Immunohistochemical expression of vascular endothelial growth factor and its correlation with tumor grade of breast ductal carcinoma among Sudanese Women

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Abstract

Background: Breast cancer is the most common cause of cancer-related death in females, after lung cancer. Angiogenesis is essential for tumor growth and metastasis; therefore, ant angiogenesis strategies for the treatment of cancer are currently an issue of interest. The role of vascular endothelial growth factor assumed to be the most potent angiogenesis factor is ambiguous in breast cancer. **Objective:** This study described the correlation between vascular endothelial growth factor expression and invasive ductal carcinoma of the breast cancer grade. **Methods:** This cross-sectional descriptive study was carried out conducted in the Radiation &Isotopes –Center of Khartoum (RICK) on 50 cases of formalin fixed paraffin embedded blocks taken from patients with invasive ductal breast carcinomas were analyzed for vascular endothelial growth factor expression by Immunohistochemical staining. **Results:** The study results revealed that vascular endothelial growth factor is expressed in 17 cases (34%) of breast cancer invasive ductal carcinoma, the expression was reported in 0/4 (0%) cases of stage I, in 10/39 (25.6%) cases of stage II, and in 7/7 (100%) of stage III. There was a significant positive

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correlation between VEGF expression and the stage of the tumor (p-value <0.000), and a higher proportion of VEGF expression were found in stages II and III. The study recommends that further research with more sample size is required.

Keywords: **Breast Ductal Carcinoma, Immunohistochemical, VEGF, Sudan**.

Introduction

Angiogenesis has been shown to be a critical aspect of tumor growth and metastasis (1,2). The induction of angiogenesis by a tumor is a controlled process, influenced by angiogenic and angiostatic factors which involve a complex interaction between tumor and endothelial cells (2,3). Among the many reported angiogenic factors, vascular endothelial growth factor (VEGF) is the most powerful endothelial cell specific mitogen that plays a key role in the complicated process of angiogenesis. VEGF a,lso known as vascular permeability factor, is a glycoprotein of 32-42 kDa. The VEGF family currently includes six known members: VEGF-A (commonly known as VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (4-5). VEGF activates tyrosine kinase receptors, VEGFR-1 (also referred to as FLT1) and VEGFR-2 (or KDR) located in the endothelium, which leads to stimulation of endothelial migration, proliferation, permeability, and survival (6). Recent evidence suggests that VEGF not only plays a role in inducing angiogenesis but also is promotes the survival of new vessels formed with in the tumor (7). Therefore the current study tries to find the expression of VEGF and its correlation with tumor stage of invasive ductal carcinoma of the breast as a possible explanation for the aggressive behavior of this tumor.

Material and Methods

This is a descriptive cross-sectional study was carried out in Khartoum state at the Radiation &Isotopes –Center of Khartoum (RICK), (50) archival formalin fixed paraffin embedded tissue blocks form Sudanese patients with breast cancer (invasive ductal carcinoma) attended RICK were enrolled.

Histopathological tissue preparation

From each tissue block two paraffin sections were cut into 3µm thickness and

floated into a preheated floating water bath at 40c, then incubated in the oven at 58˚c for over light for partial deparaffinization, then sections were placed in coated glass slide for Immunohistochemistry, and one section was placed in the clean microscopic slide for Hematoxylin and Eosin,

Hematoxylin and Eosin

Sections were stained with Hematoxylin and Eosin, then slides were examined primarily by the investigator and then results were confirmed by histopathologist to verify that an adequate number of breast cancer cells were present.

Immunohistochemistry staining technique

Sections were dewaxed in xylene and rehydrated through grade of alcohols to distilled water. Before immune staining for VEGF (cell mark) antigen retrieval by retrieval solution using PT link. Endogenous peroxidase activity will be blocked with 3% hydrogen peroxidase and methanol for 10 min, then Slides incubated with 150 µl of primary antibodies for 25 min at room temperature in a moisture chamber, and then rinsed in Phosphate buffer saline. The primary antibody for VEGF and was ready to use after washing with PBS for 3 min, binding of antibodies was detected by incubating for 25 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 to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. After that counter stained using Harris Hematoxylin for 2 minutes, then washed in water, dehydrated, cleared, and mounted in D.P.X, cover slip. The entire slide was evaluated for immune staining by a light microscope. The slide was first observed under 10X magnification to examine the staining of the whole slide, then conformed under 40Xmagnification. The positive control slides contain the antigen under investigation and the negative control slides was prepared from the same tissue block, but was incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator then the results was confirmed by consultant histopathologist and scored. Data were analyzed using a statistical

package for social science (SPSS) computer program. Significant was considered as *P. value* < 0.05.

Results:

The age distribution of the study population demonstrated in (Table 1), o most of women who enrolled in study their age range from 30 to 39 years (30%) and only one woman in the age group from 20 to 29 (2%)). histological grade of cancer 4 samples was diagnosed as grade I (8%), 39 samples were diagnosed as grade II (78%) and 7 samples were diagnosed as grade III (14%) (Table 2). The VEGF showed a positive staining reaction in 17cases (34%) and a negative staining reaction in 33cases (66%) (Table 3). They are 4 cases with grade I, 39 grades II and 9 cases of grade III. That the expression of VEGF as the following: expressed in 17 cases (34%) of breast cancer invasive ductal carcinoma, the expression was reported in 0/4 (0%) cases of stage I, in 10/39 (25.6%) cases of stage II, and in 7/7 (100%) of stage III. There was a significant positive correlation between VEGF expression and the stage of the tumor (p-value <0.000), which is statistically significant and means there is a relation between cancer grade and VEGF expression (Table 4). The patients' age in these study ranged between 20 and 80 years with a mean age of about 50 years, they are one case of (20-29) years showing no expression of VEGF, and they are 15 cases of (30-39) age group about 3 from these group show expression of VEGF and 12 show no expression in (40-49) years they have 7 cases 5 of them show the expression and 2 with no expression they are 12 cases of group ranging from 50 to 59 years show 4 from them with expression and 8 without expression in (60-69) years they are 2 from the show expression and 4 cases with no expression the last group ranged from 70 years to 80 years show 6 of them no expression and 3 cases show expression finally they are 17 cases of all group show expression and 33 show no expression of VEGF. The scoring of VEGF in grade II and grade III of histological cancer they are 10 cases of grade II 7 of them score {+} of VEGF and 3 of them score {++} and they are 7 cases of grade III 5 of them score {+} and 2 of them score {++} of VEGF which is insignificant (*P. value*=0.686) (Table 6). Also, the scoring of VEGF in the different age groups which show no

significant (P. V=0.884) scoring recorded as the following we have 2 cases show {+} and one show {++} in years (30-39), have 4 cases show {+} and one show {++} of the year (40-49), in (50-56) year group we have 2 scorings {+}and 2 scorings {++}, group (60-69) year score {+} in 2 cases in the last group scoring {+} in 2 cases and only one score {++} in (70-80) year (Table 7).

Table-1: Distribution of the age group of the study population

Age (Years)	*Number*	*Percent*
20-29 years	1	2%
30-39 years	15	30%
40-49 years	7	14%
50-59 years	12	24%
60-69 years	6	12%
70-80 years	9	18%
Total	50	100%

Table-2: The histological grade of cancer in this study

Histological grade of cancer	*Number*	*Percent*
I	4	8%
II	39	78%
III	7	14%
Total	50	100%

Table-3: VEGF expression pattern

VEGF Expression	*Number*	*Percent*
Negative	33	66%
Positive	17	34%
Total	50	100%

Table-4: Relation between cancer grade and VEGF expression

Grades	VEGF expressions		Total	*P. value*
	Positive	*Negative*		
Grade I	0	4	4	0.000
Grade II	10	29	39	
Grade III	7	0	7	
Total	17	33	50	

Table-5: Relation between cancer Age and VEGF expression

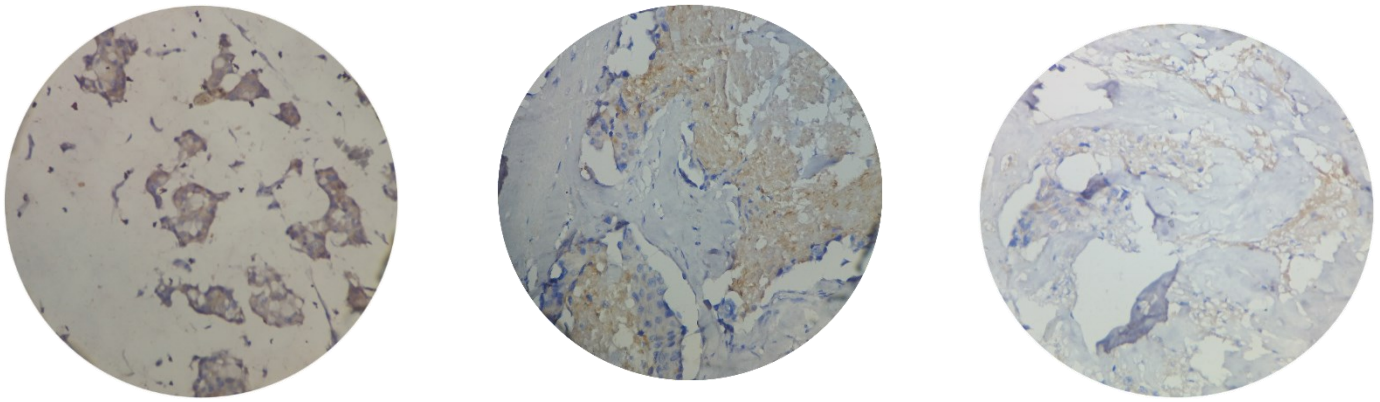
Age groups	VEGF expressions		*P. value*
	Positive	*Negative*	
20-29 *years*	0	1	0.309
	0.00%	100.00%	
30-39 *years*	3	12	
	20.00%	80.00%	
40-49 *years*	5	2	
	71.40%	28.60%	
50-59 *years*	4	8	
	33.30%	66.70%	
60-69 *years*	2	4	
	33.30%	66.70%	
70-80 *years*	3	6	
	33.30%	66.70%	

Table-6: The vascular endothelial growth factor scoring

Histological grade of cancer	VEGF Expression			*P. value*
	[+]	[++]	Total	
II	7	3	10	0.686
III	5	2	7	
Total	12	5	17	

Table-7: The VEGF expression scoring and age

Age groups	VEGF expressions		*P. value*
	(++)	(+)	
30-39 *years*	1	2	0.884
	33.30%	66.70%	
40-49 *years*	1	4	
	20.00%	80.00%	
50-59 *years*	2	2	
	50.00%	50.00%	
60-69 *years*	0	2	
	0.00%	100.00%	
70-80 *years*	1	2	
	33.30%	66.70%	



A B C

(A) Section show invasive ductal carcinoma grade 1 show positive immunohistochemically staining of VEGF.
(B) Section show invasive ductal carcinoma grade 2 show positive immunohistochemically staining of VEGF.
(C) Section show invasive ductal carcinoma grade 3 show positive immunohistochemically staining of VEGF.

Discussion

Causation of breast carcinoma is multifactorial and angiogenesis has a pivotal role (8). The biology of breast carcinoma remains poorly understood as the knowledge about individual prognostic factors provides limited information(9). Inspite of a huge number of studies role of anti VEGF therapy is still debatable in case of breast cancer (10).

VEGF was significantly increased in breast cancer grade VEGF in tumor is increased and significantly correlated with microvessel density and poor prognosis in human cancers including breast cancer (12, 13). The role of VEGF in tumor angiogenesis is well known and many of the current anti-angiogenic therapies targeting VEGF for quite some time (11, 18). VEGF activates VEGFR-2 and VEGFR- 1 (14, 15). VEGFR-2 is the primary VEGF receptor, while the VEGFR-1 is less defined and its role during tumor angiogenesis has been recently suggested (11, 17, 18), although genetic data indicate that signaling trough this receptor is not required for physiological angiogenesis (16).

This study analyzed VEGF expression by IHC in invasive carcinoma of the breast and the correlation of VEGF expression with the tumor grade and patients' age. In this study, we found the expression of VEGF in Invasive ductal

carcinoma was 34% of cases with significant positive correlation between VEGF expression and the stage of the tumor (p-value <0.000), VEGF is expressed more in those with an advanced stage which reflects the aggressive behavior of the tumor, there was an insignificant positive correlation between VEGF score and the patients age or tumor grade (p-value were 0.884, 0.868). Presence of significant positive correlation between grade of breast carcinoma and VEGF expression has been also shown by other researchers in different studies which explain the role of VEGF gene in tumor angiogenesis and tumor progression (19,20,21,22)

This study suggested the vascular endothelial growth factor (VEGF) plays an important role in the prognosis of breast cancer and supports the evidence of its role in angiogenesis and cell survival.

REFERENCES

1. Folkman J, Shing Y. Angiogenesis. *J Biol Chem,* 1992;**267**:10931-34 3. Folkman J. What is the evidence that tumors are angiogenesis dependent. *J Natl Cancer Inst,* 1990;**82**:4-6 2.Folkman J. Clinical application of research on angiogenesis. *N Engl J Med,* 1995;**333:**1757-63 5. Risau W. Mechanisms of angiogenesis. *Nature*, 1997;*386*:671-74

3. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell, 1996;86:353-64

4.Newfeld G, Cohen T, Gengrinovitch S. Vascular endothelial growth factor (Vegf) and its receptors. FASEB J, 1999;13:9-22

5. Ogawa S, OkuA, SawanoA.Anovel vascular endothelial growth factor, VEGF-E (NZ-7 VEGF), preferentially utilises KDR/Flk-1 receptor and carries a potent mitotic activity without heparin-binding domain. *J Biol Chem*, 1998;**273**:31273-82

6. hibuya M. Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. *Cell Struct Funct*, 2001;**26**:25-35

7. Benjamin LE, Keshet E. Conditional switching of vascular growth factor (VEGF) expressions in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. *Proc Natl Acad Sci USA,* 1997;**94**:8761-66

8. YenL, YouXL andAIMoustafaAE.Heregulinselectively up-regulates vascular endothelial growth

factor secretion in cancer cells and stimulates angiogenesis. *Oncogene*, 2000;19:3460-69

9. Sorli, T., Perou, C.M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., et al. Gene Expression Patterns of Breast Carcinomas Distinguishes Tumor Subclasses with Clinical Implication. *Proceedings of the National Academy of Sciences of the United States of America*,2001;98:10869-10874

10. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez E A, Shenkier T, Cella D, Davidson N. E. et al., Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer,*N Engl J Med,*2007;357(26):155-168

11. H. F. Dvorak, "Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy," *Journal of Clinical Oncology*, 2002, vol. 20, no. 21, pp. 4368–4380

12. N. Ferrara, H.-P. Gerber, and J. LeCouter, "The biology of VEGFand its receptors," *Nature Medicine*, 2003,vol. 9, no. 6, pp. 669–676

13.M. Toi, T. Matsumoto, and H. Bando, "Vascular endothelial growth factor: its prognostic, predictive, and therapeutic implications," *The Lancet Oncology*, 2001,vol. 2, no. 11, pp. 667–673

14. N. Ferrara and K. Alitalo, "Clinical applications of angiogenic growth factors and their inhibitors," *Nature Medicine*, 1999.

, vol. 5, no. 12, pp. 1359–1364

15.M. Shibuya, "Structure and function of VEGF/VEGF-receptor system involved in angiogenesis," *Cell Structure and Function*, 2001, vol. 26, no. 1, pp. 25–35.

16. S. Hiratsuka, O. Minowa, J. Kuno, T. Noda, and M. Shibuya, "Flt-1 lacking the tyrosine kinase domain is sufficient or normal development and angiogenesis in mice," *Proceedings of the National Academy of Sciences of the United States of America*, 1998, vol. 95, no. 16, pp. 9349–9354

17. J. Yao, X. Wu, G. Zhuang et al., "Expression of a functional VEGFR-1 in tumor cells is a major determinant of anti-PlGF antibodies efficacy," *Proceedings of the National Academy of Sciences of the United States of America*, 2011,vol. 108, no. 28, pp. 11590–11595

18. K. D. Lynn, C. L. Roland, and R. A. Brekken, "VEGF and pleiotrophin modulate the immune profile of breast cancer," *Cancers*, 2010 , vol. 2, no. 2, pp. 970–988

19 Adams J., Carder P. J., Downey S., Forbes M. A., Maclennan K., Allgar V., Kaufman S., Hallam S., Bicknell R., Walker J. J., CairnduffF., Selby P. J., Perren T. J., Lansdown M., Banks R. E. et al, Vascular endothelial growth factor (VEGF) in breast cancer: comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen, *Cancer Res*, 2000;60(11):2898–2905.

20. Vogl G, Bartel H, D Otto, K Cornelia et al. HER2 is unlikely to be involved in directly regulating angiogenesis in human breast cancer, *Appl Immunohistocem Mol Morphol* 2006;14(2):138-145

21. Granato AM, Nanni O, Faicini F, Folli S et al. Basic fibroblast growth factor an vascular endothelial growth factor serum levels in breast cancer patients and healthy women: useful as diagnostic tools? *Breast cancer Res* 2004;6:R38-45.

22. Ali A M, Sheta M, Mohsen M A El et al. Elevated serum and tissue VEGF associated with poor outcome in breast cancer patients, *Alexandria Journal of Medicine*, 2011;47:217-224