Correlation of VEGF and HIF-1α in breast carcinoma (an immunohistochemical study)

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Abstract

Introduction: By 2030 the incidence of breast cancer in India will increase to around 200,000 per year. During tumor growth, angiogenesis is induced by many stimuli. Reduced oxygen stimulates hypoxia induced factor 1 alpha (HIF-1α), activating proangiogenic cytokines i.e. vascular endothelial growth factor (VEGF). The best-studied factor is VEGF-A. Overexpression of HIF promotes metastasis of cancers. Higher the levels of VEGF, poorer the prognosis. HIF-1α is an independent prognostic factor for an unfavorable prognosis.

Materials and Methods: Study was conducted on 50 cases of breast cancer specimens. Histopathological typing and grading was done followed by immunohistochemistry for HIF-1α expression. Serum samples were collected for VEGF levels.

Observations and Results: Most patients were in the age group of 41-60 years. 94% of the tumors were infiltrating ductal carcinoma NOS the remaining being mucinous (4%) and tubular (2%). 16 cases were Grade II and 34 cases were Grade III. Lymph nodes were recovered in 44 cases of radical mastectomy. Metastatic carcinomatous deposits were present in 26 cases. Lynphovascular invasion (LVI) was seen in 20 cases. sVEGF levels were increased in 21 cases. No correlation was seen with grade of tumor whereas a positive correlation was seen with LVI and lymph node status. No positive correlation was seen with HIF-1α and all these parameters. No significant correlation was seen between sVEGF levels and HIF-1α.

Conclusion: sVEGF levels have direct relationship with LVI and lymph node status and should be taken as a poor prognostic factor. HIF-1α was increased more in high grade tumors but had no statistical significance with LVI and lymph node status.

Keywords: Breast cancer, VEGF, HIF-1α, Immunohistochemistry.

Introduction

Breast cancer is a complex disease resulting from a multistage process by deregulation of a number of signaling cascades. It is the second commonest cancer among women in India and accounts for 7% of global burden of breast cancer and one-fifth of all cancers among women in India.[1] A recent study estimated that by the year 2030 the incidence of new cases of breast cancer in India will increase to around 200,000 per year from the present 115,000.[2] In India, the average age of developing a breast cancer has undergone a significant shift over last few decades with almost 48% of patients being younger than 50 years of age.[3]

Even though the tumor cells can multiply indefinitely, solid tumors cannot enlarge beyond 1 to 2 mm in diameter unless they are vascularized. Neovascularization has a dual effect on tumor growth perfusion supplies needed nutrients and oxygen and newly formed endothelial cells stimulate the growth of tumor cells by secreting growth factors such as insulin-like growth factors (IGFs), platelet derived growth factor (PDGF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Angiogenesis is required not only for tumor growth but also for access to the vasculature and hence for metastasis.[2]

Angiogenesis is induced by a variety of stimuli, including pro-angiogenic growth factor, transcription factor, cell adhesion molecules and extracellular matrix proteins.[3] Relative lack of oxygen stimulates hypoxia induced factor 1 alpha (HIF-1α), an oxygen sensitive transcription factor, which then activates transcription of a variety of proangiogenic cytokines such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).[2]

The predominant proangiogenic factor is VEGF-A, that is produced in several isoforms due to alternative splicing.[4] There are two receptor tyrosine kinases (RTKs) VEGFR1 and VEGFR2 that can bind VEGFA.[5]

Hypoxia-inducible factor (HIF) transcription factors play key roles in oxygen homeostasis. HIF is a heterodimeric transcription factor, consisting of one of three subunits (HIF-1α, HIF-2α or HIF-3α) and one HIFβ subunit. Overexpression of HIF also promotes metastasis of many cancers by providing oxygen and nutrients for fast dividing tumor cells.[6]

It is now widely accepted that higher the expression levels of VEGF, poorer the breast cancer prognosis. In addition, HIF-1α is an independent prognostic factor for an unfavorable prognosis in breast cancer patients with lymph node metastases.[7]

Aims and objectives of the study were the following:
1. To find serum levels of VEGF in breast carcinoma.
2. To find expression of HIF-1α in breast carcinoma.
3. To find correlation of serum VEGF and HIF-1α in breast carcinoma.
4. To correlate the expression of these tumor markers with histological type, grade and other parameters.

Materials and Methods

1. The study was conducted on 50 cases of breast cancer received as lumpectomy or mastectomy specimens.
2. Histopathological typing and grading was done.
3. They were then subjected to immunohistochemistry for HIF-1α expression
4. Serum samples were collected and subjected to ELISA for VEGF levels

Interpretation

For HIF-1α

0= No nuclear as well as cytoplasmic staining

+1= Nuclear and/or cytoplasmic staining <1% of cells

+2= Nuclear and/or cytoplasmic staining 1-10% of cells

+3= Nuclear and/or cytoplasmic staining 11- 50% of cells

+4= Nuclear and/or cytoplasmic staining in >50% of cells

For VEGF

1. A colour change from blue to yellow is noted in the microplate on addition of the stop solution.
2. The intensity of the colour change depends on the amount of VEGF bound and is measured at 450nm.
3. Normal Range- 45-280 pg/ml

Observations and Results

The age of the patients ranged from 27 to 70 years. All patients were females. The maximum numbers of patients were in the age group of 41-60 years comprising 54% of the total. As regards the histopathological typing, 94% of the tumors were infiltrating ductal carcinoma NOS (not otherwise specified), the remaining being of mucinous (4%) and tubular (2%) type. Nottingham modification of RBB scoring was done. 16 cases were reported as Grade II and 34 cases as Grade III. (Fig IA)

Lymph nodes were recovered in 44 cases of radical mastectomy and not recovered in 1 case of simple mastectomy with rest 5 cases being lumpectomy specimens. Metastatic carcinomatous deposits were present in 26 cases, which included 11 cases of N1 Stage (1-3 positive nodes), 10 cases of N2 stage (4-9 positive nodes) and 5 cases of N3 stage (>10 positive nodes). Remaining 18 cases showed reactive pathology. Lymphovascular invasion (LVI) was seen in 20 cases (40%) whereas 30 cases (60%) were negative for LVI. When the LVI was correlated with the lymph node status, 13/19 LVI positive cases showed evidence of secondary carcinomatous deposits (1 case being that of lumpectomy) and 13/25 LVI negative cases showed evidence of secondary carcinomatous deposits (4 cases being that of lumpectomy and 1 of simple mastectomy) giving a non-significant relation (p=0.136).

Serum (s) VEGF levels were increased in 21 cases (42%). 29 cases (58%) had normal levels. On correlating tumor grade with sVEGF, 7/16 cases in Grade II and 14/34 cases in Grade III category showed increased levels of sVEGF. No significance was seen amongst the two (p= 0.863). (Table 1)

Table 1: Correlation of Grade of tumor with sVEGF

Grade	sVEGF		Total
	Increased	**Normal**	
I	00	00	00
II	07	09	16
III	14	20	34
Total	21	29	50

Regarding sVEGF level correlation with LVI, 14/20 cases that were positive for LVI, showed increased levels of sVEGF whereas only 7/30 cases which were negative for LVI showed increased levels of sVEGF which was of significance with a p value of 0.001. (Table 2)

**Table 2: sVEGF Level Correlation with LVI**

Lymphovascular Invasion	sVEGF		Total
	Increased	**Normal**	
Positive	14	06	20
Negative	07	23	30
Total	21	29	50

A significant correlation was seen between sVEGF and the lymph node status (p=0.001). Secondary carcinomatous deposits were seen in 17/21 cases with levels of sVEGF. Out of the 29 cases in which sVEGF levels were normal, 9 cases showed secondary carcinomatous deposits. (Table 3)

Table 3: Correlation of sVEGF With Lymph Node Metastasis

Lymph Node Status	sVEGF		Total
	Increased	**Normal**	
Reactive (N0)	03	15	18
N1	03	08	11
N2	09	01	10
N3	05	00	05
Not received (NR)	01	05	06
Total	21	29	50

The HIF-1α positivity was observed in 41 cases comprising 82% of the total cases. Percentage positive cells varied from 1-82% with weak, distinct and strong staining intensity. (Fig IB,IC,ID) Out of the 41 cases which were positive for HIF-1α, 6 cases were 1+, 12 cases were 2+, 19 cases were 3+ and 4 cases were of 4+ category.
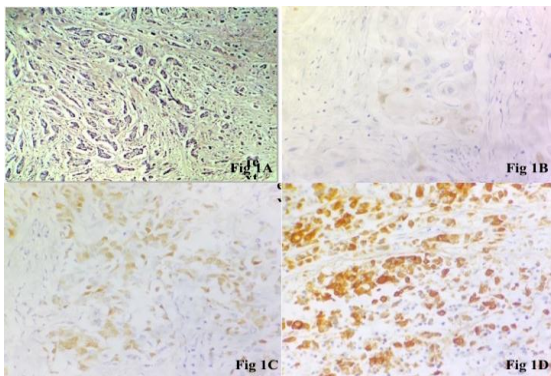
Fig. 1A: Infiltrating Ductal Carcinoma- Grade II (H&E, 100X)
Fig. 1B: HIF -1α Positivity (Mild Intensity) (H&E, 400X)
Fig. 1C: HIF -1α Positivity (Moderate Intensity) (H&E, 400X)
Fig. 1D: HIF -1α Positivity (Nuclear & Cytoplasmic Strong Intensity) (H&E, 400X)

Correlating HIF-1α with grading of tumor, 12/19 cases with a score of 3+ and 9/12 cases with a score of 2+, had grade III tumor. All the 4 cases with a score of 4+d were of grade III category giving a non-significant correlation (p=0.516). (Table 4)

Table 4: Correlation of HIF-1α with Grading of Tumor

HIF-1α Score	Grade of Tumor			Total
	I	**II**	**III**	
0	00	03	06	09
1+	00	03	03	06
2+	00	03	09	12
3+	00	07	12	19
4+	00	00	04	04
Total	00	16	34	50

A non-significant relation was noted between the HIF-1α score and the LVI (p=0.764) with a total of 16 cases being positive both for the LVI and the HIF-1α of which 9 cases were of 3+, 1 case of 4+, 4 cases of 2+ and 2 cases were of 1+ category. (Table 5)

Table 5: Correlation of HIF-1α with LVI

HIF-1α Score	LV Invasion		Total
	Positive	**Negative**	
0	04	05	09
1+	02	04	06
2+	04	08	12
3+	09	10	19
4+	01	03	04
Total	20	30	50

On correlating HIF-1α positivity with lymph node status, 21 cases were positive for HIF-1α as well as the secondary carcinomatous deposits of breast carcinoma.

18 cases which showed reactive pathology in lymph nodes had increased HIF-1α expression in 15 cases giving a non-significant correlation (p=0.852). (Table 6)

Table 6: HIF-1α Correlation with Lymph Node Status

HIF-1α Scoring	LYMPH NODE STATUS					
	N0	**N1**	**N2**	**N3**	**NR**	**Total**
0	03	03	02	00	01	09
1+	01	00	01	01	03	06
2+	05	03	02	02	00	12
3+	07	04	04	02	02	19
4+	02	01	01	00	00	04
Total	18	11	10	05	06	50

A non-significant relation was seen between sVEGF and HIF-1α score (p=0.184) with 19/21 cases showing increased sVEGF levels showed positive HIF-1α expression. Out of the 19 cases, 3 were of 1+, 7 of 2+, 8 of 3+, 1 case was of 4+ category. (Table 7)

Table 7: Correlation of sVEGF with HIF-1α Score

HIF-1α Score	sVEGF		Total
	Increased	**Normal**	
0	02	07	09
1+	03	03	06
2+	07	05	12
3+	08	11	19
4+	01	03	04
Total	21	29	50

Thus, it was observed that sVEGF had a significant correlation with the LVI and lymph node status but no definite correlation was seen with HIF-1α, which also did not show any significant correlation with LVI or lymph node status.

Discussion

The incidence of breast cancer in India is on the rise and is rapidly becoming the number one cancer in females.[1]

It is a disease with tremendous heterogeneity in clinical behaviour. Clinico-pathological variables such as tumour size, histological grade & type, lymph node metastases, vascular invasion, tumour cell proliferation, tumour necrosis, ductal carcinoma in situ, age and pregnancy may help in predicting the prognosis and the need for adjuvant therapy. However, newer prognostic factors and predictors of response to therapy are needed to look into the metastatic potential of this disease.[1,2]

In this study, age of the patients varied from 27-70 years. Maximum numbers of the patients were between 41-60 years (54%) and mean age was 51.14 years. Similar results were seen in many other studies.[8,9]

Gruber G et al in their study on 81 cases of breast carcinoma reported a mean age of 56 years.[10]

Histological type is a major prognostic factor in breast carcinoma. In this study, 94% of the tumors were infiltrating ductal carcinoma NOS (not otherwise specified) with 4% being IDC mucinous and remaining 2% were of IDC tubular type. Ambroise M et al reported 96.3% of the cases as IDC NOS.[11] Dales JP also reported 68% of the cases to be of IDC NOS.[12]

Nottingham modification of Bloom Richardson system is the most commonly followed system.[13]

Grade II was observed in 32% and grade III in 68% of the cases. Shet et al found that 70% cases belonged to grade III while 28% cases were seen in grade II and only 2% cases were seen in grade I.[14] However, Gruber G et al reported 56% of the tumors in grade II with the remaining being in the grade III category.[10]

sVEGF levels were raised in 42% (21/50) cases. Chanana P et al observed an increased level of sVEGF in 27/70 (38.57%) breast carcinoma cases.[15] Our study is in concordance with the study of Kapahi R et al and Pande et al who observed significantly increased levels of sVEGF in breast carcinoma as compared to the control groups.[16,17]

HIF-1α was positive in 41/50 (82%) of the cases. 6/50 cases were of score 1+, 12/50 cases were of score 2+, 19/50 cases were of score 3+, 4/50 cases were of score 4+. Ni X et al also observed overexpression of HIF-1α in 69.3% of the cases.[18] Bos R et al found detectable levels of HIF-1α in 75% of the cases.[19]

Out of 21 cases with levels of sVEGF, 19 cases had increased expression of HIF-1α. 22/29 cases with normal levels of sVEGF showed increased expression of HIF-1α. Although the study conducted by Ni X et al had shown a significant correlation between sVEGF and HIF-1α, in this study no significant correlation could be seen in between the two.[18] This might be attributable to the low sample size and the short period of study. The effect of geographic factors cannot be excluded. Bos R et al reported a similar study in which no correlation was found between HIF-1α and VEGF expression on multivariate analysis.[19]

In 21 cases with increased sVEGF levels, 7 cases were Grade II and 14 cases were Grade III breast carcinoma. 20/ 9 cases with normal sVEGF levels were of Grade III category.

When the grade of the tumor was correlated with the HIF-1α score, out of the 34 cases of Grade III category; 3,9,12 and 4 cases were of score 1+,2+,3+ and 4+ category, respectively with the rest of the cases being negative for HIF-1α. Both the sVEGF and HIF-1α had a non-significant correlation with the tumor grade which was in concordance with the other studies.[3,10,18] Bos R et al, on the other hand, concluded in their study that the HIF-1α levels increased as the tumor progresses from well to poor differentiation.[19]

Out of 21 cases with increased sVEGF levels, 14 cases were positive for LVI which was statistically significant (p=0.001). This is in concordance with the study done by Ni X et al and Ali EM et al.[18,20] However, LVI when correlated with the HIF-1α score, no significant correlation was seen between the two although Ni X et al reported a positive correlation between the lymphovascular density and HIF-1α expression.[18]

In the 21 cases, which had increased levels of sVEGF, 17 cases were positive for lymph node metastasis. 9 out of 29 cases with normal sVEGF levels had secondary carcinomatous deposits in the lymph nodes. This was a significant finding with p value being 0.001.

Out of the 26 cases positive for secondary carcinomatous deposits in the lymph nodes, 21 cases had increased expression of HIF-1α and out of the 18 cases which were negative for secondary carcinomatous deposits in the lymph node, 15 cases had increased expression of HIF-1α giving a non-significant correlation between the two.

The significant correlation between sVEGF and lymph node status was similar to the studies conducted by Ni X et al and Ali EM et al.[18,20] However, Viciosa L et al reported non-significant findings between the sVEGF levels and the lymph node status.[21] The results between HIF-1α and lymph node status in studies conducted by Gruber G et al and Bos R et al were in concordance with the present study.[10,19] But Ni X et al had reported a significant correlation between the two.[18]

Conclusion

To conclude, grade III carcinomas are reported more, possibly due to less awareness. sVEGF levels when increased have got direct relationship with LVI and lymph node status and thus should be taken as a poor prognostic factor. Although HIF-1α was increased more in high grade tumors but had no statistical significance with LVI and lymph node status. As its expression is increased in high grade tumors it can be taken as a poor prognostic factor.

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