and Group III, Include 61 patients with grade III of breast cancer. Tumour markers CEA, CA 15-3 and s ALCAM levels were determined and Group 2 consisted of breast cancer patients.

RESULTS: A highly significant elevation was recorded in s ALCAM, CA 15-3 and CEA. Percent change for grade II and grade III were [sALCAM (90, 127)], [CA15-3 (40, 72)] and [CEA (33, 156)]. Operating characteristic (ROC) curves were used to evaluate the diagnostic performance of the biomarkers ALCAM, CA15-3 and CEA with area under the curve (AUC) of (0.99 & 1.0) (AUC 0.947 & 0.99) and (AUC 0.88 & 0.94) for grade II and grade III respectively the incremental values of AUC were statistically highly significant ($p < 0.001$).

CONCLUSION: It could be concluded that serum ALCAM concentration represents a suitable biomarker for Saudi arabian breast carcinoma with high sensitivity and has the potential to be used as a diagnostic tool comparable to CA15-3 and CEA.

KEYWORDS: activated leukocyte cell adhesion molecules, carbohydrate antigen, carcinoembryonic antigen, breast, carcinoma

Introduction

Globally, breast cancer (BC) is considered the most frequent cancer compared to other cancer types among women. It is a major cause that would eventually lead to death or some kind of disabilities. The rate of increase of BC has not been arrested; in fact, there has been an increase.[1]

However, it has been noted that there are different escalation rates based on different geographical locations of the world. Despite that, in general, the overall escalation rates of BC continue to increase regardless of the location or the economic condition of the country. Every year, more than 1.4 million women worldwide are diagnosed with BC, and it accounts for 23% of all newly diagnosed cancers.[2]

As for Saudi Arabia, breast cancer is the most common cancer among Saudi women. It constituted around one-fourth (24.3%) of all diagnosed cancers in Saudi women in the year 2005. There were 1308 BC cases during year 2009. BC ranked first among women, accounting for 25.1% of all newly diagnosed cancers.[3] It is unfortunate that BC usually presents itself at an advanced stage and more frequently in young pre-menopausal women in comparison to Western countries.[4,5]

The most widely used tumor markers for BC are the carbohydrate antigen (CA15-3) and the carcinoembryonic antigen (CEA).[6] Additionally, between the two markers, it has become well known that CA15-3 is the more commonly serum marker.[7]

Despite the fact that there has been wide interest to use plasma CEA level measurements as an in vitro cancer test, it has been pointed out that such tests in healthy individuals may not be a suitable method for screening a population because negative results may be obtained in subjects with early carcinoma, while the increase in levels may also be associated with some nonmalignant disorders.[8]

In fact, it is recommended to use CA15-3 as well. However, the efficiency of using this marker in BC screening has not been proven conclusively. It was found that CA15-3 increased in 3% of patients with contained cancer, whereas it increased up to 70% of patients in whom the disease had spread to other parts of the body. As for the high-risk people, CA15-3 may not be a suitable marker in screening for cancer; however, it may be helpful as a predictive marker in BC patients.

Moreover, research has revealed that these cancer biomarkers are ineffective in screening early stages of the disease due to their low specificity and sensitivity.[9] Activated leukocyte cell adhesion molecules (ALCAMs) also known as CD166 or MEMD, is a member of the immunoglobulin superfamily with five extracellular immunoglobulin-like domains, which mediates cell–cell clustering through homophilic (ALCAM–ALCAM) and heterophilic (ALCAM–CD6) interactions.[10] In fact, adhesive molecules target cell adhesion to specific extracellular matrix proteins and ligands on adjacent cells. They are also involved in cell–cell and cell–substrate interactions, and may influence other processes such as cellular growth, differentiation, junction formation, and polarity. Moreover, the changes in cellular adhesion and communication can contribute to unrestrained cell growth.[11] Its altered expression in cancer has been variously associated with disease progression. Its soluble form, s ALCAM, which is formed by ADAM17/TACE metalloprotease activity, could be an important biomarker for certain types of cancer.[11] One study has indicated that ALCAMs are also expressed in several kinds of cancer, and are reportedly a marker for cancer stem cells in colon cancer.[12] Additionally, there have been several studies on the roles of ALCAMs in several kinds of cancer,[13] especially in metastatic melanoma, where they function as a cell surface sensors for cell density and control the movement from local cell spread to tissue invasion.[14]

ALCAMs are expressed at high levels in BC. High membranous expression of ALCAMs probably results in weakened adherent ability and metastasis.[15]

There are contradictory reports on the ALCAM expression in BC and its prognostic value. Little is known about the role of ALCAM levels in BC patients, as well as its comparison with other markers. A new tumor marker is greatly required to achieve more efficient screening, diagnosing, predicting, or even selecting the proper therapy of BC.

Consequently, the aim of our study was to evaluate ALCAM as diagnostic biomarker for BC with high sensitivity and specificity in serum, using quantitative methodology, and to compare it with CE and CA135 as traditional biomarkers.

Patients and Methods

Patients and specimens. This study included 119 Saudi Arabia breast cancer patients (age, 28–65 years; median, 54 years) and 42 healthy subjects matched in sex was selected as control, (age, 21–59 years; median, 39 years). Patients were selected from those attending at Princess Norah Oncology Center, Jeddah. Patients groups divided into two groups based on how abnormal the tumour cells and the tumour tissue look under a microscope. Group II, Include 58 breast cancer patient's grade II and Group III, Include 61 patients with grade III of breast cancer. Our research complied with the principles of the Declaration of Helsinki.

Patients diagnosed with BC on the basis of medical history, physical examination, and laboratory tests were included in the study. None of the subjects had a history of substance

Table 1. General characteristics of breast cancer patients.

CHARACTERISTIC	NO. OF PATIENTS
Total no. of subjects	161
Menopausal status	
Premenopausal	72
Postmenopausal	89
Clinical stage	
Grade II	58
Grade III	61
ER, PR status	
Negative	77
Positive	84
HER2 status	
Negative	83
Positive	78

abuse or dependence, serious medical conditions, severe head injury, or seizure disorders (Table 1).

Informed consent was obtained from all participants, and the protocol used in this study was approved by the Ethics Committee of the University of Dammam.

Serum collection and preparation. Eligible patients had been asked to donate blood samples for tumor marker serum level measurements. These serum samples were obtained upon initial presentation before any therapeutic intervention was undertaken. Peripheral blood samples were drawn by venopuncture after overnight fasting. Sera were collected in Becton Dickinson vacutainers, code n_367896, containing a coactivator, and kept at the ambient temperature of 20–25°C before centrifugation. The samples were centrifuged at 1,500 g at ambient temperature for 45 minutes, aliquoted within 2 hours, quickly frozen in liquid nitrogen, and directly kept at −80°C to be used for biomarkers studies.

Measurement of ALCAM, CA15-3, and CEA in serum. The concentrations of ALCAM, CIA, and CA15-3 in serum were measured by using a highly sensitive and specific "sandwich-type" enzyme-linked immunosorbent assay (ELISA). The assay was based on human ALCAM (Uscn Life Science Inc. Wuhan). The minimum detectable dose of human ALCAM was typically <0.057 ng/mL, that of of human CEA was typically <0.076 ng/mL, and that of human CA15-3 was typically <0.39 U/mL.

Statistical analysis. Statistical analysis was carried out by the aid of a digital computer, using Excel, and IBM SPSS Statistics version 21 program. Values of $P < 0.05$ were regarded as significant. Data are expressed in Figures and tables as mean ± SE.

Results

The results in (Table 2) and (Fig. 1) revealed that ALCAM was highly significant increase ($P < 0.0001$) in grade II and

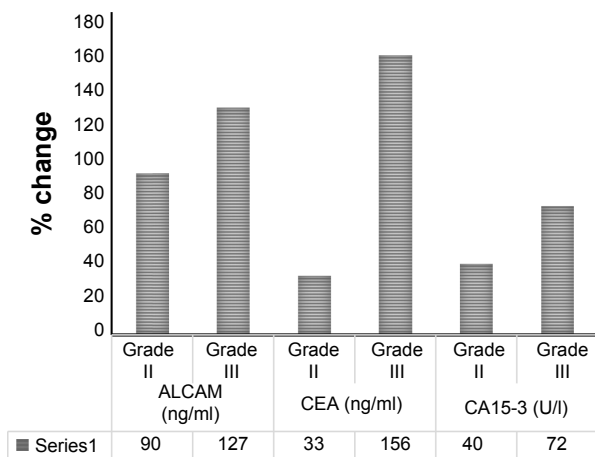**Table 2.** Statistics descriptive of ALCAM, CEA and CA15-3 levels between patients groups and control group.

PARAMETERS	GROUPS	N	MEAN	± SE	*P*-VALUE
ALCAM (ng/ml)	Control	42	48.619	2.5189	
	Grade II	58	92.416	1.7074	0.0001
	Grade III	61	110.262	2.8242	0.0001
CEA (ng/ml)	Control	42	3.581	0.0854	
	Grade II	58	4.750	0.0865	0.0001
	Grade III	61	9.166	0.4045	0.0001
CA153 (U/l)	Control	42	17.643	0.3301	
	Grade II	58	24.619	0.2237	0.0001
	Grade III	61	30.266	0.4138	0.0001

Note: *P* < 0.000: highly significant.

Table 3. Area under the curve (AUC) and cut off value of ALCAM, CA 15-3 and CEA in Grade II.

TEST RESULT VARIABLE(S)	AUC	ASYMPTOTIC SIG.	CUT OF VALUE
ALCAM (ng/ml)	0.997	0.001	72.50
CA15-3 (U/l)	0.947	0.001	21.05
CEA (ng/ml)	0.880	0.001	4.45

grade III by 90% and 127% respectively when compared with control group. CA15-3 and CEA were highly significant increase ($P < 0.0001$) in grade II and grade III by 40%, 72% and 33%, 156% respectively.

The Receiver Operating Characteristic (ROC) curve provided some guidance for determining the sensitivity and positivity of the biomarkers for diagnostic of breast cancer patients. Results of ROC curve describing the diagnostic performance of different biomarkers in grade II, where (Table 3) and (Fig. 2) showed that ALCAM provided the highest diagnostic information of these biomarkers, with AUC of 0.99, followed by CA15-3 with an AUC of 0.94 while CEA exhibited inferior diagnostic performance with an AUC of 0.88.

The results in (Table 4) and (Fig. 3) provide the highest specificity and sensitivity of ALCAM with an AUC of 1.0 ($P < 0.001$) and cut of value of 79. The AUC for CA15-3 and CEA were 0.99 and 0.94, cut off values were 23.1 and 4.5 respectively.

Discussion

Breast cancer is a major cause of death for women. To improve treatment, current oncology research focuses on discovering and validating new biomarkers for early detection of cancer; so far with limited success.[16]

Traditional prognostic factors, such as axillary lymph node status, tumor size, HG, hormone receptor expression, and HER2 expression status, multigene assay and gene expression profiling have been spotlighted. All these factors require tissue samples. Progressive size reduction of detected tumor can make it difficult to obtain samples. On the other hand, serum is easily accessible and soluble circulating tumor markers, if found to be accurate prognostic factors, would be ideal candidates for predicting outcome and monitoring treatment course. Measuring markers is simple, objective, reproducible, and cost-effective.

The current study indicates an elevated levels of both CA 15-3 and CEA associated with tumour grades, this finding is in agreement with Isa Dede et al;[17] they conclude the breast cancer subtypes are correlated with serum levels of tumour markers CEA & CA15-3 in patients with metastatic breast cancer. Tumour markers elevation may be associated with biological background of breast cancer subtypes.

Figure 1. Percent change of ALCAM, CEA and CA15-3 between patients groups and control group.

	Grade II ALCAM (ng/ml)	Grade III ALCAM (ng/ml)	Grade II CEA (ng/ml)	Grade III CEA (ng/ml)	Grade II CA15-3 (U/l)	Grade III CA15-3 (U/l)
■ Series1	90	127	33	156	40	72
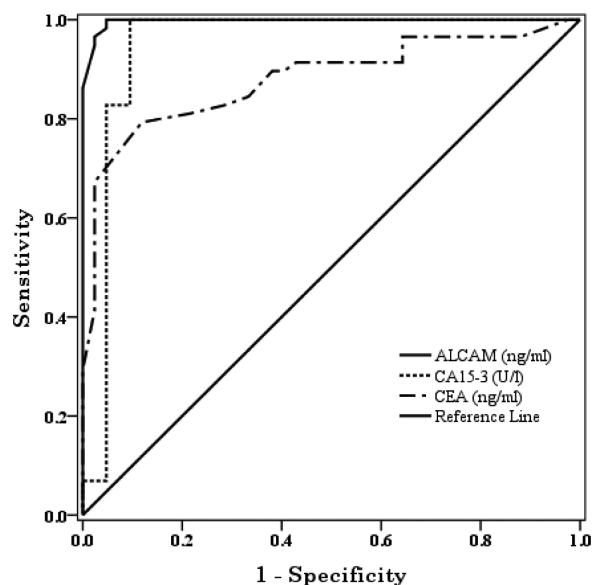
Figure 2. ROC curve of ALCAM, CA 15-3 and CEA in Grade II.

Table 4. Area under the curve (AUC) and cut off value of ALCAM, CA 15-3 and CEA in Grade III.

TEST RESULT VARIABLE(S)	AUC	ASYMPTOTIC SIG.	CUT OF VALUE
ALCAM (ng/ml)	1.00	0.001	79.0
CA15-3 (U/l)	0.99	0.001	23.1
CEA (ng/ml)	0.94	0.001	4.5

CA 15-3 may be the first independent circulating prognostic marker described for breast cancer. Preoperative CA 15-3 concentrations may thus be combined with established prognostic factors for use in deciding which lymph node-negative breast cancer patients should receive adjuvant chemotherapy.[8]

The available serum markers CA 15-3 and CEA are most frequently investigated tumor markers in breast cancer.[18] These markers also sensitive for detecting distant metastases and are of little value in diagnosing loco-regional recurrences.[19]

Our results revealed significantly increase in CEA and CA15-3 by increasing the grade of breast cancer classification. These result are in line with Soletormos et al;[20] who conclude that, expression of CEA is increased in carcinomas and it may be important to processes of intercellular recognition. It has been suggested that this might either result in disturbance of normal intercellular adhesion or provide advantages in further steps of metastasis such as conceivably facilitating establishment of a secondary tumour.

Lee et al;[21] demonstrated that both the markers could be considered for the risk evaluation and determination of adjuvant treatment strategies in clinical practice, although this

hypothesis should be further validated. Further clinical trials based on the tumour marker levels are necessary.

Activated leukocyte cell adhesion molecule (ALCAM) is an immunoglobulin superfamily cell adhesion molecule that is aberrantly expressed in a wide variety of human tumours, including melanoma, prostate cancer, breast cancer, colorectal carcinoma, bladder cancer and pancreatic adenocarcinoma. This wide spectrum of human malignancies makes ALCAM a prospective target to aid in detection and diagnosis in multiple malignancies.[22] Although ALCAM is distributed widely in tissues, its expression seems to be restricted to specific subsets of cells involved in dynamic growth and migration processes.[23]

The main goal of this study was to investigate the existence of ALCAM in serum of breast cancer patients as a prognostic biomarker.

Our results indicated a highly significant elevation level of soluble activated leukocyte cell adhesion molecule (ALCAM) which involved in cell migration and adhesion, the increased levels of ALCAM in breast cancer patients are known to correlate with early prognosis (grade II).

In the current study specifically investigated the ALCAM in breast cancer grade II and grade III, we have demonstrated that it appeared the most sensitive and specific biomarker in breast cancer detection when compared to other biomarkers like CA 15-3 and CEA in both grades. ALCAM appear to play an important functional role in metastasis, where its level closely increased with metastatic progression of the breast cancer (grade III).

In accordance to our results, Kulasingam et al,[24] were detected a significant increase in serum ALCAM in breast cancer patients. They conclude that serum ALCAM concentration represents a novel biomarker for breast carcinoma, which has potential utility as a diagnostic tool.

While previous studies reported that ALCAM could be used as a prognostic factor for several types of cancers, their conclusions differed. Some studies concluded that high levels of ALCAM expression were related to poor prognosis for breast cancer,[25] colorectal cancer,[26] pancreatic cancer and melanoma.[27] On the other hand, other studies came to the conclusion that high ALCAM expression was a favourable prognostic factor for prostate cancer,[28] breast cancer[29] and epithelial ovarian cancer.[30] This is probably because, the function of ALCAM varies depending on the cell type and the microenvironment surrounding tumour cells.[31]

Most primary cancers show loss of expression of adhesion molecules to allow a critical step in metastasis to occur: detachment of the invading cell from its neighbors.[32] However, a number of potential reasons exist for observing elevated levels of adhesion molecules such as ALCAM in cancer patients vs. normal individuals. First, increased homotypic intercellular adhesion (due to elevated levels of these molecules) may favor the metastatic process since cell aggregates, rather than single cells breaking away from the primary tumor, have a greater
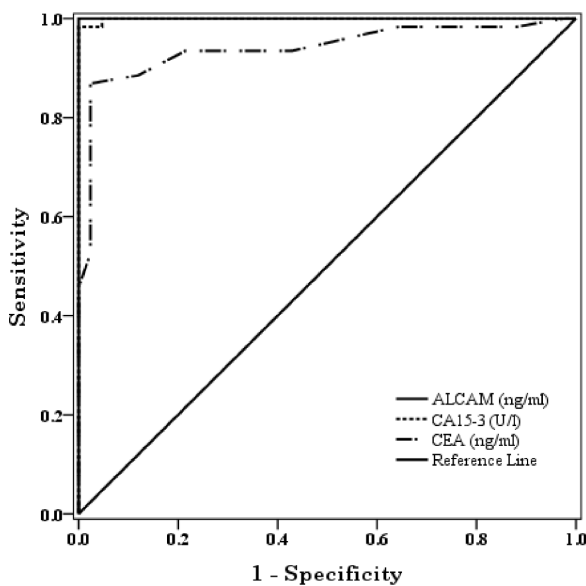
Figure 3. ROC curve of ALCAM, CA 15-3 and CEA in Grade III.

chance of survival in the circulation and of lodging in other organs.[24] Second, it is known that cell adhesion is necessary for the metastatic spread of cancer cells to new organs (secondary tumor establishment).[33]

As well, overproduction of adhesion molecules may disrupt the normally operative intercellular adhesion forces, allowing more cell movement and the adoption of a less ordered tissue architecture.[34] This findings can explore the highly significant increase of ALCAM from grade II to grade III in our present study.

The present data observed an up-regulation of ALCAM in low-grade tumour, it also provided evidence that serum ALCAM represents a prognostic biomarker for breast cancer. This biomarker displays higher diagnostic sensitivity for breast cancer than the currently used tumor markers CA15-3 and CEA according to AUC and cut off value in grade II and grade III.

The finding of decreased levels of ALCAM in breast cancer tissue compared with normal breast tissue is not contradictory to our results of elevated levels of ALCAM in serum of breast cancer patients. It is possible that ALCAM levels decrease in tissue but is elevated in serum.

The present data study observed an up-regulation of ALCAM in low-grade tumour, it also provided evidence that serum ALCAM represents a prognostic biomarker for breast cancer. This biomarker displays higher diagnostic sensitivity for breast cancer than the currently used tumor markers CA15-3 and CEA according to AUC and cut off value in grade II and grade III.

Conclusion, we show evidence that serum ALCAM concentration represents a more sensitive and specific biomarker for breast carcinoma, which has potential utility as a diagnostic tool. The combination of ALCAM with CA15-3 improved the diagnostic sensitivity. Measuring serum ALCAM may facilitate further studies to establish the clinical usefulness of this marker in breast cancer. For example, examining the levels of ALCAM in other serum samples such as those obtained from patients pre and post-surgery as well as serial serum samples collected from patients undergoing therapy may be beneficial in evaluating the biomarker potential of ALCAM as a prognostic or predictive marker of therapy.

Author Contributions
Carried out biochemical and statistical analysis: FSA-S, EMAEA. Contributed to interpretation and discussion of the results: FSA-S, EMAEA. Contributed to the writing: FSA-S, EMAEA. Both authors read and approved the final manuscript.

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