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Immunohistochemical Expression of Angiogenic Marker CD34 in Invasive Ductal Breast Carcinoma and its Correlation with Morphometric and Histopathological Parameters

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Abstract **Original Research Article**

Angiogenesis is a mechanism by which new blood vessels are developed in healing and tumor tissues, where it is necessary for regenerating growth, tumor cells survival and metastasis. CD34 is a trans membrane phosphoglycoprotein, first identified on hematopoietic stem and progenitor cells. Clinically, it is associated with the selection and enrichment of hematopoietic stem cells for bone marrow transplants. Due to these historical and clinical associations, CD34 expression is almost ubiquitously related to hematopoietic cells, and it is a common misconception that CD34-positive (CD34+) cells in non-hematopoietic samples represent hematopoietic contamination . The aim of present work was to study the angiogenic marker CD34 in invasive ductal breast carcinoma to validate its ability to be added to the traditional histopathological parameters. Immunohistochemical technique was used to examine the expression of CD34 in benign and in invasive ductal breast carcinoma IDC. Present results showed higher expression of CD34 in IDC comparing to normal and benign breast tissues. Statistical analysis showed significant correlations between the expression of CD34 and histological tumor grade, lymph node metastasis (LNM), ER and PR. Current results suggest that CD34 protein may be valuable prognostic and therapeutic marker inhuman IDC patients.

Keywords: CD34, IDC, IOD, ER, PR prognostic marker.

INTRODUCTION

Breast cancer represents a major scientific, clinical and societal problem. It is the most common malignancy and the second leading cause of cancer death in females following lung cancer [1, 2] with more than 1,000,000 new cases and 370,000 deaths yearly worldwide [3]. In many developing countries, the incidence of breast cancer is now rising sharply due to changes in reproductive factors, lifestyle, and increased life expectancy [4].

The CD34 antigen is a monomeric structure exhibiting an apparent molecular mass of 110-120 kDa, depending on the cell source from which it is immunoprecipitated [5, 6]. The human CD34 gene is found on chromosome 1q32 [7, 8] a region containing several genes encoding adhesion matrix and complement cascade binding molecules, such as L-selectin/P-selectin and E-selectin, laminin B2, and the RNA gene cluster. The genetic co-localization of CD34 with adhesion molecules suggests potential coordinate regulation of expression, and therefore may have functional relevance [9, 10].

CD34 antigen is expressed on small vessel endothelial cells [11, 12] and tumors of epithelial origin [13, 14]. On a subset of fibroblasts (including embryonic fibroblasts), bone marrow stromal progenitors, some cells in fetal and adult nervous tissue, interstitial and adventitial fibroblast-like dendritic cells from adult dermis, areolar tissue, fat somatic and visceral collagenous connective tissue express CD34 [15]. CD34 is also expressed on hematopoietic

progenitors derived from fetal yolk sac, embryonic liver, and extra hepatic embryonic tissues including aorta-associated hematopoietic stem/progenitors in the 5-week embryo [16, 17]. It is found on several myxoid, fibrovascular, fibrohistiocytic mesenchymal tumors and fatty tumors deriving from primitive fibroblast-like dendritic cells [18]. About 40% of acute myeloid leukemia and 65% of pre-B acute lymphoblastic leukemia express the CD34 molecule, whereas only 1-5% of acute T-lymphoid leukemia expresses the CD34 antigen. CD34 is often expressed on blasts from chronic myeloid leukemia patients in blast crisis; whereas chronic phase cells, other chronic leukemia and lymphomas of more differentiated phenotypes are uniformly negative [19, 20].

In the present study, expression of CD34 in an invasive ductal Breast carcinoma (IDC) was investigated using immunohistochemical technique and the intensity of CD34's immunostaining was quantitatively estimated using image optical density (IOD) analyzer.

MATERIAL AND METHODS

Tissue samples were obtained from patients diagnosed with breast tumors in the Department of Pathology, Medical Research Institute, Alexandria University, Egypt, during Janury 2011 to April 2012. Formalin-fixed and paraffin embedded tissue specimens from 60 patients, 30 patients diagnosed with benign and 30 were diagnosed as invasive ductal breast carcinoma IDC. All the cases were asked to freely volunteer to the study and informed written consents were gathered prior to their inclusion in the study. Hematoxylin and eosin (H&E) stained slides for each patient were reviewed by two pathologists. Diagnosis of the specimens was made according to the WHO classification of the Tumors. Clinical parameters included patients' age, tumor size, lymph node metastasis (LNM) status and biological markers ER and PR.

Immunohistochemical Investigation of CD34

Immunohistochemical method was utilized to study the expression of CD34 in 60 paraffin-embedded breast tissues. In brief, paraffin-embedded specimens were cut into 5µm thick sections. The sections were deparaffinized using 2 changes of xylen and rehydrated. The sections were submerged in antigen retrieval (citrate buffer saline pH 6) in an oven at 95°C for 20 minutes and then left at room temperature for 20 minutes to cool. The sections were treated with 3% H_2O_2 in PBS to quench the endogenous peroxidase activity, and then incubated with serum blocking reagent for 30 minutes to block nonspecific binding. The sections were incubated with primary antibody for CD34 (Biorbyt Company, London, UK) at 4ºC overnight. Sections were treated with conjugated 2nd antibody (ABC-HRP reagent) for 30 minutes, stained with diaminobenzedine (DAB) and counter stained with hematoxylin. For negative controls, antibody was replaced with PBS. Each step was followed by PBS washing. Evaluation of CD34's immunohistochemical results was arbitrarily graded as negative (0), weak (+1), moderate (+2) and strong (+3).

Statistical Analysis

Data were normally distributed according to the Kolmogorov-Smirnov (K-S) normality test, and then analyzed using statistical software package SPSS 20. P values≤ 0.05 were considered statistically significant.

RESULTS

A-Histopathological Results

1- Group I: Invasive Ductal Carcinoma (IDC)

Grade I (IDC)

The histopathological findings consisted of well-formed ductules having angulated contour and lined by single layer of malignant ductal cells. The tumor cells have vesicular nuclei and inconspicuous nucleoli. Mitosis was infrequent. The stroma in between was desmoplastic (Figure 1).

Grade II (IDC)

The histopathological finding consisted of malignant ductal cells having large pleomorphic vesicular nuclear with moderate number of mitotic figures arranged in trabeculae (Figure 2).

Grade III (IDC)

The histopathological finding showed large pleomorphic ductal cells having large nuclei with coarse chromatin and macro nucleoli. The cells were arranged in sheets and trabeculae (Figure 3).

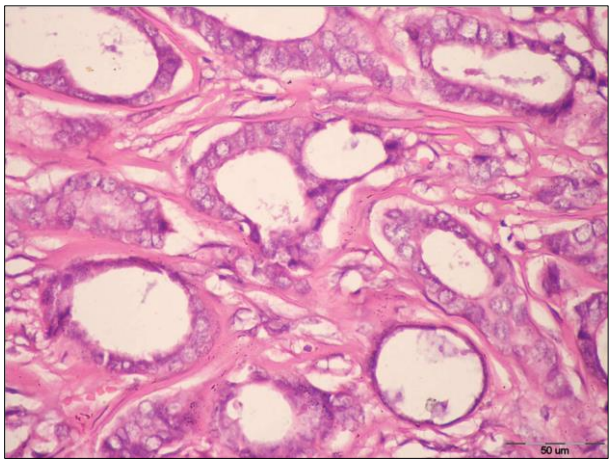

Figure 1: IDC grade I positive lymph nodes. Note: Ductules lined by pleomorphic ductal cells with vesicular nuclei (↑). H&E stain (Bar=50 µm)

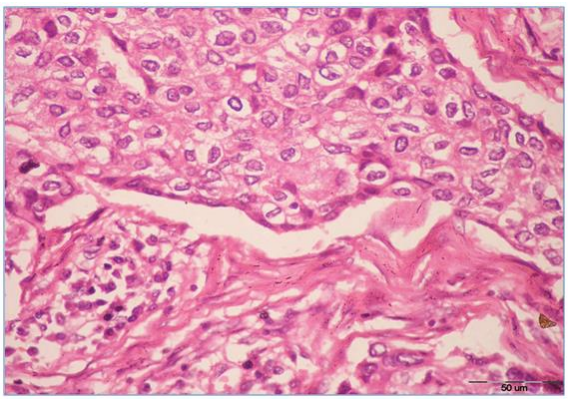

Figure 2: IDC grade II positive lymph nodes. Note: Trabeculae of malignant ductal cells in a desmoplastic stroma (↑). H&E stain (Bar=50µm)

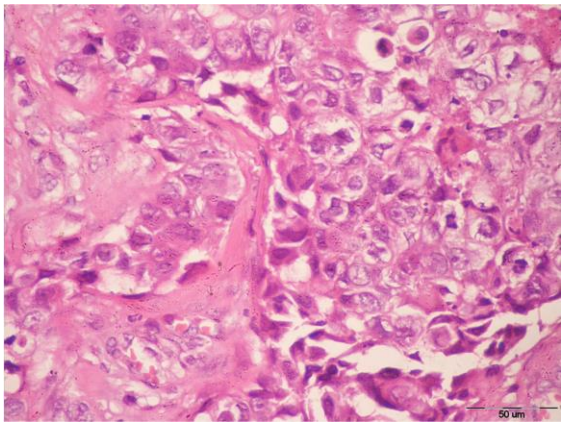

Figure 3: IDC grade III positive lymph nodes. Note: Sheets and nests of malignant ductal cells with frequent abnormal mitotic figure (↑). H&E stain (Bar=50µm)

2- Group II: Benign Breast (Fibroadenoma and Fibrocystic Disease)

Fibroadenoma, where there was a dual proliferation of both epithelial and stromal elements with the latter predominating compressing the ducts into silt like spaces (Figure 4). In fibrocystic disease, the breast parenchyma showed adenosis, cystic dilatation of some ducts and stromal collagenosis (Figure 5).

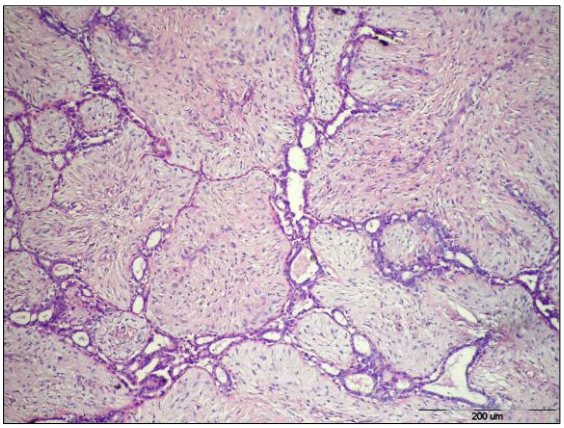

Figure 4: Fibroadenoma of the breast. Note: Compressed slit-like ducts. H&E stain (Bar=200µm)

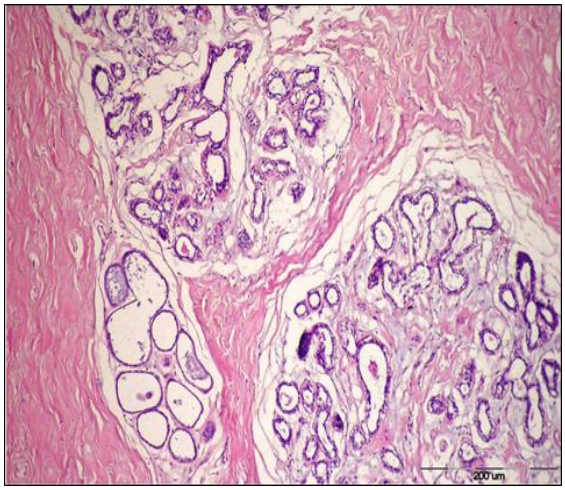

Figure 5: Fibrocystic disease of the breast. Note: Cystic dilated ducts. H&E stain (Bar=200µm)

2- Immunohistochemical Results

I. Immunostain of the Angiogenesis CD34 Marker

In the present work the CD34 immunoreactivity was in the form of diffuse brown color in cytoplasm and cell membrane of the endothelial cells of microvessel, cytoplasm of myoepithelial cells and stroma. The ductal epithelial cells were negative.

A. Group I: Invasive Ductal Carcinoma (IDC)

Grade I Positive Lymph Nodes

The immunoreactivity of CD34 marker was weak (+1) in cytoplasm and endothelial cells of microvessel, while it was moderate (+2) in the cytoplasm of myoepithelial cells and stroma (Figures 6, 7).

Grade II Positive and Negative Lymph Nodes

The immunoreactivity of CD34 marker in positive lymph nodes was moderate (+2) in cytoplasm and endothelial cells of microvessel, stroma and cytoplasm of myoepithelial cells (Figures 8, 9).

In the negative lymph nodes, the immunoreactivity of CD34 marker was intense (+3) in cytoplasm and endothelial cells of microvessel, weak (+1) in cytoplasm of myoepithelial cells and negative (‧) in stroma (Figures 10, 11).

Grade III Positive Lymph Nodes

The immunoreactivity of CD34 marker was similar to the results of grade II negative lymph nodes (Figures 12, 13).

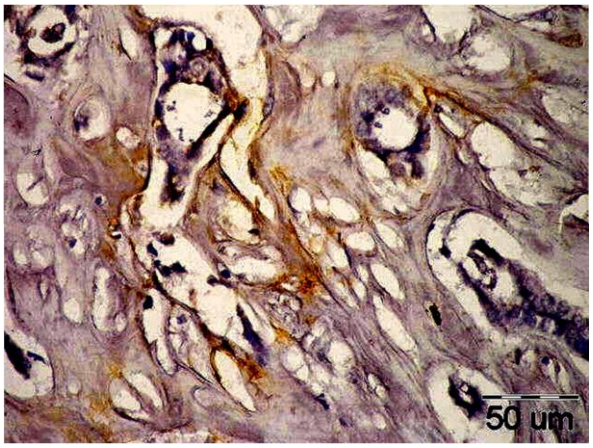

Figure 6: IDC grade I positive lymph nodes. Note: Weak immunostain (+١) of CD34 in cytoplasm and endothelial cells of microvessels (↑) and moderate (+2) in stroma (red arrow) (ABC stain, Bar= 50µm)

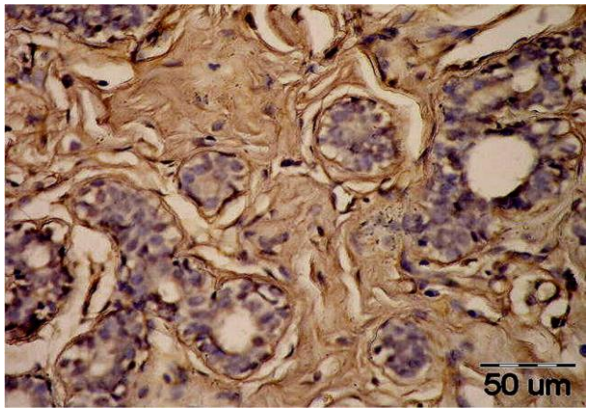

Figure 7: IDC grade I positive lymph nodes. Note: Moderate immunostain (+2) of CD34 in stroma (red arrow) and cytoplasm of myoepithelial cells (*). (ABC stain, Bar= 50µm)

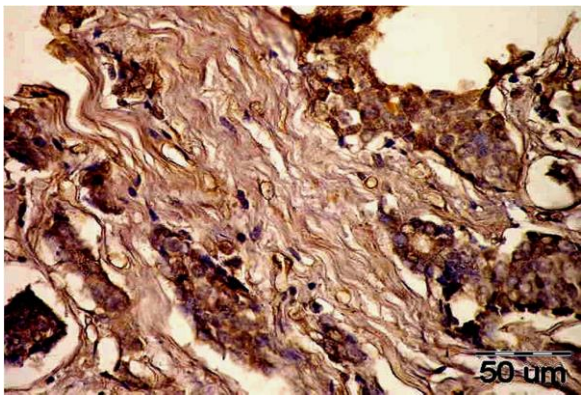

Figure 8: IDC grade II positive lymph nodes. Note: Moderate immunostain (+2) of CD34 in cytoplasm and endothelial cells of microvessels (↑). (ABC stain, Bar= 50µm)

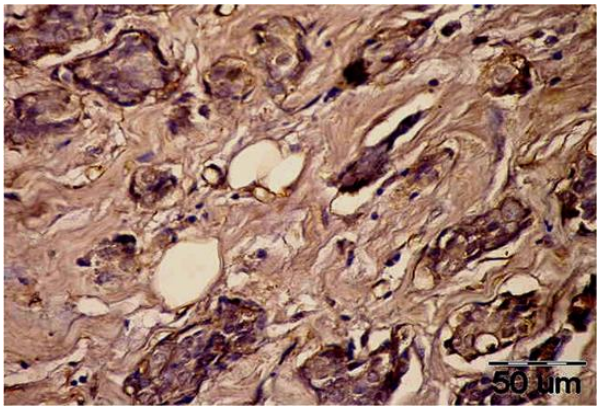

Figure 9: IDC grade II positive lymph nodes. Note: Moderate immunostain (+2) of CD34 in cytoplasm and endothelial cells of microvessels (↑) and cytoplasm of myoepithelial cells (*). (ABC stain, Bar= 50µm)

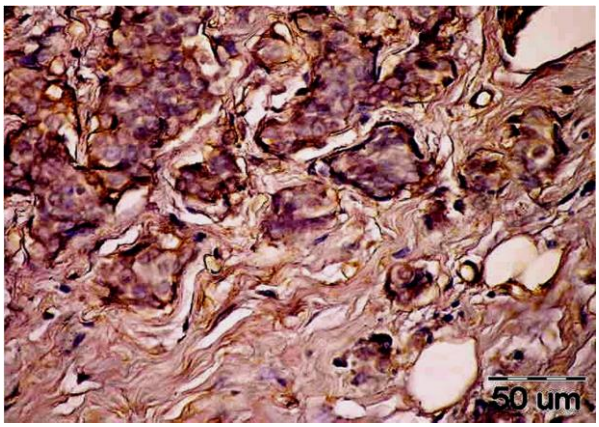

Figure 10: IDC grade II negative lymph nodes. Note: Intense immunostain (+3) of CD34 in cytoplasm and endothelial cells of microvessels (↑) and negative (٠) in stroma (red arrow) (ABC stain, Bar= 50µm)

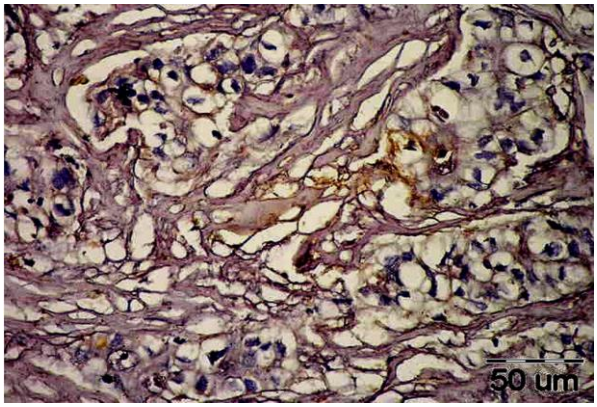

Figure 11: IDC grade II negative lymph nodes. Note: Hot Spot area where microvessels were counted (ABC stain, Bar= 50µm)

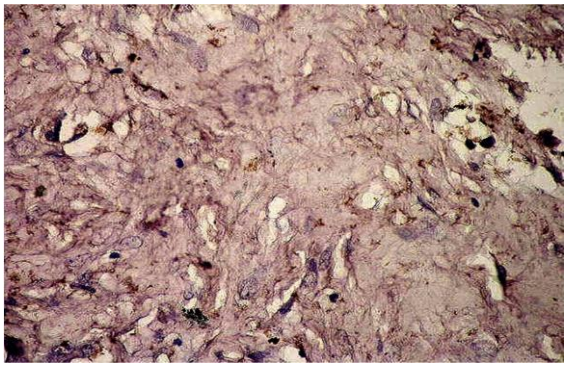

Figure 12: IDC grade III positive lymph nodes. Note: Intense immunostain (+3) of CD34 in cytoplasm and endothelial cells of microvessels (↑) and negative (·) in stroma (red arrow) (ABC stain, Bar= 50µm)

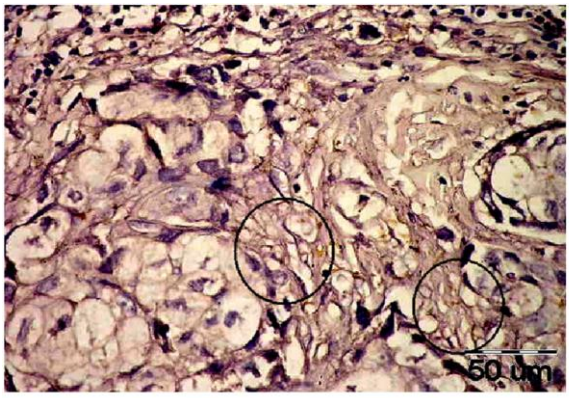

Figure 13: IDC grade III positive lymph nodes. Note: Hot Spot area where microvessels were counted (ABC stain, Bar= 50µm)

B- Group II: Benign Breast (Fibroadenoma And Fibrocystic Disease)

The immunereactivity was negative (·) in stroma and cytoplasm of myoepithelial cells (Figures 14, 15).

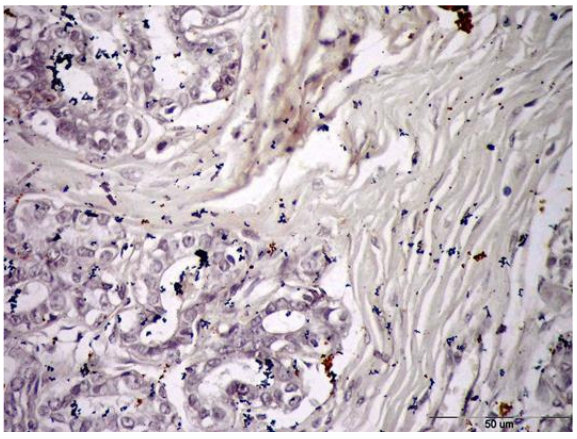

Figure 14: Fibroadenoma of benign breast cancer. Note: Negative immunosatine (·) of CD34 marker in stroma and cytoplasm myoepithelial cells. (ABC stain, Bar= 50µm)

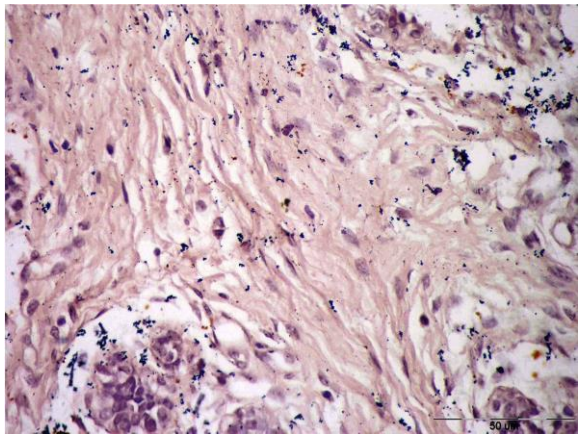

Figure 15: Fibrocystic disease of benign breast. Note: Negative immunosatine (·) of CD34 marker in stroma and cytoplasm of myoepithelial cells. (ABC stain, Bar= 50µm)

DISCUSSION

Breast cancer is the most prevalent type of cancer in the world [21]. In United States and Europe it is the most common cancer in women and the second leading cause of cancer death [22, 23]. In Arab countries breast cancer is the much more common among women with a mean age 50 years at diagnosis [24]. In Egypt, it has been reported that breast cancer in females accounting for 37.6% of all tumors [25].

Angiogenesis is the proliferation of endothelial cells to form a primitive vascular bed which is subsequently surrounded by smooth muscle to form new blood vessels [26]. Solid tumor growth and metastasis are angiogenic dependent [27]. Angiogenesis results from a complex local balance between pro and antiangiogenic agents. An imbalance of these regulators results in a switch to angiogenic tumor phenotype [28].

In recent years, several biochemical molecules have been evaluated for possible prognostic application. These include steroid receptors, C-oncogens, suppressor genes and proteases involved in metastasis and mean microvessel density (MVD), beside the traditional histopathological parameters including axillaries lymph node status, tumor size and grade [29].

The more recent use of antibodies against CD34 react not only with newly formed vessels but also normal vessels trapped within tumor tissue and thus CD34 is referred to as pan endothelial marker. CD34 a pan endothelial marker is a glycoprotein monoclonal antibody with molecular weight of 110-120 KD located on chromosome 1q3.2. Cellular expression of CD34 is seen in hematopoietic and capillary endothelial cells [30].

Microvessel density (MVD), a marker of tumor angiogenesis, has been proposed to identify patients at high risk of recurrence particularly in lymph node negative patients. The MVD assessment is the

commonly used technique to assess intratumoral angiogenesis in breast cancer [31]. Few studies have measured tumor MVD by immunohistochemical methods [32, 33]. It has been found that microvessel count by CD34 immunostaing identifies breast cancer patients with aggressive phenotype [34]. There are data suggesting that breast cancer is an angiogenic-dependant disease [35].

Computerized systems of image analysis, developed to quantify positive immunoprecipitates within tissue sections, are more reproducible than semiquantitative immunohistochemical labeling which is easy to perform but cost effective. Therefore computerized systems of image analysis are more acceptable for clinical and pathological use [36].

The current study was undertaken to determine the immunohistochemical expression of the angiogenic marker CD34 as evaluated by MVD in benign fibroadenoma, fibrocystic disease and IDC females. In addition, to correlate its expression with other established prognostic histopathological parameters (age, tumor size, histopathological grade, number and location of axillaries lymph nodes, lymph nodes status and hormonal profile ER and PR.

Competing Interests

Authors declare that they have no competing interests; financials or others.

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