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Assessment of Microvessel Density (Angiogenesis) and its Correlation with Hormonal Status of Carcinoma Breast

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Abstract: ***Background: Carcinoma Breast is the commonnest malignancy in women. Evaluation of microvessel density (MVD) provides additional information regarding the biological profile of the tumor. Aim: To detect the intratumoral MVD microscopically and tumor angiogenesis using vascular endothelial growth factor (VEGF) and ER, PR, HER2/neu status and to correlate MVD and VEGF with ER, PR, HER2/neu status. Materials and Methods: 50 cases of INVASIVE DUCTAL CARCINOMA-NOS type of breast cancers reported during the year 2014 were selected and Hematoxylin & Eosin slides were reviewed. IHC was done for VEGF, ER, PR, HER2/neu and the results were documented. Results: The Majority (50%) of cases showed ER/PR negativity while HER2/neu was positive in 34.8% of cases. Mean MVD was 33.19. However there was no significant correlation between VEGF expression and hormone receptor status in this study. Conclusion: The assessment of MVD, VEGF and hormone receptor status have applications in the evaluation of prognosis and as a therapeutic target. However study involving large sample size and computerized image analysis will help in identifying the exact association of MVD and VEGF with other prognostic factors.***

Keywords: IDC-NOS, MVD, VEGF, ER, PR, HER2/neu

1. Introduction

Carcinoma Breast is the commonest malignancy in women. It is the 2nd commonest cause in rural population [1]. It can occur at any age , Peak incidence is in 45-60 years. Breast carcinoma is a heterogenous neoplasm with diverse growth rates, different cell clones and metastatic potential. This heterogenous nature explains the different clinical behavior among patients with same pathologic or clinical stage. Research on "tumour angiogenesis in breast cancer" is one of the main field of investigation in clinical application in recent time. Newer therapeutic inhibitors of angiogenesis have been discovered and are under clinical trials. So this therapeutic inhibition of angiogenesis may be a realistic novel approach to cure breast cancer Hormone receptor and HER2/neu status are important prognostic factors and its evaluation helps in assessing the outcome of the disease and to select the appropriate treatment.

2. Aims and Objectives

1) To detect the intra tumoral MVD by counting the microvessels in the hot spot areas microscopically and to detect Angiogenesis by using VEGF
2) To detect the ER, PR & HER2/neu status and to correlate MVD and VEGF with ER, PR, HER2/neu status of the same cases.

3. Materials & Methods

The present study was a descriptive prospective study conducted in the Institute of Pathology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai, during the period between Jan toDec 2014. 50 out of 273 cases were selected for our study.

Method of data collection:
Clinical details were obtained for 50 IDC-NOS cases reported during the period of study from the Surgical Pathology records.

Hematoxylin and Eosin stained 4 µ thick sections of the paraffin tissue blocks of the specimens were reviewed.

Micro Vessel Density (MVD):
MVD scoring was performed for all 50 cases manually by light microscopy, at high power to pick up the hot-spot [2,3] (areas with intense vascularisation) .IHC analysis of markers for ER, PR, HER2/neu and VEGF were done using super sensitive HRP polymer system based on non biotin polymeric technology and slides were analysed for the presence of reaction, cellular localization, percentage of cells stained and intensity of reaction. cytoplasmic staining was assessed for VEGF. A semi quantitative method was used for VEGF with scores of 0-3.

Score Intrepretation
0 - No reaction
1 - Poor reaction
2 - Moderate reaction
3 - Intense reaction

ER, PR analysis:
46 out of 50 cases of IDC NOS type were selected for ER, PR status analysis and the immune reactivity was tabulated as positive (or) negative by using Quick Scoring System based on the summation of score for proportion of staining (score 0-5) and score for staining intensity (score 0-3).HER2/neu staining was taken as positive when intense nuclear staining of tumor cells and categorized into 2 groups as 2+(or) 3+.

4. Observation & Results

During the study period, a total of 649 breast specimens were received in the Institute of Pathology, Madras Medical College for histological examination. Breast carcinoma had a peak incidence in the age group of 41-50years and youngest age of presentation was at 25 years. The MVD ranged from 19.73 to 48.34 microvessels mm^3, median and mean MVD was 32.89, and 33.19 for all patients. Thus the cut off value was 32.89 microvessels mm^3 at 400X

Table 1: Steroid hormone receptors profile

Hormone Receptor Status	*No. of cases*	*Percentage*
ER+ PR+	8	17.4%
ER+ PR-	9	19.6%
ER- PR+	6	13%
ER- PR-	23	50%
Total No. of cases	46	100%

Hormone receptor status were evaluated by applying IHC (ER, PR) for 46 out of 50 cases. 23 cases (50%) are ER-PR- tumors, 8 cases (17.4%) are ER+ PR+ tumors and 6 cases (13 %)ER- PR+ tumors respectively (Table 1)

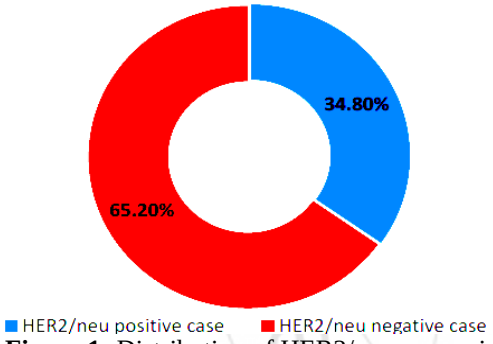


Figure 1: Distribution of HER2/neu expression

Among 46 cases, 16 cases (34.8%) were positive for HER2/neu, of which 13 cases were strongly positive and 30 cases were negative. **Distribution of Triple positive and**

Triple Negative cases. 3 cases out of 46 (8.7%) were triple positive and 15 cases (30.4%) were triple negative.

Correlation of Steroid Hormone Receptor status and MVD

In this study the median MVD for ER+ PR+,ER+PR-, status was 32.89,and 30.70, for ER- PR+,ER-PR- status was 33.98 and 32.89 respectively. High MVD was seen with ER- PR +status and low MVD was seen with ER+ PR- status. [Cut off value was defined to be less than the median value of MVD. (i.e., 32.89)] 16 out of 46 cases were positive and 30 cases were negative for HER2/neu expression with mean MVD of32.06 and 34.06 respectively. This indicated that MVD increased with HER2/neu negativity. However the P value has no statistical significance.

Table 2: Correlation of VEGF expression and ER/PR Status

ER/PR Status	*VEGF 2+*	*VEGF 3+*	*Pearson Chi Square test*	*P-value*
ER+ PR+	5	7	2.019	0.568
ER+ PR-	12	11		
ER- PR+	8	3		
ER- PR-	12	13		

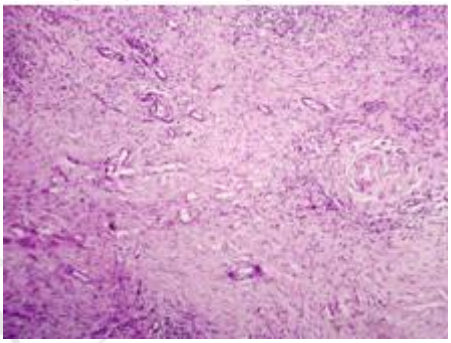
40 out of 50cases were analysed for angiogenesis by VEGF. Among the 40 cases, 23 cases showed strong positivity for VEGF and 17 cases showed moderate reaction. (Table 2)

When the VEGF expression was compared with ER/PR status, the P-value was statistically not significant and it was inferred that VEGF expression was independent of the ER/PR status. (Table 2)

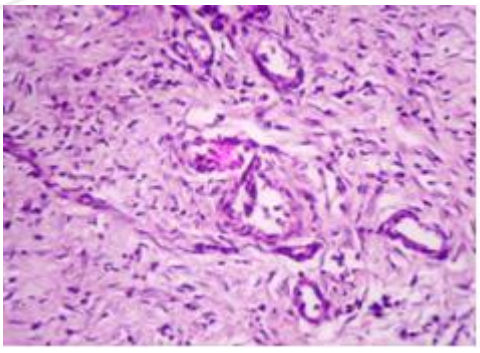
Table 3: Correlation of VEGF expression and HER2/neu Status

HER2 / neu Status	*VEGF 2+*	*VEGF 3+*	*Pearson Chi Square test*	*P-value*
Positive	7	5	*0.556*	*0.456*
Negative	8	10		

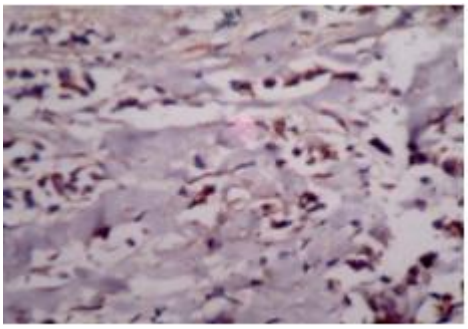
Among 12 HER2/neu positive cases, 5 showed strong VEGF reaction and 7 showed moderate reaction. Among HER2/neu negative cases, 10 showed strong VEGF expression. This indicated that strong VEGF expression was seen in HER2/neu negative cases. However the p-value was not statistically significant (Table 3).



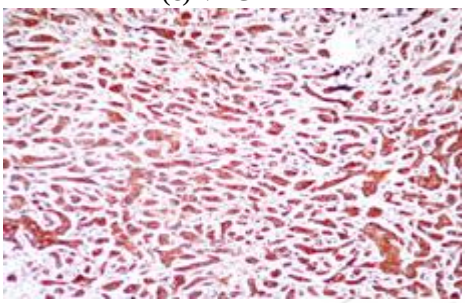
(a) Hot spot area



(b) Hot spot area

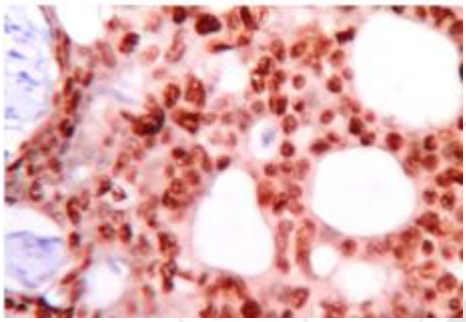


(c) VEGF 2+

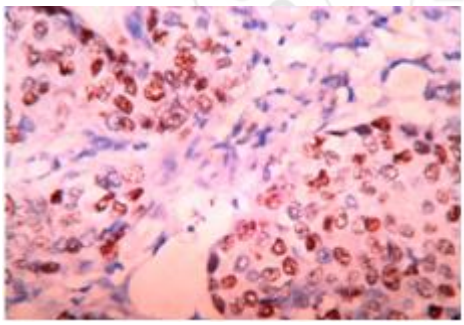


(d) VEGF 3+

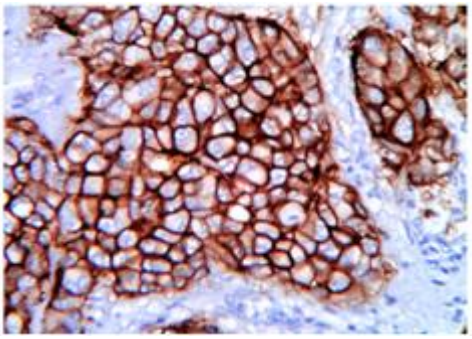
Figure 2: Microvessel Density 400x



(a) Invasive Ductal Carcinoma Positive for ER



(b) Invasive Ductal Carcinoma Positive for PR



(c) Invasive Ductal Carcinoma Positive for HER2/Neu

Figure 3: Steroid hormone and HER2/neu status of CA breast

5. Discussion

Carcinoma Breast is a heterogenous disease both clinically and pathologically. MVD and VEGF are considered as important prognostic markers which were correlated with other prognostic markers in various studies. Therefore the evaluation of MVD might provide additional information regarding the biological profile of the tumor and may have applications in evaluation of prognosis and as a therapeutic target in primary breast carcinoma. In the present study, angiogenesis was assessed by counting MVD in the hot spot areas microscopically and IHC was done for VEGF,ER,PR and HER2/neu and an attempt was made to correlate MVD and VEGF expression with ER,PR and HER2/neu status.

Microvessel density in primary breast cancer

Median MVD was 32.89 and mean MVD was 33.19 for all patients. In total there were 46% in low and 54% in high MVD group.

Table 4: Comparison of microvessel density by various studies

Authors	*MVD*
Vamesu et al (microscopic count)	35.29
Lysa Ryden et al (CD31)	33 in excised tumors 32 in CNB
Current study (microscopic count)	32.89

Vamesu et al did micro vessel counts and density scoring manually as a single microvessel count by light microsopy in areas of invasive tumor.[21]

Lysa Ryden et al did MVD scoring by using CD31 antibody and they compared the MVD in core needle biopsy and subsequent excised specimens and found that there was no difference in distribution of MVD between the 2 types of specimens.[20]

The basic problem in assessing the MVD in all these various methods was selection of the areas with high vascularisation (hot spot areas), because of the heterogenous nature of vascularisation in breast carcinoma. In our study, majority of the cases (50%) were ER- PR- tumors,19.6% & 13% were ER+ PR- and ER- PR+ tumors respectively. 34.8% of cases were HER2/neu positive, 65.2% of cases were HER2/neu negative.

Slamon et al reported that amplification of HER2/neu is seen in approximately 20-30% of breast cancers(.22) 8.7% of cases were triple positive (ER+ PR+ HER2/neu+) and 30.4% were triple negative (ER- PR- HER2/neu -) in this study.Vamesu et al analysed MVD in tumors with various hormone receptor status and showed high MVD in ER-PR+ and low MVD in ER+ PR- tumors and they also demonstrated statistically significant correlation between MVD and various groups defined by ER/PR status.[20]

The results of our study showed high MVD in ER negative and low MVD in ER positive tumors. However in concurrence with JB Parenters et al study, there was no statistical association between MVD and ER status in our

study.16 out of 46 cases were positive and 30 cases were negative for HER2/neu expression with mean MVD of32.06 and 34.06 respectively. This indicated that MVD increased with HER2/neu negativity.How ever the P value has no statistical significance.

The reason for low accuracy in our study may be due to various types of specimens (needle biopsy, trucut biopsy, wedge biopsy, incisional biopsy, excisional biopsy, lumpectomy and MRM specimens) selected for our study and other reason is heterogenous nature of vascularity in breast carcinomas which was found to be the recognized methodological problem. Weidner et al demonstrated MVD by using F VIII & showed that high MVD was seen in, 154HER2/neu expression & ER negativity. But Axelsson et al reported no significant association was seen between MVD, HER2/neu and, ER status. This indicates that angiogensis is an independent prognostic factors. The various methodologies used in assessing the MVD (CD31, FVIII, and CD34) is the main reason for failure of published studies to demonstrate association between other prognostic factors and angiogenesis. Another major factor limiting the strength of association was high inter-observer variability in MVD counting and scoring.

Overall the clinical significance of high MVD remains uncertain and variability in methodologies, difficulties in differentiating lymphatic and blood vessels appears to contribute to this uncertain nature of the angiogenesis. MVD measurements are not universally reproducible. To improve the accuracy of our study, use of multi parametric computerized image analysis system is necessary.

To avoid the problem of the heterogenous nature of vascularisation , MVD should be quantified over the entire histologic section rather than over the hot spot areas.

VEGF expression in primary breast carcinoma

This study shows that MVD is increasing with strong VEGF expression. Toi et al evaluated MVD and found strong correlation between MVD and VEGF expression, which correlates with our study.[18]

When the VEGF expression was compared with ER/PR status, more number of cases with ER- PR- status showed strong VEGF expression followed by ER+ PR- status, ER+ PR+ and ER- PR+. But there was no statistical significant association between VEGF expression and ER/PR status. Hence VEGF expression is independent of ER/PR status. Results of our study suggest that VEGF is one of the main angiogenic factor and is a valuable prognostic indicator in patient with ER- negative tumors. Among 12 HER2/neu positive cases, 5 showed strong and 7 showed moderate VEGF expression. Among 18 cases of HER2/neu negative cases, 10 cases showed strong VEGF expression. This indicated that strong VEGF expression was seen in HER2/neu negative cases. However the p-value was not statistically significant.

6. Summary

An increased MVD was noted in ER- PR+, HER2/neu - and triple negative cases. But their association was not statistically significant. Strong VEGF expression was seen in ER- PR- and HER2/neu – cases. The correlation between MVD and VEGF with ER, PR, HER2/neu status are not statistically significant.

These findings indicated that the angiogenic factors (i.e.) MVD and VEGF were independent factors.

The reason for this statistical non correlation may be due to;

1. Heterogeneity of the vascularity within breast tumors.
2. Inter-observer variations in manual counting methods of MVD.
3. Less sample size.
4. Lack of standardization of VEGF grading system.
5. Various types of samples included in our study.

The overall accuracy of methods of MVD estimation and VEGF expression could be further validated by identification of better endothelial markers, the use of multi-parametric computerized image analysis system for counting MVD and standardizing the grading system for VEGF evaluation like that of ER/PR status analysis, before it can be used in clinical practice. Further a study with uniform type of samples, could be more helpful for planning treatment modalities.

Thus, a large sample size and the above mentioned computerized image analysis will help in identifying the exact association of MVD and VEGF with other prognostic factors of breast carcinoma to formulate treatment strategies and possible targeted therapy.

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