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Significance of Blood Microvessel Density Quantitation in Breast Carcinoma

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Abstract
Background: *Metastasis is the leading cause of mortality in patients diagnosed with breast cancer. The common pathways of cancer cell dissemination are via the lymphatics and the bloodstreams.*
Objective*: The aim of the present study was to assess the significance of blood microvessel quantitation using CD105 in breast carcinoma and its correlation with tumor grade.*
Methods: *The study included 50 cases of invasive breast carcinoma. Microvessels were identified by using immunohistochemical stain CD105 in tumoral and peritumoral area.*
Result: *There was a sigtnificant correlation of Peritumoral microvessel density in node positive and node negative cases was. Intratumoral and peritumoral microvessel density in node positive cases was also significantly correlated. However, no correlation was seen with different grades of tumor.*
Summary: *A significant intra and peritumoral microvessel density in node positive patients indicate that their expression have clinical utility in defining risk for nodal metastasis in breast cancer.*
Key words*: Breast, carcinoma, microvessel density, CD105, metastasis*

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I. Introduction

Breast carcinoma is the most common malignant tumor and leading cause of carcinoma death in women, mainly due to metastasis. Angiogenesis is essential for tumor growth and metastasis. The extent of neovascularization in invasive breast carcinomas is an independent predictor of metastasis either to axillary lymph nodes, distant sites or both. [1] Several markers of blood vessel endothelium have been developed for routine use, including CD31/PECAM-1, CD34, and Factor VIII-related antigen (von Willebrand Factor or vWF).[2] However, CD105 is overexpressed on endothelial cells of tissues undergoing neovascularisation and is strongly expressed in breast cancer. Quantification of microvessel density has been suggested to have prognostic significance in selected neoplasias.[3] The aim of the present study was to assess the significance of blood microvessel quantitation using CD105 in breast carcinoma and its correlation with tumor grade.

II. Material And Methods

The study was conducted on specimens from 50 cases of breast cancer undergone radical or modified radical mastectomy.. Specimens were examined grossly for tumor size, consistency, margins, and cut surface along with axillary lymph node status. Specimens were fixed and processed by routine histological technique for paraffin embedding. Representative blocks were prepared from tumor, peritumoral tissue, tumor margins, overlying skin, deepest resection margin and axillary lymph nodes. Histopathological diagnosis was established on routine haematoxylin and eosin stain (H & E) and all the histologic prognostic parameters including histologic type, histologic grade, tumor necrosis, lymphatic vessel invasion and lymph node metastasis were assessed. Histologic grading was done by Modified Bloom-Richardson system (MBR). Angiogenic profile of the tumor and peritumoral tissue was assessed by CD105 immunostain. IHC was performed by peroxidase-antiperoxidase method. Positive and negative controls were run with each batch of immunohistochemical stain. Positive control for CD105 was section from tonsillar tissue.

Interpretation of Results

Blood microvessel density was calculated using Olympus BX51 microscope with image analysis software, image Pro Plus Version 6.3. The four most vascularized areas within tumor, peritumoral areas were identified at low magnification (40X) and vessels were counted in a representative high magnification field (400X), in each of these four areas. Mean blood microvessel density was calculated by taking the average of four counts. Microvessel was defined as any highlighted endothelial cell or endothelial cell cluster clearly

separated from adjacent microvessels, tumor cells and other connective tissue elements. Vessel lumen was not necessary for a structure to be defined as microvessel. Blood vessel endothelium showed brown membranous positivity with CD105.

III. Results

The patients included were in the age group of 21 to 80 years with a mean age of 48.3±11.8 years. 54% of the patients were premenopusal and 46% were postmenopausal. 66% of the cases belonged to grade II followed by grade I (30%). Grade III tumors were least common constituting only 4% of cases. Sixty percent cases were lymph node positive with 32% falling into stage II (1-3 positive lymph nodes) and 28% falling into stage III (≥4 positive lymph nodes). 40% of cases did not show lymph node involvement.

The mean peritumoral microvessel density (P-MVD) of lymph node positive and lymph node negative breast carcinoma was 54.82±20.37 and 43.82±16.20 vessels per mm^2 respectively with a p value of 0.04, which was statistically significant. The mean intratumoral microvessel density (I-MVD) of lymph node positive and lymph node negative breast carcinomas was 52.59±20.47 and 53.01±18.42 vessels per mm^2 respectively with a p value of 0.94, which was statistically not significant.

The mean I-MVD and P-MVD of lymph node positive breast carcinoma was 52.59±20.47 and 54.82±20.37 vessels per mm^2 respectively. There was a positive correlation between I-MVD and P-MVD with a p value of 0.000, which was statistically significant. The mean intratumoral and peritumoral MVD of lymph node negative breast carcinoma was 53.01±18.42 and 43.82±16.20 vessels per mm^2 respectively with a p value of 0.10, which was statistically not significant..

The mean I-MVD of Grade I, Grade II and Grade III breast carcinoma was 61.86±23.28, 47.59±15.64 and 69.78±22.09 vessels per mm^2 respectively with a p value of 0.15, which was statistically insignificant. The mean P-MVD of Grade I, Grade II and Grade III breast carcinoma was 50.80±22.35, 50.09±18.62 and 53.12±19.14 vessels per mm^2 respectively with a p value of 0.94, which was statistically not significant.

IV. Discussion

Metastasis relies heavily on development of new blood vessels (angiogenesis) and lymphatics (lymphangiogenesis).[4] Angiogenesis is crucial for tumor development and progression and antiangiogenic therapy represents a promising approach for cancer treatment. The original concept of antiangiogenic therapy as an alternative adjuvant to traditional anti-cancer therapies has attracted enormous attention.[5]

Microvessel density (MVD) as a measurement for angiogenesis, has been found to be strongly associated with features of tumor aggression as larger size and poor differentiation, however its role as a prognostic factor has not been firmly established.[6] Microvessel quantitation may be used as an indicator of the existence of occult systemic metastasis in breast cancer patients with no clinical evidence of metastatic disease as well as a predictor of death in breast cancer patients.[7]

In present study CD105 stained microvessels were seen both in the intratumoral region as well as in peritumoral region. The vessels in intratumoral region were flattened which was in agreement with Dales et al[8] who observed that the CD105 immunostaining was distributed regularly along the small vessels as thin, linear deposits. It has been hypothesized that higher angiogenic state have an increased probability of metastasis. Without it tumor cells are rarely shed into the circulation. New proliferating capillaries have fragmented basement membrane and are leaky which makes them permeable to tumor cells than mature vessels.Thus, angiogenesis involves interaction between a variety of cells, growth factors and components of the extracellular matrix, regulated by pro-angiogenic and anti-angiogenic factors.

Present study showed that mean intratumoral MVD of lymph node positive and negative breast carcinomas was 52.59±20.47 and 53.01±18.42 vessels per mm^2 respectively with a p value of 0.94, which was statistically not significant. Our study was concordant with Visscher et al[9], Kumar et al[1], Ogawa et al[10] and Boneberg et al[11] suggesting that intratumoral microvessel density in invasive carcinoma breast did not correlate with lymph node metastasis.

However our study was in discordance with Choi et al[2], Hansen et al[12] and El gohary et al[13] who observed that intratumoral vascular density in invasive carcinoma breast correlated with lymph node metastasis supporting the theory that angiogenesis is necessary for tumor cells to metastasize.

TABLE XXIV

	I-MVD		**P-value**
	LN +	**LN -**	
Present Study	52.59±20.47	53.01±18.42	0.94
Visscher et al[65]			>0.05
Ogawa et al[61]			>0.05
Boneberg et al [72]			>0.05
Kumar et al[9]			>0.05

Choi et al[4]		<0.05
Hansen et al[67]		<0.05
El Gohary et al[56]		<0.05

In present study the mean peritumoral MVD in lymph node positive and negative cases was 54.82±20.37 and 43.82±16.20 vessels per mm^2 respectively with a p value of 0.04, which was statistically significant. This was in concordance with El Moeim et al[14] who observed that the vascular hot spots were most frequently seen at the margins of the invasive carcinoma and exhibited a significantly greater density of vessels compared to adjacent normal lobules. They confirmed that the tumor vascular counts obtained are at least to some extent due to neovascularization and that angiogenesis is important for tumor growth and progression.

However Ch'ng et al[15] stressed that there was no significant difference in microvessel density between the groups for number of lymph node metastasis, tumor size and grade. They observed that out of 282 hotspots evaluated, 197(69.9%) hotspots coincided with the tumor invasive front. The remaining 85(31.1%) hotspots were situated within the centre of the tumors. This phenomenon is perceived as the tumor angiogenic activity at the invasive front which is controlled mainly by angiogenic factors and proliferation of endothelial cells to form new vessels. This is opposed to the inner tumor areas where anti-apoptotic ability might become more crucial for existing blood vessels to survive and the tumor angiogenesis might act as prerequisite permit for vessel invasion.

TABLE XXV

	P-MVD		**P-value**
	LN +	**LN -**	
Present study	54.82±20.37	43.82±16.20	0.04
El Moeim et al[69]			0.006
Ch'ng et al[81]			>0.05

In present study the mean intratumoral MVD of Grade I, Grade II and Grade III breast carcinoma was 61.86±23.28, 47.59±15.64 and 69.78±22.09 vessels per mm^2 respectively with a p value of 0.15 which was statistically not significant. Our study was in agreement with the study of Ogawa et al[10] who observed that the capacity of tumor cells to induce angiogenesis does not always correlate with malignancy and found no correlation between I-MVD and histological grades using Factor VIII immunostain.

However our results were in disagreement with the studies of Mohammed et al[6], El-gohary et al[13] and Hansen et al[12] who observed a positive correlation of microvessel density with histological grade.

TABLE XXVIII

	I-MVD			**P-value**
	Grade I	**Grade II**	**Grade III**	
Present study	61.86±23.28 (n=15)	47.59±15.64 (n=33)	69.78±22.09 (n=2)	0.15
Ogawa et al[61]	52.3±20.9 (n=80)	50.4± 15.0 (n=52)	56.7 ±15.8 (n=23)	>0.05
Mohammed et al[80]				<0.001
El-gohary et al[56]				0.008
Hansen et al[67]				<0.001

n - number of cases

In present study the mean peritumoral MVD of Grade I, Grade II and Grade III breast carcinoma was 50.80±22.35, 50.09±18.62 and 53.12±19.14 vessels per mm^2 respectively with a p value of 0.99, which was statistically not significant.

Our study was in concordance with the study of Ch'ng et al[15] and El moeim et al[14] who observed that there was no significant difference in microvessel density between tumor grades.

TABLE XXIX

	P-MVD			**P-value**
	Grade I	**Grade II**	**Grade III**	
Present study	50.80±22.35 (n=15)	50.09±18.62 (n=33)	53.12±19.14 (n=2)	0.99
Ch'ng et al[81]				0.44
El moeim et al[69]				0.24

n - number of cases

This difference in the studies could be attributed to inter-study variation due to patient selection criteria, staining techniques, methodology used in counting mitosis, blood vessels and in selection of the cut-off level for MVD assessment.In present study the mean intratumoral and peritumoral MVD of lymph node positive

breast carcinoma was 52.59±20.47 and 54.82±20.37 vessels per mm^2 respectively with a p value of 0.000. However the mean intratumoral and peritumoral MVD of lymph node negative breast carcinoma was 53.01±18.42 and 43.82±16.20 vessels per mm^2 respectively with a p value of 0.10. Thus, there was a significant positive correlation between intratumoral & peritumoral MVD in lymph node positive breast carcinoma suggesting role of angiogenesis in metastasis.

V. Conclusion

Peritumoral MVD was significantly associated with lymph node metastasis suggesting that microvessel invasion should only consider tumor emboli outside the tumor margin to be reproducible. However, no such relationship was found with intratumoral MVD. A significant intra and peritumoral lymphatics and microvessel density in node positive patients indicate that their expression have clinical utility in defining risk for nodal metastasis in breast cancer and the identification of these features in early stage of disease in node negative patients would be of great interest allowing for a more effective antiangiogenic drugs.

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LEGENDS

Fig.1 Photomicrograph showing intratumoral area of infiltrating duct carcinoma.(H&E, 400X)
Fig.2 Photomicrograph showing intratumoral microvessels stained with CD105.(IHC, 200X)
Fig.3 Photomicrograph showing peritumoral area of infiltrating duct carcinoma.(H&E, 100X)
Fig.4 Photomicrograph showing peritumoral microvessels stained with CD105.(IHC, 200X)

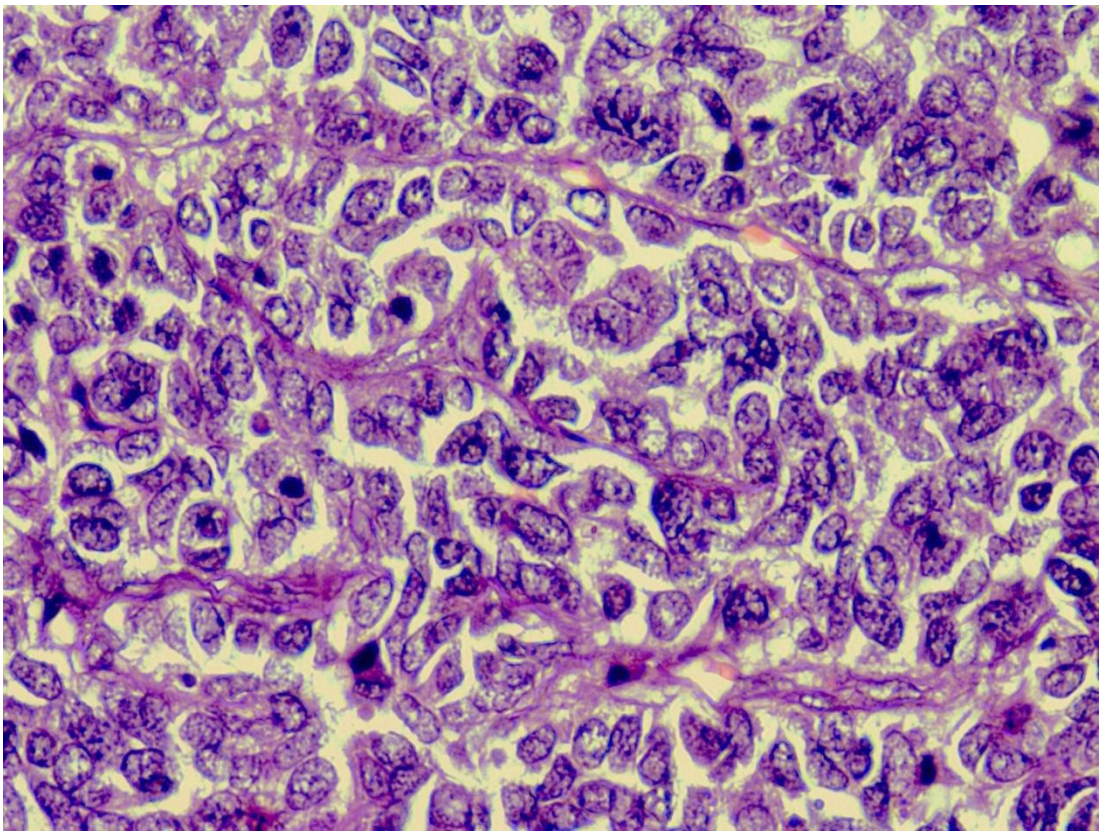

Fig 1

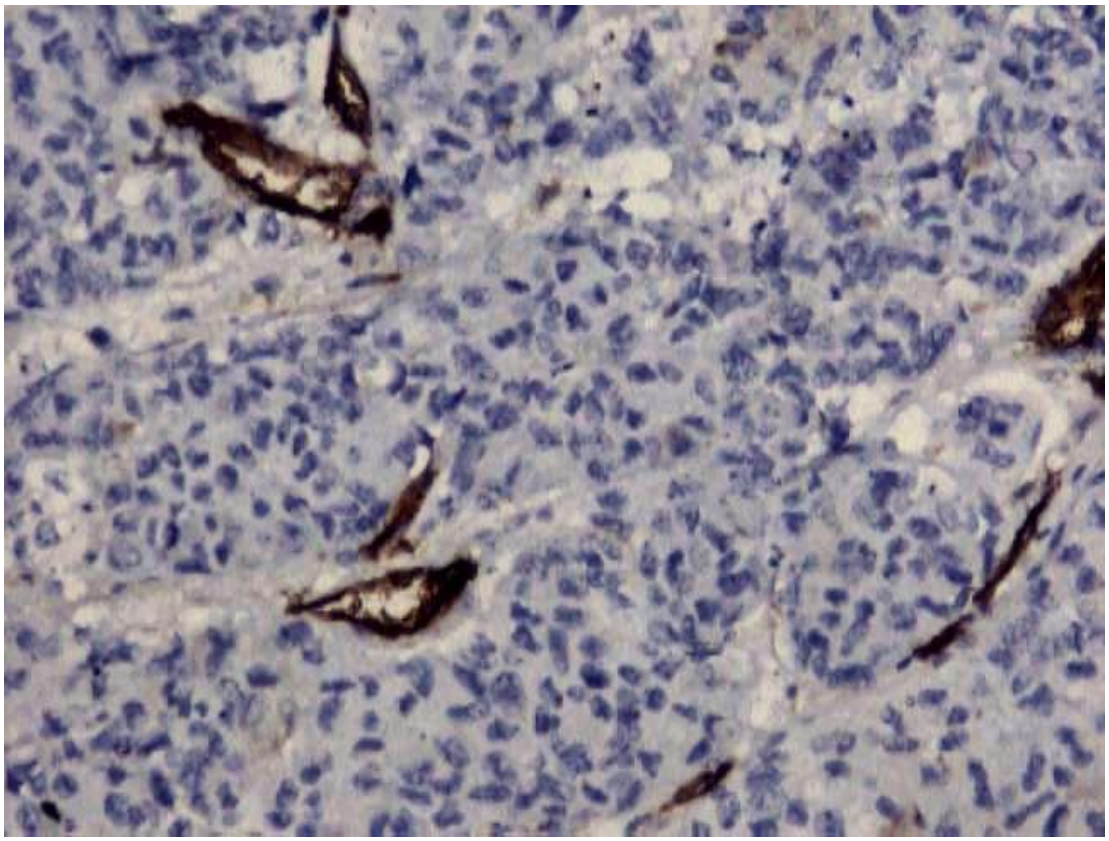

Fig2

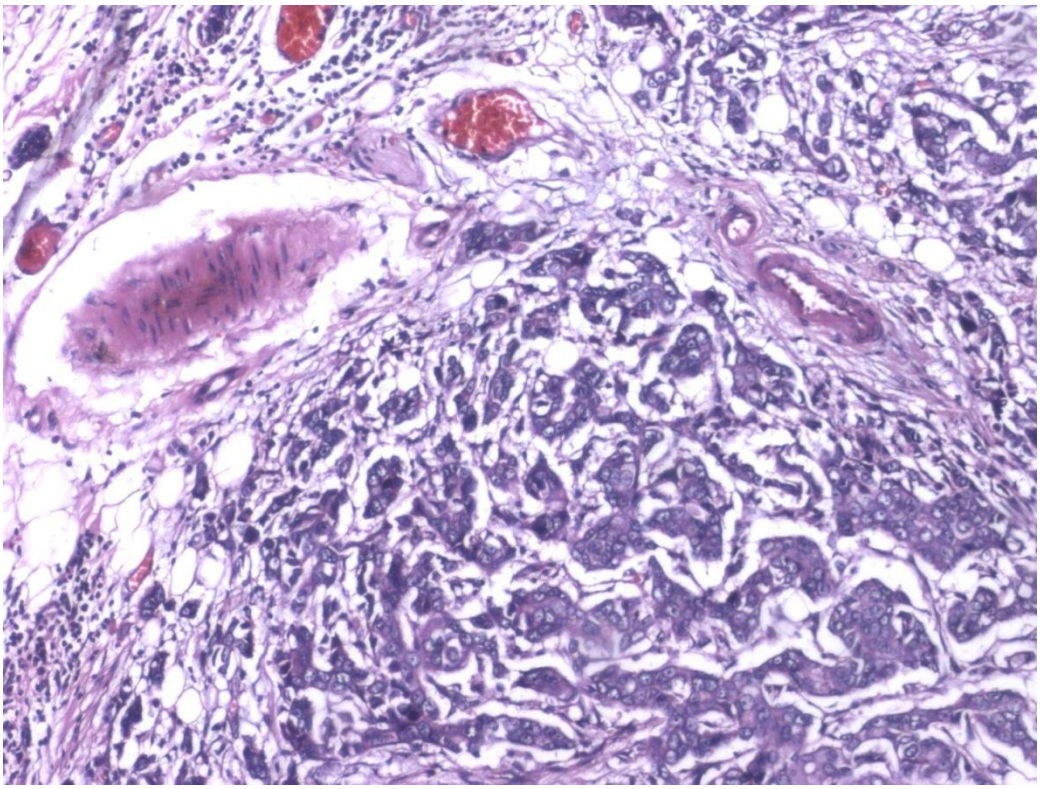

Fig 3

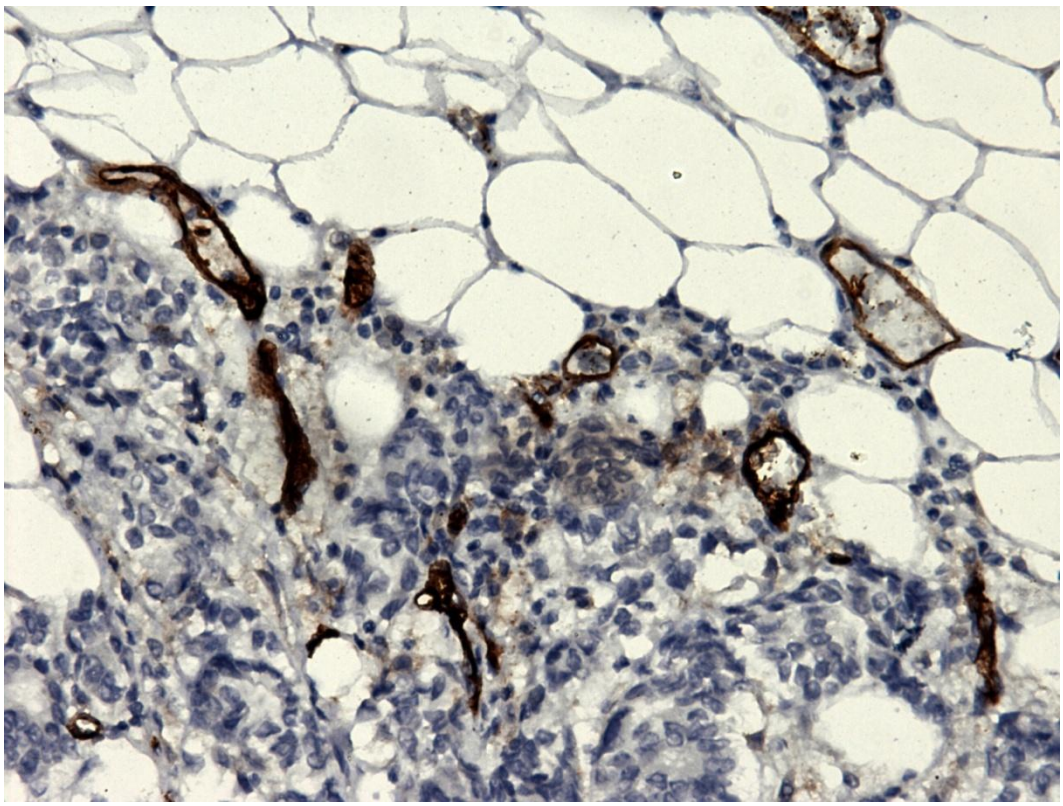

Fig4

Dr Shruti Semwal. "Significance of Blood Microvessel Density Quantitation in Breast Carcinoma." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 3, 2019, pp 14-19.