Expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) in Invasive Ductal Carcinoma of the Breast

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Abstract

Background and Aim: VCAM-1 (CD106) expressed mainly on activated endothelial cells in response to pro-inflammatory cytokines. Recent evidences suggest that it is closely correlated with tumor formation, angiogenesis and cancer progression. The aim of the current study was to evaluate expression status of VCAM-1 in mammary invasive ductal carcinoma (IDC), and to correlate its expression with some known clinicopathological data.

Methods: Formalin-fixed paraffin embedded tissue blocks of 58 specimens of IDC were evaluated for VCAM-1 expression by immunohistochemistry (IHC). Correlation of VCAM-1 expression with different clinicopathological data was measured statistically.

Results: Up-regulation of VCAM-1 expression was frequently observed in less differentiated tumors and within advanced stages with poor Nottingham prognostic index (NPI). VCAM-1 overexpression was significantly associated with the presence of lymph node metastasis (LNM), lymphovascular invasion (LVI), and prominent lymphocytic infiltration. There was no significant association of VCAM-1 expression with patients' age, tumor laterality, tumor size, muscle or skin invasion, and presence of *in situ* component.

Conclusion: VCAM-1 molecule could promote LVI and LNM. VCAM-1 is a potential independent prognostic factor for breast carcinoma.

Abbreviations: CD: Cluster of differentiation, **IDC:** Invasive ductal carcinoma, **IDC-NST:** Invasive ductal carcinoma of no specific type, **IHC:** Immunohistochemistry, **LNM:** lymph node metastasis, **LVI:** lymphovascular invasion, **NPI:** Nottingham prognostic index, **VCAM-1:** Vascular cell adhesion molecule.

Keywords: VCAM-1, Breast carcinoma, IDC, CAMs, Tumor progression

Introduction:

VCAM-1 is a 110 kDa trans-membrane sialoglycoprotein and a member of immuneglobulin superfamily. It is expressed on many different types of endothelial and stromal cells mediating cellular adhesion. Recent findings suggest that VCAM-1 is closely correlated with tumor formation and cancer progression. It is also proved as an important factor to predict the prognosis and to determine the patients at high risk for metastasis [1].

VCAM-1 is aberrantly expressed in various cancer cells promoting invasion and metastasis to distant organs. It also promotes angiogenesis, metastasis and survival of breast cancer cells into lung, bone, and brain. It has emerged as a

diagnostic and prognostic marker in breast carcinoma and as a novel target in anti-cancer therapy [2]. Previous studies evaluating VCAM-1 expression focused mainly on its circulating level. Few studies were conducted to evaluate VCAM-1 expression in solid tumor tissues that is suspected to be more reliable indicator in elucidating the relationnship between VCAM-1 expre-ssion levels and prognosis [3].

Materials and Methods

Tissue samples:

Approval to perform this work was obtained from the Institutional Research Ethical Committee. Formalin-fixed paraffin-embedded tissue blocks of 58 specimens of IDC were retrieved in the period from January 2018 to June 2019, from Pathology Department, Sohag University Hospitals, Egypt. Specimens that fulfilled complete clinical data were included. Cases with history of pre-operative anti-cancer therapy or with insufficient clinical data were excluded. Clinical and pathological da-ta were obtained from the patients` clinical files and pathology reports. Histopathological grading was done according to the Nottingham Histolo-gical Score System (NPI); modification of Scarff-Bloom-Richardson grading system. Tumors were staged according to eighth edition of AJCC TNM staging system [4,5].

Immunohistochemistry:

4µm thick sections were deparaffinized in xylene and hydrated by graded alcohols. Endogenous peroxidase activity was blocked by incubation in 0.3% H2O2 for 20 minutes at room temperature, followed by washing in two changes of phosphate buffer solution (PBS). Heating tissue section at 70º C for 20 minutes in Ethylenediaminetetraacetic acid (EDTA) was done for antigen retrieval. Following washing twice in PBS, the sections were incubited with mouse monoclonal anti-VCAM-1 antibody (Catalog # 0335, Activated Endothelial Marker, Clone 1.4C3, ScyTek Corporation, Fremont, USA) at dilution 1/50 for overnight at 4º C. After washing in PBS, the sections were incubated with a biotinylated secondary antibody for 30 minutes at room temperature. Finally sections were incubated with streptavidin peroxidase for 10 minutes, also preceded and followed by washing twice in PBS for five minutes each. DAB chromogen was freshly prepared by adding DAB chromogen to DAB substrate at a concentration of 1:25. Fifty microliter from the freshly prepared solution was applied to each tissue section and incubated at room temperature until the positive control showed staining then the sections were washed in distilled water. The sections were counterstained by hematoxylin stain for half a minute then rapidly washed in tap water to remove extra dye. The sections were dehydrated, cleared and mounted as usual. ***Positive and Negative Controls,*** placenta sections worked as positive control for the IHC process **(Fig. 1A)**. Negative control sections were prepared from breast carcinoma sections by adding PBS instead of primary antibody.

Scoring of immunoreactions:

VCAM-1 positivity was identified as membranous or cytoplasmic brown staining of neoplastic cells with ignoring the stromal staining. The expression level of VCAM-1 was measured by calculating a total immunoreactive score (IRS) which is defined as the product of multiplying of a proportion score and intensity score. The proportion score is the estimated proportion of positively stained tumor cells (0: < 10%, 1: 11%-20%, 2: 21%-75%, and 3: > 75%). The intensity score represents the estimated staining intensity (0: no

staining, 1: weak, 2: moderate, and 3: strong). The total score ranges from 0 to 9 with VCAM-1 –ve/low or overexpression defined as a total score <3 and ≥3, respectively [6].

Statistical analyses:

The statistical software SPSS (Version 20) was used for data analysis. Quantitative data was represented as mean, standard deviation (SD), median and range. The Chi-square test was used to compare categorical features. p value was considered significant if it was less than 0.05.

Results

The current study included 58 cases of IDC treated surgically by modified radical mastectomy. The patients' ages ranged from 22-70 years with mean ±SD and median was 49.83 ± 9.34 and 50 years, respectively. The size ranged from 1-10 cm with mean ±SD and median were 5.39± 2.36 cm and 5cm, respectively.

Histologically, the tumors were classified as IDC-NST in 44 (76%) and rare special subtypes in 14 (24%). Among the investigated cases, 7 (12%) of tumors were grade I, 29 (50%) were grade II, and 22 (38%) tumors were grade III. Histologically normal mammary tissue and in situ component was found adjacent to the invasive tumor tissue in 20/58 (34%) and 27/58 (47%) cases, respectively. The clinical and pathological findings of the cases included in the present study were summarized in **(Table 1)**.

Predominantly cytoplasmic expression of VCAM-1 was observed with some cases showed associated membranous expression **(Fig 1)**. VCAM-1 was overexpressed in 32/58 (55%) of cases of IDC from them 26/32 (81%) cases were IDC-NST. VCAM-1 expression was dichotomized into 2 groups, overexpressing cases (total score >3) and –ve/low expressing cases (total score, 0-3). Using these appropriate cutoff points, VCAM-1 was overexpressed in 32/58 (55%) of specimens, and showed –ve/low expression in 26/58 (45%) of specimens. Different clinical and pathological variables of IDC cases were summarized in **(Table 1)**.

Among the studied parameters; VCAM-1 showed statistically significant positive correlation with tumor grade (p=0.008), stage (p=0.002), NPI (p<0.001), LNM (p=0.012), tumor lymphocytic infiltration (p=0.021) and LVI (p=0.007) as illustrated in **(Graph 1A&1B)**. There was no significant association between VCAM-1 expression and patients' age, tumor laterality, size of tumor, and histologic subtype. Also, no significant correlation was found between its expression and invasion of muscle, skin or surgical resection margins.

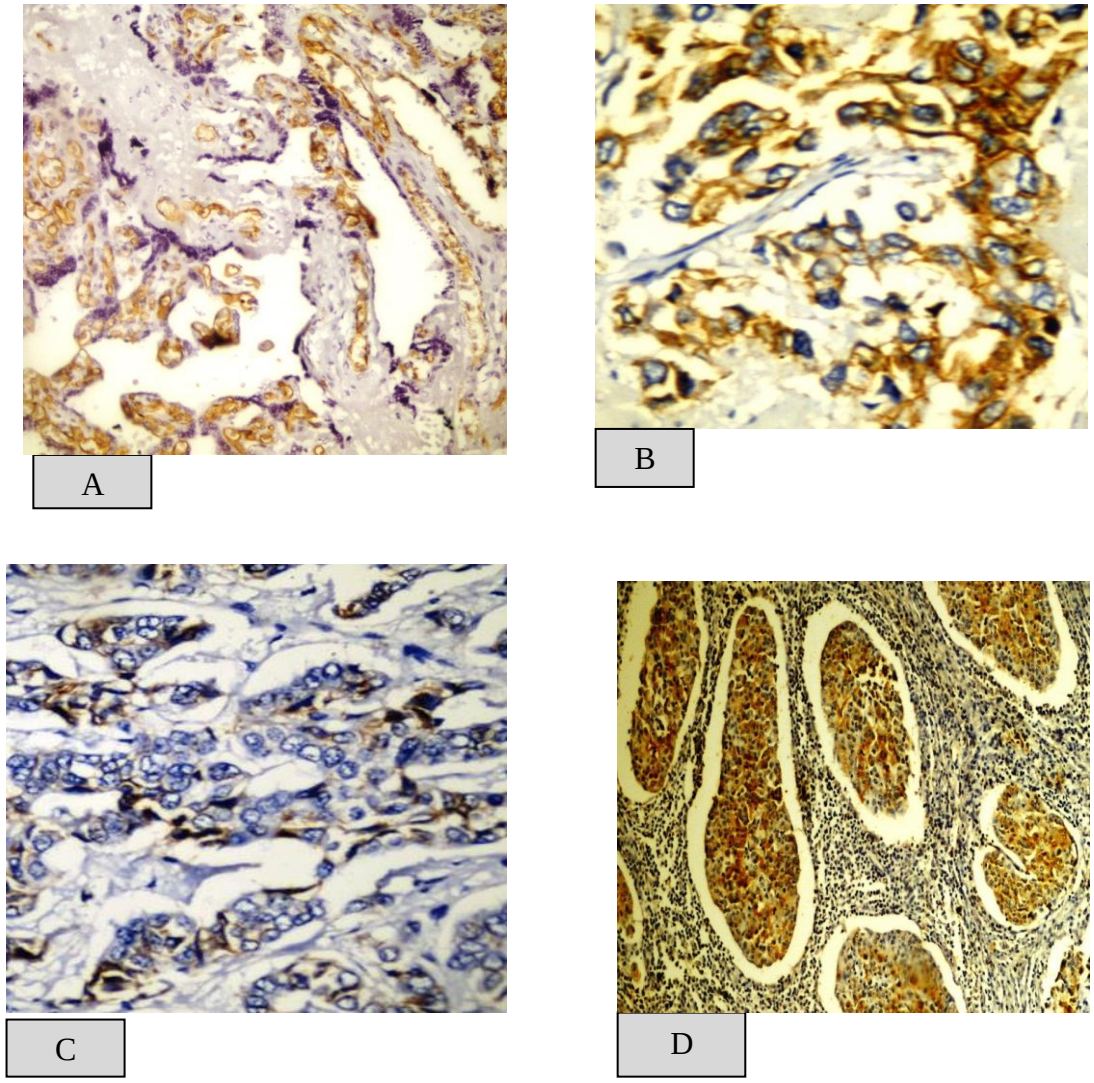
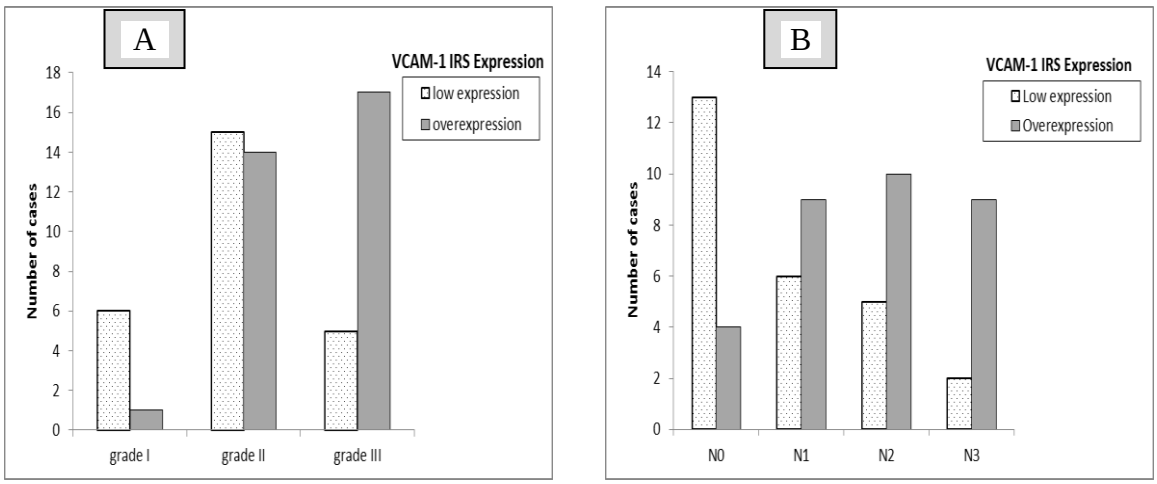


Figure 1: Representative IHC Staining of VCAM-1 **(A)** Placenta as positive control with VCAM-1 overexpression, **(B)** IDC-NST (grade III) showing cytoplasmic VCAM-1 overexpression, **(C)** IDC-NST (grade II) with –ve/low expression of VCAM-1, **(D)** Medullary carcinoma with cytoplasmic VCAM-1 overexpression. Original magnification is 200X in D and 400X in others.

Clinicopathological Parameter	No. of cases (58)	VCAM-1 Expression		p value
		-ve/low expression N=26 (45%)	Over expression N=32 (55%)	
Age				
≤50 years	28	13 (46%)	15 (54%)	**0.592 (NS)**
>50 years	30	13 (43%)	17 (57%)	
Laterality				
Right breast	24	12 (50%)	12 (50%)	**0.506 (NS)**
Left breast	34	14 (41%)	20 (59%)	
Histologic subtype				
IDC-NST	44	18 (41%)	26 (59%)	**0.073 (NS)**
Other subtypes	14	8 (57%)	6 (43%)	
Tumor grade				
I	7	6 (86%)	1 (14%)	**0.008****
II	29	15 (52%)	14 (48%)	
Tumor size				
T1	6	5 (83%)	1 (17%)	**0.133 (NS)**
T2	28	11 (39%)	17 (61%)	
T3	24	10 (42%)	14 (58%)	
LN status				
N0	17	13 (76%)	4 (24%)	**0.012***
N1	15	6 (40%)	9 (60%)	
N2	15	5 (33%)	10 (67%)	
N3	11	2 (18%)	9 (82%)	
AJCC stage				
I	6	6 (100%)	0	**0.002****
II	24	13 (54%)	11 (46%)	
III	28	7 (25%)	21 (75%)	
NPI				
Good	13	11 (85%)	2 (15%)	**<0.001****
Moderate	24	12 (50%)	12 (50%)	
Poor	21	3 (14%)	18 (86%)	
Lymphocytic infiltration				**0.021***
Minimal	26	16 (62%)	10 (38%)	
	32	10 (31%)	22 (69%)	
Tumor margin				
Free	36	19 (53%)	17 (47%)	**0.119 (NS)**
Infiltrated	22	7 (32%)	15 (68%)	
Muscle invasion				
Present	23	7 (30%)	16 (70%)	**0.074 (NS)**
Absent	35	19 (54%)	16 (46%)	
Skin invasion				
Present	28	11 (39%)	17 (61%)	**0.412 (NS)**
Absent	30	15 (50%)	15 (50%)	
LVI				
Present	17	3 (18%)	14 (82%)	**0.007****
Absent	41	23 (56%)	18 (44%)	

Table (1): Statistical Correlations between VCAM-1 Expression and Clinicopathological Parameters in IDC cases

P value was calculated by Chi-square test, *= significant, **= highly significant, and NS= non-signif icant



Graph 1: Correlation of VCAM-1 Expression with Tumor Grade (**A)** and Lymph Node Status (**B).**

Discussion

Breast carcinoma is a life threatening malignant tumor of females with an increa-sing incidence worldwide. Met-astasis always impairs the curative effect of chemotherapeutic agents [7]. VCAM-1 expression has received increasing attention as a potential diagnostic and prognostic factor. The invasive capacity of breast cancer cells is determined by events in the micr-oenvironment such as angiog-enesis and inflammation. VCAM-1 is suspe-cted to be involved in inflamm-ation-mediated cancer development, migra-tion and tumor invasion; VCAM-1 activity is increased in cancer cells. It plays an important role in leukocytes recruitment to inflammation sites that is essential process for carcinogenesis in-cludeing enhanced cell proliferation, alterations in epigenetic events, and in-appropriate gene expression, so incre-asing resistance to apoptosis. It also pr-omotes neovascularization, inva-sion, and metastasis [8].

In the present study, VCAM-1 immun-oreactivity was detected mainly in the cytoplasm of tumor cells. By contrast, there was very low or no detectable VCAM-1 expression in histologically normal mammary tissue adjacent to invasive tumor. These findings were consistent with the pattern observed in previous reports [8,9]. According the current study; significant positive correlation was found between VCAM-1 expression in breast carcin-oma and tumor grade and histologic stage. These findings were consistent previous findings that demonstrated that the highest VCAM-1 expression levels were associated with moderately or poorly differentiated tumors and advanced stages of breast carcinoma [2,10].

According to our findings; increased VCAM-1 expression was significantly associated with high incidence of LVI, high rates of LNM suggesting that VCAM-1 is a potential independent prognostic factor for breast carcinoma. Previous reports indicated that the overexpression of VCAM-1 was assoc-iated with tumor invasiveness and poor prognosis in breast cancer [11]. Howe-ver, several studies concluded that it is not the up-regulation of VCAM-1 but the aberrant expression of this molecule was associated with progression of breast carcinoma [12]. The current

study did not find any significant correlation between VCAM-1 expression and patients' age, necrosis, presence of *in situ* component, and invasion of skin and muscles. There was no significant difference in VCAM-1 expression in relation to histological type.

Conclusion: VCAM-1 expression is significantly correlated with prognosis of breast carcinoma, where higher expression levels tend to be associated with worse prognosis referring to tumor progression, and occurrence of metastasis. This suggests that VCAM-1 is a potential independent prognostic factor for breast carcinoma and could be a suitable target for anti-cancer therapy.

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