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The expression of Vascular Endothelial Growth Factor (VEGF) and Ki67 in Correlation to Stage of Breast Cancer

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Abstract: Background: *Breast cancer (BC) is a common malignant neoplasm comprising a large heterogeneous group of cancers with variable histological type, biological and clinical characteristics.* ***Aim:*** *This study was aimed to detect over expression of vascular endothelial growth factor and Ki67 in correlation to stage of breast cancer.* ***Materials and Methods:*** *This was descriptive cross sectional hospital based study conducted in Radiation &Isotopes –Center of Khartoum (RICK). Included Sudanese women with breast cancer during the period* ***between (October 2020 To January 2021).*** *Purposive sampling techniques was used in this study included 50 subject, from each tissue sample paraffin block was prepared two paraffin sections it was cut into 3µm thickness section floated into preheated floating water bath at 40˚c, two sections were placed in coated glass slide for Immunohistochemistry, one section was placed in clean microscopical slide for Hematoxylin and Eosin , incubated in oven at 58˚c.* ***Results****: The study results revealed that expression of VEGF in invasive ductal carcinoma was showed positive staining reaction in 14 cases (28%). No expression of VEGF was reported in 4 cases of stage I, in 7 out of 32 cases of stage II, and in all 7 cases out of 7 of stage III .There was significant positive correlation between VEGF expression and the stage of tumor (p value <0.000), and a higher proportion of cases were found in stage II and III. There was significant positive correlation between VEGF expression and Ki67(p value <0.000).* ***Conclusion****: This study concluded that there was relationship between VEGF expression and cancer stage and Ki67 expression.* ***Recommendations****: The study recommends that further study with more sample size is required.*

Keywords: VEGF, Ki67, Stage, Breast Cancer

Introduction

Breast cancer (BC) is a common malignant neoplasm comprising a large heterogeneous group of cancers with variable histological type, biological and clinical characteristics. Breast cancer refer to cancers originate from breast tissues, most likely from inner lining of milk ducts or the lobules which supply the duct with milk, cancer originate from duct called ductal carcinoma and that originate from lobes called lobular carcinoma. The story of breast cancer is told in the acts and artefacts of the human struggle against disease. The oldest description of breast cancer was in Egypt and dates back approximately to 1600 BC, it was the most commonly diagnosed cancer in women worldwide (1), and is second only to lung cancer as a leading cause of mortality. It is found in high rates in developing countries as well as low-middle income countries. 1 in 9 women is diagnosed with BC worldwide (2).

The incidence of breast cancer in Sudan during the period from 2000 to 2009 is 6622 (17.2%) cases; from these cases females represented 6299 (95.1%), while male represented 323 (4.9%). Registrations of breast cancer in Sudan (1068) in 2010, (1036) in 2011 representing 16% of total patients and (1242) in 2012 representing 17% of total patients according to radiation and isotope center-Khartoum records (3).

Breast cancer incidence and mortality has been rising. Together with cervical cancer, breast cancer comprises 50% of women's cancer in the Sudan (4). Breast cancer in Sudanese women was characterized by a younger age at onset, and about 78% present with advanced stages of disease at diagnosis (stages III, IV) (2). Some breast cancer requires hormones for its growth such as estrogen and progesterone which has receptor; Prognostic factors of breast cancer include tumor size, lymph node stage, tumor grade; tumor type and vascular lymphatic invasion (5).

There are multiple factors associated with increased risk of breast cancer include race, age, family history, previous medical history, menstrual history, reproductive history and Genetics (6).

Immunohistochemical studies in sporadic cancers have led to identification of novel targets with roles in diagnosis, prognostic and therapeutics. During the last few years, several investigators have focused on tumor angiogenesis as a critical step in cancer development and progression. Among these, vascular endothelial growth factor (VEGF) is emerging as a prognostic marker with several type of cancer including breast cancer (7).

Much of the prognostic information and treatment options are related to tumor subtype, and due to the limited availability and accuracy of prognostic and predictive markers, many patients don't benefit from the treatment (8). Triple negative breast cancer (TNBC) demonstrates poor prognosis because of aggressive tumor biology, and lack of targeted agents .TNBC has a pattern of rapid recurrence following diagnosis, and the peak risk of recurrence is within three years, therefore, much researches has investigated the significance of diagnosing the basal-like carcinoma in terms of prognostic value.

Basal like breast cancer remains a great challenge because of its clinically aggressive nature and poorly characterized molecular pathogenesis. In this study we aimed to classify breast cancer into basal and non-basal-like and to study their biological behavior using immunohistochemically markers.

The study aimed To detect expression of vascular endothelial growth factor and Ki 67 in correlation to stage of breast cancer in Sudanese women.

Materials and Methods

Study Design

This was descriptive cross sectional hospital based study.

Study Area

This study was conducted in Radiation &Isotopes –Center of Khartoum (RICK).

Study population

Sudanese women with breast cancer.

Selection criteria

Sudanese women with breast cancer who attended to (RICK).

Inclusion Criteria

Sudanese women with breast cancer .

Exclusion Criteria

Sudanese women with metastatic breast cancer

Study duration

Study was conducted during the period between (October 2020 To January 2021)

Sampling and Samplesize:

Purposive sampling techniques was used in this study
This study include 50, subject, this study enrolled according to the equation:

$n = z^2 * pq/d^2$

n = sample size
Z = power (1.096)
P = prevalence of disease d = stander deviation (0.05) q = 1-p

Data collection

Formalin fixed tissue sample from patients diagnosed with breast cancer was used in this study.

Sampling

Histopathological tissue preparation

From each tissue sample paraffin block was prepared two paraffin sections itwas cut into 3µm thickness section floated into preheated floating water bath at 40˚c, two sections were placed in coated glass slide for Immunohistochemistry, one section was placed in clean microscopical slide for Hematoxylin and Eosin , incubated in oven at 58˚c. The staining procedure was as follows

Hematoxylin and Eosin:

Sections were stained with hematoxylin and eosin as described by Mayer's in Bancroft (9), then slides were examined primary by the investigator and then, results were confirmed by histopathologist to verify that an adequate number of breast cancer cells were present also the morphological classification**. Immunohistochemical staining**

Procedure have been carried out using the following antibodies

Primary antibody	Specification
KI67	Monoclonal mouse anti human ki67
VEGF	Mono clonal mouse anti human VEGF

The immunohistochemicall procedure have been done as follows

Two sections (3µm) from formalin-fixed, paraffin-embedded tissue were cut and mounted onto positive charge slides (Dako). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and were placed in running water. Samples were steamed for antigen retrieval for VEGF and KI67 using PT link. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase for 10 min, and then slides were incubated with 50-60 µl of primary antibodies for 25-30 min at room temperature in a moisture chamber, and then were washed in Phosphate buffer saline three times. The primary antibody for VEGF and KI67 were ready to use after washing with PBS for 3 min, binding of antibodies were detected by incubating for 25-30 minutes with dextran labelled polymer (Dako- EnVision TM Flex kit). Finally, the sections were washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) (Dako) as a chromogen for 5-10 minutes to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. Slides were counterstained with hematoxylin. For each run of staining, positive and negative control slides were also prepared. The positive control slides were contain the antigen under investigation and the negative control slides were prepared from the same tissue block, but were incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator then the results was confirmed by consultant histopathologist and scored. The method is performed according to manufacture.

Data analysis:

Data were analyzed using statistical package for social science (SPSS) computer program. Significant considered as (P.value <0.05), all results expressed as mean + SD, Chi2 was calculated.

Ethical Considerations

Ethical approval were obtained from research committee in National University —Sudan for this research,

Results

Samples recruitment and histological types

Fifty cases of breast cancer were included in this study; all of this cases were invasive ductal carcinoma.

Patient age:

The patients' age ranged between 20 and 80 years with mean age of about 50 years.

VEGF expression pattern:

The VEGF showed positive staining reaction in 14 cases (28%) and negative staining reaction in 36 cases (72%) **Table (1)**.

Ki67 expression pattern:

The Ki67 showed positive staining reaction in all cases 50 (100%).

Relation between cancer stage VEGF expressions:

The (P value 0.000) which is statistically significant, that means there was relation between cancer stages of the breast cancer and VEGF expression. **Table (2)**.

Relation between Ki67 and VEGF:

The (P value 0.000) which is statistically significant that means there are relation between Ki67 in the breast cancer and VEGF expression **Table 3**.

Table 1: VEGF expression pattern

VEGF expression	Frequency	Percentage
Positive	**14**	**28**
Negative	**36**	**72**
Total	**50**	**100**

Table 2: Relation between cancer stage and VEGF expressions

Grades	VEGF expressions		Total	P.value
	Positive	Negative		
Grade I	**0**	**4**	**4**	**0.000**
Grade II	**7**	**32**	**39**	
Grade III	**7**	**0**	**7**	
Total	**14**	**36**	**50**	

Table 3: Relation between Ki67 and VEGF

Ki67 expressions	VEGF		Total Percentage	P value
	Positive	Negative		
5-10	**1**	**17**	**18**	**0.000**
10-20	**1**	**12**	**13**	
20-30	**2**	**6**	**8**	
30-40	**2**	**1**	**3**	
40-50	**4**	**0**	**4**	
50-60	**4**	**0**	**4**	
Total	**14**	**36**	**50**	

Discussion

The expression of VEGF in invasive ductal carcinoma was showed positive staining reaction in 14 cases (28%), this finding was lower than those of Shamim Shera *et al*., (10) who reported that 75% were VEGF positive. It was lower than a study conducted by Al Harris *et al*.,(11) who reported. 61.5% were VEGF positive.
VEGF expression was not reported in 4 cases of stage I, in 7 out of 32 cases of stage II, and in all 7 cases out of 7 of stage III .There was significant positive correlation between VEGF expression and the stage of tumor (p value <0.000), and a higher proportion of cases were found in stage II and III. These findings correlated well with Shamim Shera *et al*., (10), Callagy *et al.*, (12), Bolat *et al*.,(13), AL-Harris E *et al*(11) and Xu W *et al*., (2007).
VEGF is expressed more in those with advanced stage which reflects the aggressive behavior of the tumor.

There was significant correlation between VEGF expression and Ki67(p value <0.000),this result agreed with Shamim Shera *et al*., (10), Xie *et al*.,(15) ,and disagreed with Li *et al*.,(16) who did not reach similar result.

This study concluded that there was relationship between VEGF expression, cancer stage and Ki67 expression.

This study recommends that:

- Further study with large sample size should be done.

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