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Original Research Article

Expression of VEGF in breast lesions: an immunohistochemical study

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ABSTRACT

Background: Breast cancer is the most frequent cancer in India. 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 in patients with several type of cancer including breast cancer. The aim of the study was to analyse the expression of VEGF in human breast cancer as compared to normal breast tissue and benign breast lesions by immunohistochemistry. Also, to assess the usefulness of VEGF as a predictor of aggressiveness of breast lesions.
Methods: Formalin fixed paraffin embedded sections of 10 cases of normal breast tissue, 20 cases of benign breast lesions and 20 cases of malignant breast lesions were taken up for the study and subjected to immunohistochemistry using VEGF.
Results: The intensity of VEGF immunostaining in normal breast, benign and malignant breast lesions was evaluated and scoring was graded as 0, 1+, 2+, 3+ and 4+. Statistical analysis was performed with Chi-Square test and significant differences were noted between these 3 groups (p value <0.05).
Conclusions: VEGF expression correlated well with the grade and stage of tumor indicating that VEGF positive tumors are biologically aggressive and are associated with poor prognosis but little is known about the implication of genetic alterations of VEGF in benign breast lesions.

Keywords: Breast lesions, Immunohistochemistry, VEGF

INTRODUCTION

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 1600BC. Breast cancer is the most frequent cancer in India and mortality rates associated with it is higher in India. In India, the incidence of breast cancer is almost one third to one fourth that in USA but it is still a leading cause of cancer among women in many regions. Mortality from breast cancer in India ranges to 11.1 as compared to 14.7 in USA which is higher (Globocan).[1] Many genetic alterations and oncogene protein products which interfere with the mechanism of proliferation and differentiation of tumor growth have been discovered and investigated.

Candidate prognostic biomarkers in breast cancer include elevated levels of expression of proliferation indices such as Ki67 and proliferating cell nuclear antigen, expression of Estrogen Receptor (ER) and Progesterone Receptor (PR), amplification and overexpression of HER2, cyclin

D1, c-Myc, p53 nuclear protein accumulation, bcl-2 expression and alteration in angiogenesis proteins such as Vascular Endothelial Growth Factor (VEGF).

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.[2]

VEGF produced and secreted by a number of normal cells are polyfunctional molecules that have been implicated in vasculogenesis, endothelial cell proliferation and migration, vascular permeability and stromal degradation through the activation of some proteolytic enzymes involved in tumor invasiveness and angiogenesis.[2] VEGF is required for the initial stages of breast cancer tumorigenesis and this initial effect is related to the development of neovascular stroma. Keeping all these alterations in mind, in this present study author have characterized the pattern of VEGF expression in normal breast, benign and malignant breast lesions.

METHODS

The study was conducted at a Tertiary Care Hospital. Fifty cases were selected, out of which 10 cases of normal breast tissue, 20 cases of benign breast lesions and 20 cases of malignant breast lesions were taken. All female cases irrespective of their age and other physical conditions during the period of June 2010 to June 2012 were taken up for the study.

- Study group: twenty cases of female breast cancer patients were selected randomly, their ages ranging from 20 to 70years. All of them underwent modified radical mastectomy.
- Control group: ten cases of normal breast tissue from patients presenting with breast mass, other than tumor were selected and regarded as a control group.
- Comparative group: twenty cases with benign breast lesions were taken as comparative group to compare the rate of VEGF immuno-expression with that in malignant breast lesions. Of the benign cases: 10 cases were fibroadenoma, 10 cases were fibrocystic change.

The diagnosis was reconfirmed on Hematoxylin and Eosin (HandE) stained sections and the appropriate blocks were subjected to IHC using VEGF antibody (BIOGENEX, AR483-5R polyclonal antibody 6ml, ready-to-use USA).

The criteria for positive immunoreaction were dark brown precipitate (cytoplasmic for VEGF). Scoring according to Apple SK, et at objective 40x (Table 1).[3] The intensity of the staining was assessed as follows:

Table 1: Scoring for VEGF.

Score	Results	Interpretation
0	Negative	None or <5% cells positive.
1+	Weak or Mild staining	Weak or mild staining, 5-10% of tumor cells are positive.
2+	Moderate staining	<25% of tumor cells are positive.
3+	Strong staining	Strong staining, 25-50% of tumor cells are positive.
4+	Highly strong staining	Highly strong staining, >50% of tumor cells are positive.

Statistical analyses of all results were done by using Chi square test at level of significance $p \leq 0.05$ was done.

Ethical clearance was obtained from the Ethical Committee Meeting conducted at Meenakshi Medical College and Research Institute, Kanchipuram, Tamil Nadu, India.

RESULTS

The cases included in the study were selected irrespective of their age differences. This study included 50 cases of breast lesions which included infiltrating ductal carcinoma, fibroadenoma, fibrocystic change and normal breast tissue. Of the 50 cases taken up for the study, 20 cases were infiltrating ductal carcinoma, 10 cases were fibrocystic change, 10 cases were fibroadenoma, 10 cases were normal breast tissue (Table 2).

Table 2: Cases included in the study.

Type of breast tissue	No. of cases	Percentage
Normal	10	20
Benign	20	40
Malignant	20	40
	50	100

The cases included in the study were selected irrespective of their age differences. Analysis of the study sample showed that majority of the cases were in the age range of 15 to 45years which comprised 76% of the sample size (Table 3).

Table 3: Age wise distribution of cases.

Serial no.	Age group	No. of cases	Percentage
1	15-30 yrs	19	38
2	31-45 yrs	19	38
3	46-60 yrs	8	16
4	>60 yrs	4	8
Total		50	100

The malignant lesions in the study were seen in all histological grades of the tumor with majority of the cases having a higher grade. These also presented with

different stages with predominant cases having a higher stage with a presentation of stage III being more common followed by stage IV. None of the cases presented in stage 0 or stage I in this study. The axillary lymph nodes were involved in 70% of these cases with only 6 cases showing no axillary lymph node involvement (Table 4).

Table 4: The characteristic features of all malignant breast lesions.

Features	Grade			Stage					Axillary lymph nodes	
	I	II	III	0	I	II	III	IV	+ve	-ve
No. of cases	2	8	10	0	0	4	12	4	14	6

In immunohistochemical analysis of VEGF protein it was observed that none of the 10 cases of normal breast tissue showed immunoreactivity for VEGF.

Out of the 20 cases of benign breast lesions, 10 cases were fibroadenoma and 10 cases were fibrocystic change. Expression of VEGF was noted in 3 (15%) of the 20 cases of which 2 cases were fibroadenoma and 1 case was fibrocystic change (Figure 1).

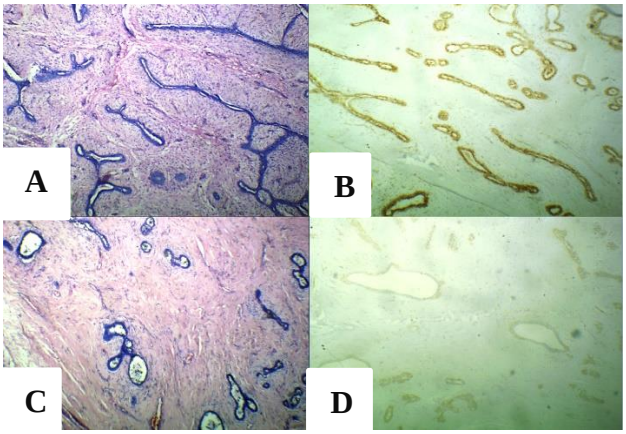

Figure 1: VEGF in benign breast lesions (10x). A) Fibroadenoma of breast, B): Positive immunostaining of VEGF in fibroadenoma, C): Fibrocystic change of breast, D): Positive immunostaining of VEGF in fibrocystic change.

Though only 3 cases of benign breast lesions were positive for VEGF but among these, 2 cases that of fibroadenoma showed a strong positivity (3+) whereas 1 case was that of a fibrocystic change of breast which showed positivity and one case of fibrocystic change showed a moderate staining (2+) for VEGF (Table 5). VEGF expression in malignant breast lesions was seen in higher proportion of cases in comparison to benign breast lesions. Out of the 20 cases of malignant breast lesions, expression of VEGF was noted 75% of cases in the current study (Figure 2).

Table 5: Percentage expression of intensity of staining in benign breast lesions for VEGF.

Intensity of staining of VEGF	No. of cases	%
1+	0	0
2+	1	33
3+	2	67
4+	0	0

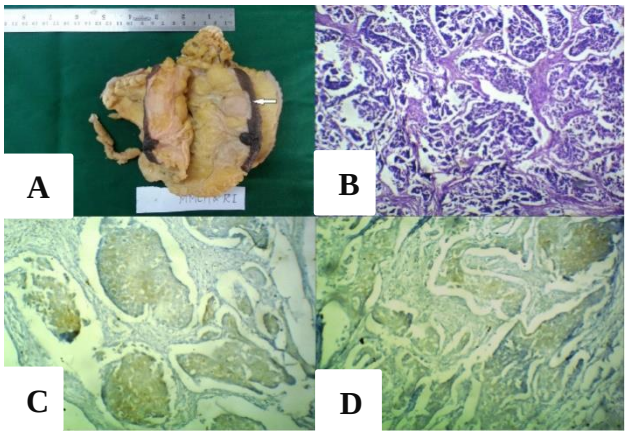

Figure 2: VEGF in IDC Breast. A): IDC breast with a large firm grey white area, B): H and E of IDC breast (10x), C) and D): Positive immunostaining of VEGF in IDC breast (10x).

Among the 15 cases, 5 cases showed a weak (1+) positivity for VEGF whereas 7 cases showed a moderate positivity (2+), 3 cases showed a strong (3+) positivity and none of the cases showed a highly strong (4+) positivity (<p=0.05) (Table 6).

Table 6: Percentage of expression of intensity of staining in malignant breast lesions for VEGF.

Intensity of staining of VEGF	No. of cases	%
1+	5	33
2+	7	47
3+	3	20
4+	0	0

VEGF immunohistochemical analysis in relation to grade of tumor revealed that none of grade I was positive, 6 (75%) out of 8 cases of grade II were positive, 9 out of 10 (90%) cases of grade III were positive for VEGF. It seems that the detection rate of VEGF was well correlated to the grade of tumor with higher grade tumor showing positivity in a higher proportion whereas the tumor with lower grade I, VEGF expression was not detected p<0.05 (Table 7).

VEGF immunohistochemical expression was reported all 4 cases of stage IV with 9 out of 12 cases in stage III showed positivity and among the 4 cases in stage II, 2 cases were positive with 9 out of 12 cases showed expression of VEGF. There was significant positive

correlation between VEGF overexpression and the stage of tumor (p value <0.05) and a higher proportion of cases were found in stage III and IV (Table 8).

Table 7: Expression of VEGF in relation to grade of the tumor.

Grade of tumor	Positive staining	Negative staining	Total
I	0	2	2
II	6	2	8
III	9	1	10

Table 8: Expression of VEGF in relation to grade of the tumor.

Stage of Tumor	Positive Staining	Negative Staining	Total
I	0	0	0
II	2	2	4
III	9	3	12
IV	4	0	4

About 13 out of 14 cases of node-positive breast cancer found to have VEGF overexpression (91%), while only two out of 4 cases of node-negative breast cancer showed VEGF overexpression (33%) with significant difference between these two groups (p value <0.05).

DISCUSSION

In immuno-histochemical analysis of VEGF protein, it was observed, that none of the 10 cases of normal breast tissue showed immunoreactivity for VEGF. This fact corresponds to studies done by Noranizah W et al, Esraa A et al, Yasushi Nakamura et al, who also reported the absence of expression of VEGF in normal breast tissue.[4-6]

Out of the 20 cases of benign breast lesions, expression of VEGF was noted in 15% of the cases. This finding is slightly lower than those of Li Liquin et al, ES Al Harris et al, who reported that 20%, 19% respectively of benign breast lesions were positive for VEGF protein but differs from the study which was carried out by Esraa A et al, who found that expression of VEGF was negative in benign breast lesions.[5,7,8] Positive over-expression of VEGF in paraffin- embedded tissue of infiltrating ductal carcinoma was found in 75% of cases. This finding is higher than those of Melanie Schmidt et al, ES Al Harris et al, who reported that 60% and 61.5% respectively of primary breast carcinoma were VEGF positive and lower than those of Anca Maria Cimpean et al, Yasushi Nakamura et al, who reported that 87.1% and 83.7% respectively of primary breast carcinoma were VEGF positive.[6,8-10]

In this study, VEGF overexpression was detected in 75% of breast cancer patients and 15% of benign breast lesions was found to be VEGF immunoreactive with a significant difference between these groups (p<0.05).

VEGF immunohistochemical analysis in relation to grade of tumor revealed that none of grade I was positive, 75% of grade II were positive, 90% of grade III were positive for VEGF. There was highly significant positive correlation between VEGF overexpression and grade of breast cancer (p<0.05). This finding agreed with Linderholm B et al, Gottfried et al, Konecny GE, Shankar R et al, and AL-Harris E et al.[8,11-13]

VEGF immunohistochemical expression was reported in 2 out of 4 cases of stage II in 9 out of 12 cases of stage III and in all 4 cases of stage IV. There was significant positive correlation between VEGF overexpression and the stage of tumor (p value <0.05) and a higher proportion of cases was found in stage III and IV. This finding was in agreement with Collagy G et al, Bolat F et al, AL-Harris E et al, and Xu W et al, who found that there was a significant correlation between tumor VEGF and stage of breast cancer.[8,14-16]

However, differs from that of Linderholm B et al, and Mac Conmara M et al, who proposed that there is no significant difference between VEGF and the stage of breast cancer.[11,17] VEGF may be more expressed in those with advanced stage which reflects the aggressive behavior of the tumor, which was explained by the degree of differentiation, that is reduced as the tumor stage advances.

The results revealed that VEGF immuno-expression was not always increased with increasing age. There was no significant difference among these age groups (p>0.05). This may be corresponding to the natural frequency of breast cancer. This finding is consistent with that of Gasparini G et al, Obermair A et al, and Li J et al, who suggested that there is no correlation between age of the patient and VEGF expression and against that reported by Greb R et al, and Fuckar D et al, who proposed that VEGF expression is higher in younger age groups.[18-22]

VEGF overexpression is higher in node positive breast cancer than in node negative breast cancer with significant difference between these two groups (p value <0.05).

This finding agreed with that reported by Yi WJ et al, Gottfried et al, Wang X et al, Xu W et al, and Hao L et al, and this may be attributed to the aggressive behavior of node positive breast cancer and from that of Li J et al, who reported that VEGF expression is not significantly associated with lymph node involvement.[12,16,20,23-25]

CONCLUSION

Genesis of breast cancer is a multi-stage process involving progressive accumulation of genetic alterations, but little is known about the implication of genetic

alterations in benign breast lesions. VEGF immunopositivity in benign breast lesions is thought to be associated with increased risk of subsequent breast cancer development.

Results of this study showed that no normal epithelial or stromal breast tissue expressed immunohistochemically detectable levels of VEGF protein. However, expression of VEGF was noted in benign breast lesions immunohistochemically. The clinical significance of VEGF expression in benign breast lesions remains to be determined. Further research will be necessary to evaluate whether these markers could serve as useful adjunct in evaluation of the malignant potential of benign breast lesions.

In this study, VEGF protein overexpression was significant in all grades and stages of breast cancer ($p<0.05$). VEGF overexpression correlated well with the grade and stage of tumor indicating that VEGF positive tumors are biologically aggressive and are associated with poor prognosis. Nonetheless resolution of the role of VEGF in the genesis of breast cancer requires larger sample size with long term follow up studies.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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