Original Article

Microvascular Density of Invasive Breast Carcinoma as an Independent Prognostic Factor in Comparison with Already Established Ones : A Study from Northeast India

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Background and Objectives : Breast Carcinoma is the most common cancer in women Worldwide. This study was done to assess the tumour angiogenesis using CD34 immuno-reactivity and thereby see the relationship of the same with already established prognostic factors like age, histological type, tumour size, tumour grade (Modified Bloom Richardson Scoring System) and lymph nodal metastasis.

Materials and Methods : The 19 Breast Carcinoma cases included in this study were diagnosed in the Department of Pathology, Gauhati Medical College and Hospital within 1 year and Tumour Angiogenesis was assessed using CD34 immunoreactivity. The results were then compared with the already established prognostic factors.

Results : The relationship of MVD/HPF with tumour size, MBR tumour grade and lymph nodal metastases was found to be statistically significant at p-value <0.05. MVD/HPF with patient age was not statistically significant. Grade I &II IDC and medullary carcinoma had low MVD/HPF, whereas grade III IDC, metaplastic carcinoma and invasive papillary carcinoma had high MVD/HPF.

Conclusion : Tumour angiogenesis assessed by MVD/HPF following CD34 immunostaining can be used as a prognostic factor in Breast Carcinoma alongwith other known factors with more studies and standardized methods to calculate the MVD/HPF and can also be a potential targeted therapy with anti-angiogenic factors.

Breast Carcinoma is the most common cancer in women worldwide, second only to lung cancer as a cause of cancer related deaths[1] with increasing prevalence in India[2]. It is seen in all age groups with peak incidence in 45-60 years age group[3]. For every two women affected by breast cancer in India, one is dying[4]. Breast cancers staged on the basis of tumour size, lymph nodal metastases and distant metastases seem to show different etiology and clinical behaviour even among those presenting in the same stage[5]. A number of prognostic factors, namely histologic type, tumour size, lymph node metastases, distant metastases, locally advanced disease, molecular subtype, ER/PR receptors, HER2 expression and proliferation rate (Ki-76 index) have been extensively studied and established. Recent studies suggest tumour angiogenesis (assessed by microvessel density) as an independent predictive and prognostic factor in breast carcinoma[6].

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CD34 is an endothelial marker, sensitive to tumour angiogenesis representing the clear state of neovascularization during tumour growth[7]. Previous studies have suggested the importance of CD34 in cancer prognosis[8]. Some studies have, however, denied this association[9]. So, this study was done to assess tumour angiogenesis using CD34 immuno-staining to see the relationship of tumour angiogenesis with other prognostic parameters.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted in the surgical specimens obtained in the Department of Pathology, Gauhati Medical College and Hospital, Guwahati in a one-year period.

Inclusion Criteria : All invasive Breast Carcinoma, irrespective of age and sex.

Exclusion Criteria : All in-situ Breast Carcinoma.

The specimens were subjected to macroscopic examination, followed by histopathological

examination and immuno-histochemistry with CD34. Microvessel Density (MVD) was calculated in the tumour sections by the technique developed by Weidner, *et.al* and mean MVD per high power field (MVD/HPF) was calculated which was found to be 11.7. Any value lower than the mean was regarded as "low MVD" and any value above the mean was regarded as "high MVD". The p-value was calculated using Chi-square method and p-value <0.05 was considered as statistically significant.

RESULTS

Most common age group of the patients studied was 50-59 years (42.1%), followed by 40-49 years age group (21%). IDC was the most common histological type (78.9%). Most tumours were of MBR grade III (57.9%) with tumour size >5cm (T3) (57.9%). Lymph nodal metastases was seen in majority of the cases (73.6 %).

All the 19 cases were divided into high and low based on microvessel density/ HPF with respect to tumour angiogenesis based on CD34 immuno-reactivity. The relationship between tumour angiogenesis with patient age, histological type, MBR score, tumour size and lymph nodal metastases was analyzed.

Most of the cases were IDC (15 out of 19). IDC and Medullary carcinoma mostly showed low MVD/ HPF, whereas the high grade ones like metaplastic carcinoma, sarcoma and invasive papillary carcinoma had high MVD/HPF (Tables 1-5)(Figs 1-4).

DISCUSSION

In this study, statistically significant relation (p<0.05) was noted between MVD/HPF and MBR tumour grade, tumour size and lymph nodal metastases. Similar findings were noted in studies done by Pyakurel, *et al*[10], Kwatra, *et al*[11], Bhawna Agnani, *et al*[12] and Horak, *et al*[13].

No statistical significance was noted between patient age and MVD/HPF in this study, which could be compared to studies done by Ebru, *et al*[14] and Wang G, *et al*[15].

So, it has been observed that tumour angiogenesis assessed by MVD/HPF following CD34 immuno-staining has a strong relationship with tumour size, MBR tumour grade and lymph nodal metastases. Thus,

Table 1 — *Microvessel Density (MVD) / HPF versus Patient Age*

MVD / HPF	Age Groups						
	20-29	30-39	40-49	50-59	60-69	70-79	80-89
Low (<11.7)	0	2	3	6	0	0	0
High (>11.7)	1	1	1	2	1	1	1
Total	1	3	4	8	1	1	1
The p-value >0.05							

Table 2 — *MVD / HPF versus Histological type*

MVD / HPF	Histological Type				
	IDC	Medullary	Metaplastic	Sarcoma	Invasive papillary
Low (<11.7)	10	1	0	0	0
High (>11.7)	5	0	1	1	1
Total	15	1	1	1	1

Table 3 — *MVD / HPF versus MBR Tumour Grade*

MVD / HPF	MBR Grade		
	Grade I	Grade II	Grade III
Low (<11.7)	2	6	3
High (>11.7)	0	0	8
Total	2	6	11
The p-value <0.05.			

Table 4 — *MVD / HPF versus Tumour Size*

MVD / HPF	Tumour size		
	T1 (<2 cm)	T2 (2-5 cm)	T3 (> 5 cm)
Low (<11.7)	1	4	1
High (>11.7)	0	3	10
Total	1	7	11
p=0.02.			

Table 5 — *MVD / HPF versus Lymph Nodal Metastases*

MVD/HPF	Lymph nodal metastases	
	Present	Absent
Low (<11.7)	3	4
High (>11.7)	11	1
Total	14	5
p=0.01.		
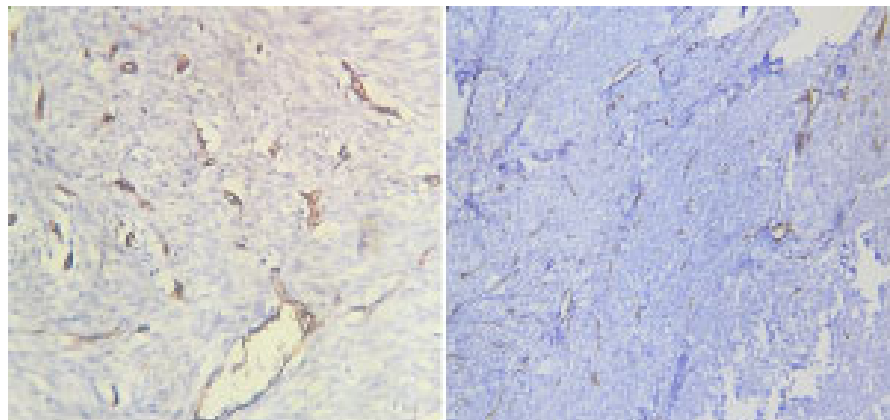
Figs 1 & 2 — Breast Carcinoma with High Microvessel Density after CD34 immuno-staining, IHC Staining CD34, 10X10
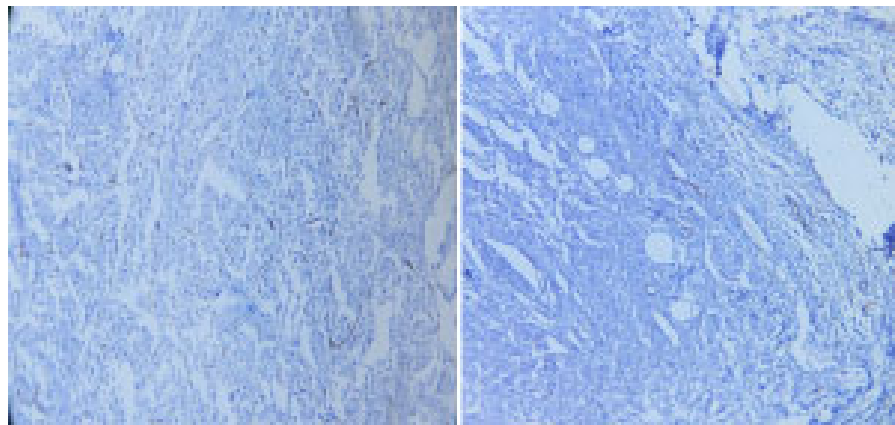
tumour angiogenesis can be used as a specific prognostic marker in breast carcinoma.

CONCLUSION

Breast Carcinoma continues to carry its havoc inspite of the tragic fact that it occurs in an exposed organ accessible to self-examination and continuous high clinical surveillance. With larger number of samples, newer prognostic parameters like tumour angiogenesis and more detailed studies on targeted therapies with anti-angiogenic factors, we will hopefully be able to treat and manage cases of Breast Carcinoma in better ways in the upcoming future.

Figs 3 & 4 — Breast Carcinoma with Low Microvessel Density after CD34 Immuno-staining, IHC Staining CD34, 10X10

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