IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 3 Ver. XI (March. 2017), PP 15-19
www.iosrjournals.org

Assessment of Microvessel Density (Angiogenesis) And Its Correlation with Tumor Grade

Dr. M.Gunasundari[1], Dr. P.Vijaya Bhaskar[2]
[1](Institute Of Pathology, Madras Medical College, Chennai, Tamilnadu, India.)
[2](Institute Of Pathology, Madras Medical College, Chennai, Tamilnadu, India.)

Abstract :
Background: *Breast Carcinoma is the most common cause of cancer related mortality among females. Various prognostic factors have been studied among which evaluation of microvessel density provides additional information regarding the biological profile of the tumor.*
Aim : *To detect the intratumoral Microvessel Density of breast carcinoma microscopically and to detect the tumor angiogenesis using the endothelial marker, Vascular Endothelial Growth Factor,. Also to correlate VEGF and MVD with tumour grade.*
Materials and Methods: *50 cases of Invasive Ductal Carcinoma-Nos type of breast cancers reported during the year 2012 were selected and appropriate clinical details were obtained . Corresponding Hematoxylin & Eosin slides were reviewed. Immunohistochemistry was done for VEGF and the results were documented.* ***Results:*** *The percentage of breast carcinoma during the one year period was 41.91% with a peak incidence in 41-50 years of age group. Mean MVD was 33.19. Significant association between MVD & histological groups defined by Blooms Richardson grading was seen while strong VEGF expression was seen in large sized tumors (P-value : 0.001).*
Conclusion : *The assessment of MVD and VEGF may have applications in the evaluation of prognosis and as a therapeutic target in primary breast carcinoma.*
he abstract should summarize the content of the paper. Try to keep the abstract below 200 words. Do not make references nor display equations in the abstract. The journal will be printed from the same-sized copy prepared by you. Your manuscript should be printed on A4 paper (21.0 cm x 29.7 cm). It is imperative that the margins and style described below be adhered to carefully. This will enable us to keep uniformity in the final printed copies of the Journal. Please keep in mind that the manuscript you prepare will be photographed and printed as it is received. Readability of copy is of paramount importance.
Keywords: *Idc-Nos, Mvd, Vegf*

I. Introduction

Carcinoma of Breast is the most common malignancy in women. The incidence is 30-33 per 100000 women in urban Indian population and 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 of breast carcinoma 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.

II. Aims And Objectives

1. To detect the intra tumoral microvessel density by counting the microvessels in the hot spot areas microscopically in breast carcinoma.
2. To detect Angiogenesis by using VEGF in breast carcinoma.
3. To correlate VEGF expression with MVD and histopathological grading of breast carcinoma

III. Materials & Methods

The present study was a descriptive prospective study of breast carcinomas conducted in the Institute of Pathology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai, during the period between January to December 2013.

Out of 273 cases of breast cancer 50 cases were selected for our study.

Method of data collection:

Clinical details, were obtained for all the 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 and histomorphological grading was done by using Scharff-Bloom-Richardson (SBR) grading system which was based on parameters including percentage of Tubular pattern ,Nuclear pleomorphism and mitotic activity.

Micro Vessel Density (MVD):

Micro vessel density 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). VEGF expression in the tumour cells was analysed for 40 cases and evaluated based on the degree of staining of the cytoplasm of tumor cells. A semi quantitative method was used with scores of 0-3.

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

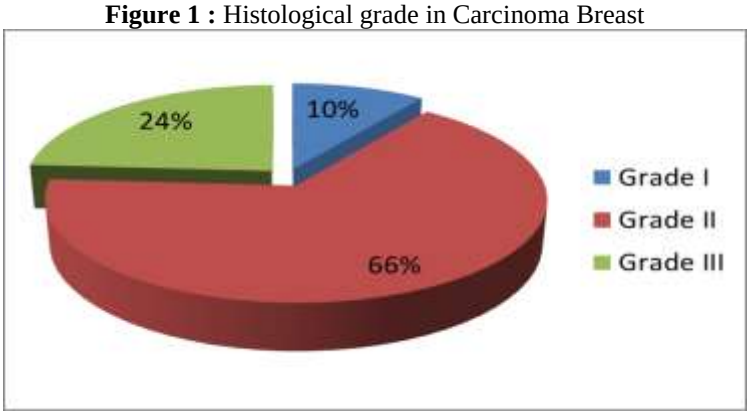
IV. Observation & Results

In the study period of 12 months from January 2012 to December 2012, a total of 649 breast specimens were received in the Institute of Pathology, Madras Medical college for histological examination. Among which the benign breast tumours, malignant breast tumors and non-neoplastic lesions were 276,273 and 88 cases respectively.

Breast carcinoma had a peak incidence in the age group of 41-50 years. The youngest age of presentation was at 25 years in this study. The mean age at diagnosis of breast cancer was 45.62 years, ranging from 25 to 70 years for all patients.

This study showed that the size of the tumor ranged from 2.5 to 4 cm. 23 cases (46%) showed 2.5 cm, and 13 cases (26%) were > 4 cm respectively Among 50 cases of invasive ductal carcinomas, 5 cases were Grade- I. 33 cases were Grade II.

Figure 1 : Histological grade in Carcinoma Breast



The MVD ranged from 19.73 to 48.34 microvessels mm^3 ,median MVD was 32.89,mean MVD was 33.19 for all patients.Thus the cut off value was 32.89 microvessels mm^3 at 400X

Table 1 : Comparison of MVD and Grading of Carcinoma Breast

Grading	Mean MVD	Median MVD	Std. deviation MVD	P-Value
Grade – I	23.68	24.12	2.86	0.001
Grade – II	32.09	30.70	3.09	
Grade – III	40.19	40.56	6.56	

Significant difference has been noted when the mean values of MVD of the various groups defined by Bloom Richardson grading system were compared.

One way ANOVA test was used to evaluate the correlation between grade of breast carcinoma and Cases with high MVD were significantly more numerous in Grade 3 tumors where as Grade I tumors showed low MVD, when the tumors were classified as high or low MVD based on cut off value 32.89 MVD showed a significant relationship with groups defined by Bloom Richardson grading system.

Correlation of Tumor size with MVD

In this study, 14 of them had tumor size of 1-2 cm. This mean MVD level was 35.39 with the standard deviation of 6.06. 23 cases had tumor size 2.5x4 cm and their mean MVD level WAS 31.15 with SD 6.22 and 13 cases had tumor size > 4cm and their mean MVD level was 33.67 with SD 5.86.The non significant P value infers that MVD is independent of the tumor size.

Correlation of VEGF expression and MVD in primary carcinoma breast

Out of total 50 cases, 40 cases were analysed immunohistochemically for angiogenesis by VEGF. Among the 40 cases, 23 cases showed strong positivity for VEGF and 17 cases showed moderate reaction.

Table 2 : Comparison of MVD and Grading of Carcinoma Breast

VEGF Expression	Mean MVD	Median MVD	Std. deviation MVD	P-Value
3+++ strong positivity	33.68	32.89	6.43	0.398
2++	33.41	30.70	5.40	

When the median MVD and VEGF expression status was compared, high MVD was observed in tumors with strong VEGF expression than those with moderate reaction. This indicates that MVD is increased proportionately with strong expression of VEGF. But the p-value was not significant statistically.

Among 29 Grade-II patients, 15 showed strong VEGF reaction and 14 showed moderate reaction. Among 9 Grade-III patients, 4 were strongly positive for VEGF and 5 were moderate positive. The non significant. p-value implied that VEGF expression was independent of tumor grade . since the p-value was not statistically significant.

Micro Vessel Density

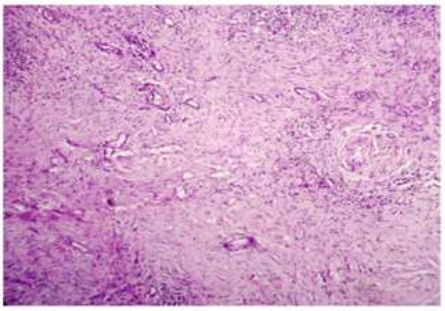


Figure 1- HOT SPOT AREA

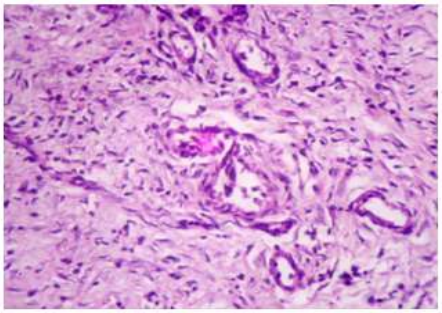


Figure 2- HOT SPOT AREA

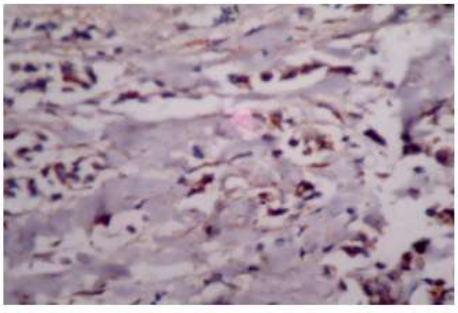


Figure – 3 VEGF 2+

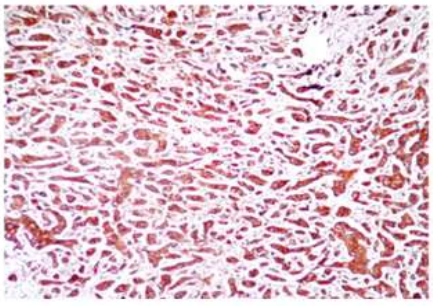


Figure – 4 VEGF 3+

V. 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 in breast carcinoma (IDC NOS Type) was assessed by counting MVD in the hot spot areas microscopically and immunohistochemical evaluation was done by using VEGF and an attempt was made to correlate theMVD and VEGF expression . This study showed that 10% of tumors were Grade I,66% and 24% were Grade II and Grade III respectively.

Lysa Ryden et al found more number of Grade 2 tumors in their study of 54 cases [20]. Bloom Richerdson grading identified a greater proportion of tumors as Grade 3 and Nottingham grading identified as Grade I tumors.

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 MVD group and 54% in high MVD group.

Table 3 : 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 they 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 CNB and subsequent excised specimens and they 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. Grade 3 tumors showed high MVD and tumors with high MVD are significantly more numerous. Grade I tumors showed low MVD. Significant difference has been noted when the mean value of the MVD of Grade I, Grade II and Grade III were compared. In our study MVD showed a relationship with groups define by Bloom Richardson grading system. Hence the prognostic factors like tumor grading (Bloom Richardson Grading system) and MVD assumed clinical significance. Our study results indicated that angiogenesis assessed by counting of MVD and MVD scoring strongly correlates with tumor grade. Hence the quantification of angiogenesis by MVD score at first diagnosis in patients with primary breast carcinomas might be useful in predicting the prognosis of these patients.

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.[152] Among 9 patients with Grade III tumors, 4 showed (3+) VEGF positivity & 5 showed (2+) positivity. But VEGF expression was found to be independent of tumor grade, since the p-value was statistically insignificant.

Our study showed strong VEGF expression with increased MVD and larger tumor size. This was almost similar to the study by Toi et al[,152] and Ludini et al[.157] This study also showed that VEGF expression was low in tumors with high histological grade (Grade III). But these correlations were not found to be statistically significant. Hence it was inferred that VEGF is an independent angiogenic factor.

VI. Summary

Grade 3 tumors showed high MVD & Grade I tumors showed low MVD. MVD showed statistically significant correlation with groups defined by Bloom Richardson grading system. The above results of our study indicates that MVD correlates significantly with tumor grade and VEGF expression correlates significantly with tumor size. These findings show that the quantification of MVD and VEGF expression in primary tumor may be useful in predicting the prognosis of the patient at the time of first diagnosis.

References

[1]. Agarwal G, Ramakant P, Breast Cancer Care in India: The Current Scenario and the Challenges for the Future. Breast Care 2008; 3(1):21-27.
[2]. Breast cancer incidence and mortality worldwide in 2008, Cancer fact sheet, Globocan 2008, P1-3.
[3]. Mansour EG, Ravdin PM, Dressler L: Prognostic factors in early breast carcinoma. *Cancer* 1994; 74:381-400.
[4]. Uzzan B, Nicolas P, Cucherat M, et al. Microvessel density as a prognostic factor in women with breast cancer : A systematic review of the literature and meta-analysis. Cancer Res 2004;64:2941-2955.
[5]. Angiogenesis of Breast Cancer : JCO (2005) 23(8): 1782-1790.
[6]. Baillie CT, Winslet MC, Bradley NJ, Tumor vasculature : A potential therapeutic target. Br J Cancer 1995; 72:257-267.
[7]. Yancopoulos GD, Klagsfrun M, Folkman J. Vasculogenesis, angiogenesis and growth factors : Ephrins enter the fray at the border. Cell 1998 ; 3:661-664.
[8]. LEEK R. D., The prognostic role of angiogenesis in breast cancer,Anticancer Res, 2001, 21(6B):4325–4331.
[9]. GASPARINI G., Clinical significance of determination of surrogate markers of angiogenesis in breast cancer, Crit Rev Oncol Hematol, 2001,37(2):97–114.
[10]. Goulding H., Abdul Rashid N. F., Robertson J. F., Bell J. A., Elston C. W.,Blamey R. W., Ellis I. O., Assessment of angiogenesis in breast carcinoma:an important factor in prognosis?, Hum Pathol, 1995, 26(11):1196–1200.
[11]. Vascularization in primary Breast Carcinomas : Its prognostic significance and relationship with tumor cell dissemination. Clin.Cancer Res. (2008) 14(8):2341-2350.
[12]. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;146:1029-39.
[13]. Toi M, Hoshina S, Takayanagi T, Tominaga T. Association of vascular endothelial growth factor expression with tumor angiogenesis and with early relapse in primary breast cancer. Jpn J Cancer Res 1994;85:1045-9.
[14]. Connolly DT, Heuvelman DM, Nelson R et al. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989;84:1470-1478.
[15]. Nicosia RF. What is the role of vascular endothelial growth factor-related molecules in tumor angiogenesis? [comment]. Am J Pathol 1998;153:11-16.
[16]. Senger DR, Van de Water L, Brown LF et al. Vascular permeability factor (VPF, VEGF) in tumor biology. Cancer Metastasis Rev 1993;12:303-324.
[17]. Salven P, Lymboussaki A, Heikkilä P et al. Vascular endothelial growth factors VEGF-B and VEGF-C are expressed in human tumors. Am J Pathol 1998;153:103-108.
[18]. Guidi AJ, Schnitt SJ, Fischer L et al. Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in patients with ductal carcinoma in situ of the breast. Cancer 1997;80:1945-1953.
[19]. Anticancer Research 4:371-375(2004) Assessment of Microvessel Density in Core Needle Biopsy Specimen in Breast Cancer. Lisa Ryden1, Poul Boiesen2 andPer-Ebbe Jonsson1.
[20]. Lisa Ryden et al. Assessment of MVD in Core Needule Biopsy specimen in breast cancer, Department of Surgery, Helsing Horg, Sweden.
[21]. Angiogenesis and tumor grading in primary breast cancer patients – Vamsu et al, Department of Histology, Faculty of Medicine, Ovidius University, Constanta.
[22]. Breast Cancer – New Horizon in research and treatment – Jeffreys 2001.
[23]. Ludovini V, Sidoni A, et al. Evaluation of the prognostic role of VEGF and MVD in breast carcinoma patients 2003; 81(2) : 159-168.