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Immunohistochemical Expression of Angiogenic Marker CD34 in Invasive Carcinoma of the Breast

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Abstract:

Background: *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. It has been found that microvessel count by CD34 immunostaining identifies breast cancer patients with aggressive phenotype. There are data suggesting that breast cancer is an angiogenic- dependent disease.*

Objectives: *This study aimed to investigate CD34 expression in breast carcinoma of different grades of differentiation and correlated the expression patterns with their tumor size and metastasis using immunohistochemical staining.*

Materials and methods: *Formalin fixed-Paraffin embedded blocks from 50 female patients with breast carcinoma were used, immunohistochemistry staining for CD34 was performed and*

expressed low levels of CD34 ≤15/HPf and high levels of CD34 >15/HPf in all 50 cases of different grades of breast carcinoma and correlated with their histological parameters.

Results: *8 (16%) cases of breast cancer expressed low levels of CD34 and 42(84%)cases expressed high levels of CD34 >15/HPf . The expression of CD34 had no significant correlation with tumor size p value (0 .65) and metastasis with p value (0.80)*

Conclusion: *We conclude that there is no statistical significant between CD34 expression and histological parameters like tumor size and mtastasis in breast cancer in Sudanese patients.*

Key words: Immunohistochemical Expression, Angiogenic Marker CD34, Invasive Carcinoma of the Breast

INTRODUCTION:

Breast cancer is the most prevalent type of cancer in the world [(1)]. In United States and Europe it is the most common cancer in women and the second leading cause of cancer death [(2,3)]. In Arab countries breast cancer is the much more common among women with a mean age 50 years at diagnosis [(4).]

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 [(6)]. Solid tumor growth and metastasis are angiogenic dependent [(7)]. 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 [(8)].

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 micro vessel density (MVD), beside the traditional histopathological parameters including axillary lymph node status, tumor size and grade [(9)].

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 [(10)].

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 [(11)]. Few studies have measured tumor MVD by immunohistochemical methods [(12,13)]. It has been found that microvessel count by CD34 immunostaing identifies breast cancer patients with aggressive phenotype [(14)]. There are data suggesting that breast cancer is an angiogenic- dependent disease [(15)].

Figure (1)

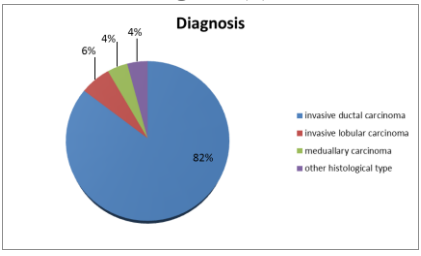

Figure (2)

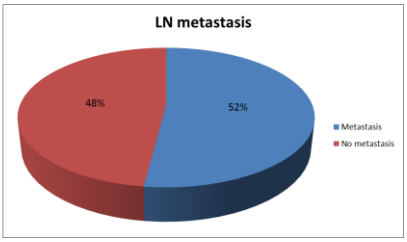

Figure (3)

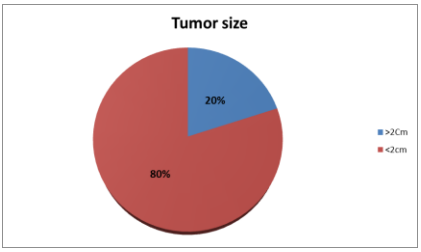

Figure (4)

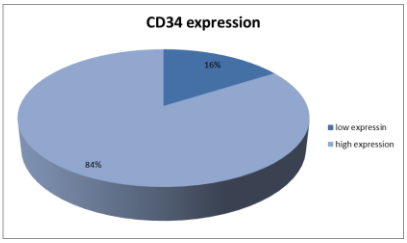

Table (1) correlation between CD34and Tumor metastasis

Metastasis	CD34 expression		Total
	Low	High	
Yes	8%	44%	52%
No	8%	40%	48%
Total	16%	84%	100%

P.value:0.8

Table (2) correlation between CD34and Tumor sizes

Metastasis	CD34 expression		Total
	Low	High	
<2cm	4%	16%	20%
>2cm	12%	68%	80%
Total	16%	84%	100%

P.value:0.65

MATERIALS AND METHODS:

Fifty (50) formalin-fixed -paraffin embedded blocks of Sudanese female with breast carcinoma were taken from archive of Histopathology department in Radiation and isotope center –

Khartoum (RICK), evaluation of histopathology was made on 3 micron thick sections stained with hematoxylin and eosin, and the grading assessed by consultants Histopathologist using Nottingham grading system of Elston and Ellis (16).

Immunohistochemistry:

Paraffin embedded blocks were cut at 3Mm and mounted on salinized slides, deparaffanization in xylene and then rehydrated through graded series of alcohol. Antigen retrieval was performed by using PT link, endogenous peroxidases activity was blocked with 3% hydrogen peroxidase and methanol for 10 minutes, then incubated with 100\200 µL of primary antibody for CD34 for 20 minutes at room temperature , rinsed in phosphate buffer saline, and binding of antibody was detected by incubating 20 minutes with system(Thermo -ultra vision) , finally, the sections were washed in three changes of PB , visualization of the positive reaction with 3,3 diaminobenzidine and counter-stained with hematoxylin.

Statistical analysis:

Cross tabulation was used during the statistical analysis using SPSS package version 18 and chi-square test was assessed to correlate between expression of CD34 and tumor size and metastasis. Ethical clearance for this study is provided by ethical committee of Faculty of medical laboratory sciences – AL-Neelain University, Khartoum, Sudan.

RESULTS:

All patients included were females, the average age of the invasive ductal carcinoma was 52 year (range 24- 80 years), and total number were 50 cases of different grades of invasive ductal carcinoma.

Expression of CD34 in breast cancer. As determined by immunohistochemical analysis,8 specimens of breast cancer expressed low levels of CD34 ≤15/HPf, accounting for 16% of cases. By contrast, 42 specimens expressed high levels of CD34 >15/HPf, accounting for 84% of cases. Figure (4)

The expression of CD34 had no significant correlation with histopathological findings such as tumor size p value (0 .65) Table (2) and tumor metastasis with p value (0.80) Table (1)

DISCUSSION

Cluster of differentiation (CD) 34 is a specific marker of vascular endothelial cells; an endothelial cell-specific marker, predominantly expressed on endothelial and hematopoietic progenitor cells,. In particular, CD34 is particularly sensitive to tumor angiogenesis, as it can clearly represent the state of neovascularization during the growth of a tumor (Chen, et al. 2015).

In our study we found that the expression of CD34 had no significant correlation with histopathological findings such as tumor size with p value (0 .65) Table (2) and tumor metastasis with p value (0.80) Table (1)

This finding same to Ch'ng et al in 2012 ,they analyzed CD34 expression in 94 invasive ductal breast cancer tissue samples and identified that CD34 was highly expressed in the youth group (aged ≤55 years), but was not associated with other clinicopathological factors.

Chen et al in 2015 had found that high CD34 expression levels (microvessel density, >15/HPF) were identified in 27.3% (12/44) of cases, exhibiting no significant correlation with the clinicopathological characteristics of the patients. However, Kaplan-Meier analysis demonstrated that the survival time of patients with high CD34 expression was significantly shorter

than that of patients with low CD34 expression (50.0 vs. 90.6%; P=0.003).

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