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Research Article
Potential Value of ICAM-1 as a Biomarker for Detection of Progression and Prognosis in Breast Carcinoma

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Abstract

Background and Objective: Breast cancer is reported as one of the most frequent type of cancer all over the world and its incidence is increasing. Tumorigenesis and its progression to metastatic disease are accompanied by changes in the expression of Cell Adhesion Molecules (CAMs). The intercellular adhesion molecule-1 (ICAM-1) is expressed on the surface of a wide variety of cells which are intimately involved in inflammatory reactions and progression of tumor. To date, no serum biomarkers can discriminate benign from malignant breast lesions, this study aimed to identify the role of ICAM-1 using enzyme linked immunosorbant assay (ELISA) as a biomarker in breast cancer and study whether it could be used in assessment of progression of tumor. **Materials and Methods:** Serum ICAM-1 was determined in 92 women diagnosed as primary breast carcinoma (mean age, 49.04±12.67 years, range, 26-76 years) compared with 25 benign breast disease cases. **Results:** Data showed that ICAM-1 were higher in primary breast cancer than in benign tumor but the difference did not reach the significant level ($p = 0.771$). A significant elevation was observed in concentration of ICAM-1 in patients more than 50 years old ($p = 0.05$). Moreover, ICAM-1 concentration did not differ based on clincopathological parameters. **Conclusion:** Serum levels of ICAM-1 seem to be not associated with tumor burden, histological type, grade, size and hormonal receptors status in patients with breast cancer. So serum ICAM-1 is not a useful marker for diagnosis of breast cancer in Egyptian patients.

Key words: Adhesion molecules, ICAM-1, intercellular adhesion molecule-1, enzyme-linked immunosorbent assay, breast carcinoma, tumor grade, prognosis, HER2 neu

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer among women in 140 of 184 countries worldwide and the 5th leading cause of cancer death around the world with increasing reported incidence (522000 deaths in 2012)[1,2].

Serum biomarkers are effective and non-invasive for the prognostic assessment and early recognition of most cancer types. However, to date, a limited number of biomarkers have been confirmed for the clinical management of breast cancer[3]. Therefore, the identification of novel biomarkers related to breast cancer is highly valuable.

The intercellular cell adhesion molecule-1 (ICAM-1) also known as CD54 is one of the most important members of the immunoglobulin (Ig) superfamily of proteins expressed in several cell types. Its molecular weight varies from 80-114 kDa[4]. The ICAM-1 plays a key role in inflammatory conditions, nervous system development, immune responses through antigen recognition and lymphocyte circulation and activation[5]. The ICAM-1 is expressed in carcinoma cells of breast, kidney, prostate, pancreas, small intestine and colorectum but not in the normal epithelia of these organs[6].

Several types of cancers are associated with up-regulation of ICAM-1, which expression has been associated with aggressive tumor phenotypes in breast cancer, prostate cancer and myeloma. Experimental evidence revealed that ICAM-1 stimulates multiple cell-signaling pathways that promote cancer cell proliferation, migration, resistance to apoptosis and development of cell adhesion molecule-induced drug resistance[7].

This study aimed at investigating the influence of benign breast cancer and primary breast cancer on serum profile of ICAM-1 and their diagnostic value as useful marker in development of breast cancer.

MATERIALS AND METHODS

Patients: All breast cancer patients enrolled in this study were diagnosed based on histopathological evaluation for the first time at Pathology Department, National Cancer Institute, Cairo University in the period between December 2013 and August 2015. Tumor tissue as well as benign breast control tissue were preserved in formalin and embedded in paraffin wax. Sections were stained with routine hematoxylin-eosin staining and diagnosed according to the criteria of the World Health Organization[8] and graded according to the modified Scarff-bloom and Richardson method[9].

Table 1: Relation between serum concentration of ICAM-1 and clinicopathological characteristic of the 92 studied cases

Characteristics		Cases (%)	*ICAM-1 median (IQR**)	p-value
Age	<50 years	48(52.2)	210(103-323.3)	0.050
	≥50 years	44(47.8)	330.6(184.8-441.2)	
Histology	IDC#	78(84.8)	263.7(122.1-349.8)	0.169
	ILC##	6(6.5)	155.9(98.3-209.1)	
	Mixed IDC and ILC	6(6.5)	351.9(341.2-482.4)	
	Mixed IDC and IPC	2(2.2)	211.76	
Tumor size	pT1	10(10.9)	262.3(98-307.8)	0.656
	pT2	60(65.2)	262(86.3-359.3)	
	pT3	16(17.4)	255.1(157.8-361.8)	
	pT4	6(6.5)	321.1(288.2-449.8)	
Grade	1	2(2.2)	298.5	0.649
	2	74(80.4)	260.3(119.6-340.2)	
	3	16(17.4)	297.1(220.1-412.3)	
Lymph nodes	pN0	44(47.8)	280.4(98-340.2)	0.634
	pN1	2(2.2)	359.3	
	pN2	30(32.6)	210.8(96.8-466)	
	pN3	16(17.4)	323(237.7-335.3)	
ER###	-ve	74(80.4)	260.3(124.5-340.2)	0.658
	+ve	18(19.6)	298.5(210.8-430)	
PRΦ	-ve	70(76.1)	260.3(125.7-327.7)	0.207
	+ve	22(23.9)	359.3(132.4-466.2)	
HER2neuΦΦ	-ve	64(69.6)	281.1(96.8-380.1)	0.905
	+ve	28(30.4)	257.8(130.8-340.2)	

Statistical analysis was performed by using Mann-Whitney test and Kruskal-Wallis test, *ICAM-I: Intercelluclar cell adhesion molecule-1, **IQR: Inter-quartile range, #IDC: Invasive ductal carcinoma, ##ILC: Invasive lobular carcinoma, ###ER: Estrong receptors, ΦPR: Progesterone receptor, ΦΦHER2neu: Human epidermal growth factor receptor

A number of clinical pathological parameters were shown including details on age, tumor stage, tumor histology, nodal status, hormonal analysis. The detailed clinical characteristics of patients and benign controls were shown in Table 1.

A total of 125 serum specimens were included in this study, including 25 samples from patients with benign breast diseases, 100 samples that were collected from patients with breast cancer. Among the 100 studied non-metastatic breast carcinoma cases, few (8) patients were excluded from the early beginning when their bone scan was positive.

Most of the cases (65) were initially diagnosed by fine needle aspiration cytology (FNAC), 22 cases by core biopsy and 13 cases by excision biopsy prior to surgery. Selected patients were subjected to breast surgery which was either radical or conservative surgery.

All patients had undergone full clinical examination, routine laboratory investigations: complete blood count, liver and kidney function tests, chest x-ray, mammography, breast and abdominal ultrasonography and bone scan.

The criteria for selecting the patients were: (a) Presence of breast lump which diagnosed as breast carcinoma, (b) Availability of collected venous blood samples of the same patients, (c) No distant metastasis and (d) No neoadjuvant therapy.

Exclusion criteria: Any patients with history of systemic disease such as diabetes mellitus, hypertension, chronic inflammatory disease, liver, renal or heart failure are excluded from the study.

This study was approved by the Medical Research Ethical Committee of National Research Center, Cairo, Egypt (Approval No.14-031) in accordance with the Helsinki declaration 1975 (as revised in 2008). All individuals provided the informed consent before participation in the study.

Sandwich ELISA for ICAM-1 in sera: Blood samples were obtained from patients with breast cancer and benign breast cancer patients (n = 117) by venipuncture and were clotted at room temperature.

The sera were collected following centrifugation at 3000 rpm for 10 min after a minimum time span of 30 min and frozen immediately at -80°C until analysis.

The serum concentration of ICAM-1 was detected by enzyme linked immunosorbent assay (ELISA) kit (R and D Systems-Abingdon, UK, catalog number DCD540) according to the manufacturer's instructions.

Statistical analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical software package version 16. Data were tested for normality and were found to be non-normally distributed. Accordingly, all data are presented as medians (interquartile range). The Kruskal–Wallis analysis of variance (ANOVA) and the Mann–Whitney U-test were used to evaluate differences between multiple groups, unpaired observations, respectively. Correlations were evaluated using the Spearman rank test. Significance was presumed at $p<0.05$ and all reported p-values were 2 sides.

RESULTS

The relationships between serum levels of cell adhesion molecules and clinicopathological variables are shown in Table 1.

Serum ICAM-1 levels in patients with malignant disease was examined according to tumor size. The levels of ICAM-1 in patients with different size of tumor showed no significant difference ($p = 0.656$), (the median levels were pTI = 262.3, pT2 = 262, pT3 = 255.1 and pT4 = 321.1 pg mL^{-1}). Meanwhile, the serum levels of ICAM-1 in the >50 years old patients were significantly elevated than in <50 years old patients ($p = 0.05$) (the median levels of ICAM-1 was 210 pg mL^{-1} in patients less than 50 years old and 330.6 pg mL^{-1} in patients more than 50 years old). There was no significant difference in serum ICAM-1 levels between patients who were node positive and node negative (Mann-Whitney U>0.05), pN0 = 280.4, pNI = 359.3, pN2 = 210.8 and pN3 = 323 pg mL^{-1}. There was also no significant difference between ICAM-1 levels in patients who were ER, PR and HER2-neu positive and negative (Mann-Whitney U>0 05). The median levels of ICAM-1 in ER positive and ER negative was 298.5 and 260.3 pg mL^{-1}, respectively ($p = 0.658$). The median levels of ICAM-1 in PR positive and PR negative was 359.3 and 260.3 pg mL^{-1}, respectively ($p = 0.207$). By comparing the results in positive and negative HER2neu, the serum level of ICAM-1 was 257.8 in patients with positive HER2neu, while it was 281.1 in patients with negative HER2neu ($p = 0.905$).

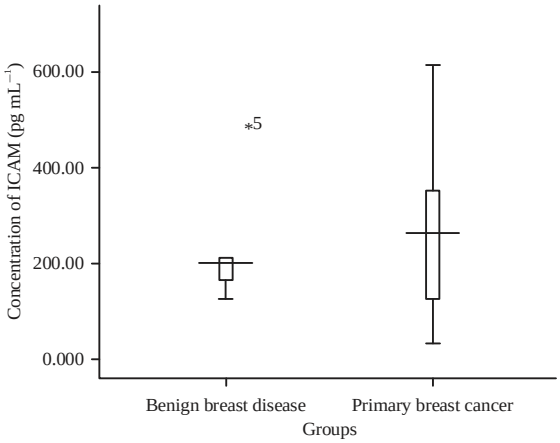

Fig. 1: Serum levels of ICAM1 in patients with malignant breast disease (n = 92), patients with benign breast lesions (n = 25). Data is expressed as median

As regards grades of the malignant breast cancer, serum levels of the studied cell adhesion molecule were 298.5 pg mL^{-1} in grade I, 260.3 pg mL^{-1} in grade II and 297.1 pg mL^{-1} in grade III and there were no significant differences between serum levels of the studied cell adhesion molecule and tumor grade ($p = 0.649$).

According to the type of the malignant lesions, the median levels of ICAM-1 was 263.7, 155.9, 351.9 and 211.76 pg mL^{-1} in invasive ductal carcinoma, invasive lobular carcinoma, mixed invasive ductal and lobular carcinoma and mixed invasive ductal and Intracystic papillary carcinomas, respectively ($p = 0.169$).

Also there were no significant differences in serum ICAM-1 levels in patients with primary breast cancer and benign breast cancer controls ($p = 0.771$) (Table 2). The median level of ICAM-1 in serum of the malignant group was 262.25 pg mL^{-1} and in the benign control group 199.5 pg mL^{-1} as assessed by Mann-Whitney U-test (Fig. 1).

Table 2: Serum ICAM-1 level (pg mL^{-1}) in different studied groups

	Study groups		
			Mann-whitney test
Markers	Benign breast	Primary breast	
ICAM1 (pg mL^{-1})	disease (N = 25)	cancer (N = 92)	p-value
Minimum	125.98	32.353	0.771
Maximum	481.86	612.745	
Median(IQR)	199.5(164.2-210.8)	262.25(124.5-352)	

IQR: Inter-quartile range

DISCUSSION

Adhesion molecules play a crucial role in the progression of cancer by the enhancement of biological processes related to cancer- like survival, migration, extravasation, homing and metastasis.

Many adhesion molecules are dysregulated in human cancer and the development of therapeutic anti-adhesion strategies is ongoing. Previous studies have demonstrated that ICAM-1 plays roles in a variety of malignant tumors, including colorectal, gastric, breast, prostate, lung, bladder carcinoma and malignant melanoma[10].

The ICAM-1 plays an important role in inflammation. Logically, anti-ICAM-1–targeted interventions were developed for the treatment of chronic inflammatory disorders. The role of ICAM-1 in oncology also has been under deep study. ICAM-1 up-regulation is observed in several types of cancers associated with advanced disease, poor survival and resistance to chemotherapy[11]. The ICAMs recognize leucocyte and high concentrations of it destruct tumor cells by making tumor cells more liable to lysis by leucocyte-activated killer cells[12]. Ozer *et al.*[13] and Coskun *et al.*[14] showed that serum levels of ICAM-1 were found to be significantly higher in bladder carcinoma patients compared to those in controls and at all T-stage's and grade compared to controls. However, its level was not different among patients at different T-stages or grades also it was not correlated with T-stage and grade. Okamoto *et al.*[15] observed significant increase in s-ICAM-1 levels in patients with cervical cancer compared to healthy controls and patients with benign diseases. However, no correlation was found between the s-ICAM-1 levels and either the clinical stages or the histological type[15].

There are a little published data about the significance of serum ICAM-1 In breast cancer patients by ELISA most studies measured this marker by immunohistochemistry or gene expression but these methods are costly and need a several steps prior to the final staining of the tissue antigen and many potential problems affect the outcome of the procedure. The major problem areas in IHC staining include strong background staining, weak target antigen staining and autofluorescence. Some investigators have demonstrated the expression of ICAM-1 in breast cancer by immunohistochemistry[6,16].

Schroder *et al.*[16] demonstrated that high ICAM-1 expression was significantly associated with a poorly differentiated phenotype, a negative Estrogen Receptor (ER) status and positive lymph node involvement when detected by IHC[16].

In contrast, Ogawa *et al.*[6] who reported that in 50.3% of patients expressed ICAM-1 and its expression had negative correlation to tumor size ($p = 0.003$), tumor infiltration ($p = 0.003$), lymph node metastasis ($p<0.0001$), nuclear pleomorphism ($p = 0.004$) and nuclear grade ($p = 0.042$), also they found that patients with ICAM-1-positive tumors had better relapse-free and overall survival than others with negative tumors ($p<0.0001$ and $p = 0.0003$, respectively)[6]. Also Guo *et al.*[11] exhibited a significant increase in ICAM-1 expression in Triple Negative Breast Cancer (TNBC) compared with various other subtypes of breast cancers and normal epithelium. They found that ICAM-1 is overexpressed in 26 human TNBC tissues provide clinical evidence supporting ICAM-1 as a potential molecular target for TNBC[12].

In this study, breast cancer patients showed higher level of ICAM-1 when compared with benign breast disease, similar to the reported results in many different types of tumors. However, elevated levels of ICAM-1 had no prognostic significance by increasing the severity of disease. Moreover, they did not show any association between serum ICAM-I levels and pathologic characteristics of tumors such as tumor stage, tumor grade, tumor size, presence of lymph node and the hormone receptors like estrogen receptors, progesterone receptor and human epidermal growth factor receptor. But there was a significant difference between ICAM-1 and age. These findings are in agreement with Fersching *et al.*[17]. They did not find a significant difference in serum ICAM-I levels between benign and locally confined breast cancer before receiving neoadjuvant chemotherapy[17]. In another study, O'Hanlon et al.[12] reported that soluble levels of ICAM-1 were significantly elevated only in stage 4 breast cancer patients compared with benign controls also they found that there was no significant elevation in the soluble adhesion molecules associated with histological evidence of lymphovascular invasion[12].

While our findings are in disagreement with the observations of Klein *et al.*[18] who demonstrated that serum ICAM-I levels were significantly higher in breast cancer patients than healthy control[18].

CONCLUSION

It demonstrated that ICAM-I are not clinically useful biomarker for differentiating primary breast cancer from benign breast disease or for predicting progression of disease. Its value should be further explored in prospective trials with more patients.

SIGNIFICANT STATEMENTS

Breast cancer is the most common female cancer both in the developed and less developed world. In Egypt, breast cancer ranked first among cancers in females. Angiogenesis is an essential step for breast cancer growth, progression and dissemination.

The objective of this study is evaluation the role of ICAM-1 using enzyme linked immunosorbant assay (ELISA) as a biomarker in breast cancer and study whether it could be used in assessment of progression of tumor.

Determination of new effective low cost and non-invasive biomarkers may be more valuable for the early diagnosis, prognosis and staging of the disease and can support clinicians in their daily routine. However, analysis tools need to be standardized and simplified in order to be useful, reliable and widely available.

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